FIELD AND LABORATORY STUDIES ON SOME COMPOUNDS AGAINST COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.)

El- SHEIKH T. ABDEL-HAMID AND AZIZA E.ABDEL-AAl
Plant Prot. Res. Inst., Agric. Res. Cent., Dokki, Giza, Egypt.

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INTRODUCTION

The cotton leafworm (CLW), *Spodoptera littoralis* (Boisd.) is a highly polyphagous insect pest infesting about 73 host plants including important field crops such as maize, clover, vegetables and various fruits .The extensive use of insecticides to control *S. littoralis* has led to its resistance to the action of various classes of insecticides (Tabashink *et al.*, 1987), residual toxicity and environmental pollution (Frank *et al.*, 1990) and negative effects on non-target organisms (Franz, 1974). The foregoing problems and hazards that have arisen as a result of using conventional insecticides are incentives for researching alternative control agents with new mode of action. Among these agents are the insect growth regulators (IGRs) and the plant extracts. IGRs are group of insecticides have developed chitin synthesis inhibitors (CSI's) such as Hexaflumuron (consult) and Teflubenzuron (No moult). Many IGR's have shown potentiality against lepidopterous insects including *S. littoralis* (Farag, 2001 and Abdel-AAl, 2003). This work aimed to evaluate field and laboratory studies of two plant extracts (Azadirachtin A , Soybean seed oil (30%)) and two insect growth regulators Triflumuron (Alsystin,SIR 8514), Flufenoxuron (10% EC) against cotton leafworm, *Spodoptera littoralis*.

MATERIAL AND METHODS

1. Tested compounds

1.1.Plant extracts

1.1.1. Azadirachtin A, an active ingredient in the extract of seed kernels of neem tree, *Azadirachta idica* A.juss, produced by Trifolio-M Gmbh Company. Lahnau, Germany. The extract is formed as Neemazal1%containing 10000ppm Azadirachtin A.The recommended rate is 200 ml. / 100 L.

1.1.2. Soybean seed oil (30%) produced by INT"Lfor Warding Co., Ltd . The recommended rate is 325 ml./feddan.

1.2. Insect growth regulators

1.2.1.Triflumuron (Alsystin,SIR 8514)

2-chloro-N-{(4-(trifluromethoxy)-phenylamino)carbonyl}-benzamide.

1.2.2 Flufenoxuron (10% EC)

1-[4-(2-chloro-α-α-α-trifluoro-p-tolyloxyl]-2,fluorophenyl]-3-[2,6- difluorobenzoyl) urea.

2- Field experments

Field experments were caried out on Egyptian clover, *Trifolium alexandrinum* L. cultivated in Quisna region ,Menofia Governorate during the two successive seasons 2005 and 2006 .The tested plant extracts and insect growth regulators were applied at the recommended field rate , while control was sprayed with water using solo sprayer .The efficacy of the tested compounds against *S.littoralis* was measured after 3 days of spray .The percentage of reduction in the population density of insects was estemated according to Henderson and Tilton (1955).The initial and residual activity of the tested plant extracts and insect growth regulators against *S.littoralis* in Egyptian clover field were determined .An area of 1.5 feddan divided into 10 equal plots were used .Each plot was divided into 5 subplots as replicats .

3- Rearing technique

A laboratory strain of cotton leafworm *S.littoralis* was maintained under constant conditions and kept away from any contamination caused by chemicals for about 10 generations till the time of study .A field strain was collected as egg -masses of *S.littoralis* from Menofia Governorate and the strain was reared on castor bean leaves according to El-dafrawy *et al.* (2002) under laboratory conditions of 25°C and 65 ± 5% RH.

4-Toxicological studies

For the detection of the median lethal concentration (LC_{50}) values, a series of concentrations dissolved (in water) for each compound was prepared using the commercial formulation of each plant extracts (Neemazal and soyabean)and insect growth regulators compounds (Triflumuron and Flufenoxuron). Castor-bean leaves were dipped for 15 seconds in each concentration then left for one hour to dry. Newly molted 4th instars of both field and laboratory strains larvae were fed on the

treated leaves in glass jars (1 lb.) covered with muslin for 24 hr for each compound, treated leaves were then removed and fresh untreated leaves were provided for another one day. Five replicates (each of 20 larvae) were used for each concentration. Mortality percentages were recorded after 48 hr and corrected according to the control mortality (Abbott, 1925). To estimate the LC_{50} values, the corrected mortality percentages were subjected to probit analysis according to the method of Finney (1952).The resistance ratio between the two comparative strains was calculated according to the equation:

Resistance ratio (R.R) = LC_{50} of Field strain/ LC_{50} of Laboratory strain

5-Biological studies

The 4th instar larvae of *S.littoralis* fed on castor oil leaves treated with LC_{50} of the tested compounds were examined daily and the following biological aspects were determined: larval duration, pupal duration, percentage of pupation, pupal weight, percentage of adult emergence, and life span of both sexes of adult moths.

6-Determination of biochemical aspects

A-Preparation of samples for biochemical studies

The 4th instar larvae of both field and laboratory strain of cotton leaf worm were fed on castor oil leaves treated with LC_{50} of tested compounds till the 6th instars . The collected larvae were placed in clean jars, and then starved for 4 hours. Samples of haemolymph were collected from the starved larvae.

B. Determination of non-specific esterases activity

Alpha- and beta-esterases (α-E, β-E) were determined according to the method of Van Asperen (1962) using α-naphthyl acetate and β-naphthyl acetate as substrates, respectively. Naphthol produced as a result of substrate hydrolysis can be measured by the addition of diazoblue sodium lauryl sulphate solution which produces a strong blue colour in case of α-naphthol or strong red colour in the case of β-naphthol. The colour was measured spectrophotometrically using Milton Roy Spectronic model 1201 spectrophotometer.

C. Determination of acetyl-cholinesterase (AchE) activity

Acetyl-cholin esterase was measured according to method described by Simpson *et al.* (1964) using acetylcholine bromide (AchBr) as substrate. The activity of AchE in the haemolymph was expressed as μg Acetyl-choline bromide/min/ml.

D- Determination of phosphateses activity

a- Acid phosphatase activity was measured according to the method of Laufer and Schin (1971).

b-Alkaline phosphatase activity:The method for acid phosphatase activity was applied for alkaline phosphatase activity determination, but instead of acid buffer (pH 4.8) an alkaline buffer of pH 10.5 (5 ml of 0.2 M glycine + 3.86 ml of 0.2 N NaOH, diluted with 20 ml distilled water) was used. The yellow color releasd by interaction with alkaline phosphatase was then measured (Laufer and Schin, 1971).

E- Determination of Total soluble proteins

Total proteins content was determined in the late 6^{th} instars of *S. littoralis* haemolymph with the folin phenol reagent according to the method of Lowry *et al.* (1951).

RESULTS AND DISSCUTION

1. Initial and residual effect of the tested plant extracts against *Spodoptera littoralis*

Results presented in table (1) indicated that the initial effect of the (Azadirachtin A, Soybean seed oil (30%)) tested plant extracts, expressed as the reduction rates in the percent of infestation recorded 10 ± 0.34, 9.8 ± 0.23 in the first conducted season (2005); whereas, these values were 18.5 ± 0.78, 15.7 ± 0.54 where conducted in the second one (2006) using Neemazal and soyabean extract treatments; respectively. According to the residual effect; the percent of reduction in *S. littoralis* infestation caused after five days of spray recorded 17.24 ± 0.25 and16.3 ± 0.54 in the season of 2005; as compared with 27.5 ± 0.67 and 20.8 ± 0.87 carried out in the second one 2006 using Neemazal ,soyabean extract treatments respectively. While after seven days of spray; the infestation reduction rates recorded 34.5 ± 0.52 and 22.2 ± 0.63 in the season of 2005 30.5 ± 0.51 in comparable with 25.5 ± 0.54 in the second one (2006) using Neemazal ,soyabean extract respectively .It is obvious from the obtained results in table (1) that the reduction percentages in *S. littoralis* infestation after nine days of spray recorded 40.7 ± 0.36 and 31.4 ± 0.54 in the season (2005) and 35.2 ± 0.85 in comparable with 31.9 ± 0.56 in the second one (2006) using Neemazal ,soyabean extract respectively. The general mean residual effect of the tested botanical extract recorded for Neemazal was higher than that which recorded by Soyabeen. This may be

attributed to the higher volatility of Soyabeen extract (Desuky *et al.* (2005). While the higher potent of Neemazal attributed to its toxic effect against the pest .On the other hand, the toxic effect of the plant extract used against 4th instar larvae of *S. littoralis* in the present study is similar to the findings of Hashem *et al.* (1998) using neem extract against *S. littoralis,* and Abd El-Wahab (2002) using acetone extract of *M. azedarach* seeds against *S. littoralis.*

TABLE (I)

Effectiveness of plant extracts on the cotton leafworm, *S. littoralis* expressed as percentage reduction of larval population and residual toxicity after spraying on Egyptian clover at Kwisna region Menofya Governorate, Egypt, seasons 2005 and 2006.

Seasons	Treatment	Rate/feddan	No of Larvae Before spray	No. of larvae and reduction % in larval population at indicated days after spray								Residual toxicity
				Initial effect after		Residual effect						
				3 days		5 days		7 days		9 days		
				No.	Red %	No.	Red %	No.	Red %	No.	Red %	
2005	Neemazal	400ml.	145	130	10.3 ±0.34	120	17.24 ±0.25	95	34.5 ±0.52	86	40.7 ±0.36	25.5 ±0.51
	Soyabeen	325ml.	153	138	9.8 ±0.23	128	16.3 ±0.54	119	22.2 ±0.63	105	31.4 ±0.54	19.4 ±0.52
	control		146	140		135		152		165		
2006	Neemazal	400ml.	200	163	18.5 ±0.78	145	27.5 ±0.67	139	30.5 ±0.51	130	35 ±0.85	27.9 ±0.84
	Soyabeen	325ml.	235	198	15.7 ±0.54	186	20.8 ±0.87	175	25.5 ±0.54	160	31.9 ±0.56	15.57 ±0.54
	control		242	256		248		230		241		

2. The initial and residual effect of tested insect growth regulators against *S. littoralis* infesting clover fields

The results obtained in table (2) indicate that the initial effect expressed as percent of reduction in *S. littoralis* infestation after five days of spray recording 96.1 ± 0.45 and 94.7 ± 0.025 in season 2005 as compared with, 96.8 ± 0.056 and 98.2 ± 0.025 in season 2006 using Triflumuron and Flufeoxuron treatment respectively. The percent reduction in *S. littoralis* infestation after ten days of spray recorded 98.3 ± 0.035 and 97.9 ± 0.075 in the season 2005, 98.8 ± 0.027 and 98.8 ± 0.078 in the second season (2006) using Triflumuron and Flufeoxuron trteatment; respectively. The percent reduction in the rate of *S. littoralis* infestation after fifteen days of spray recorded 99.1 ± 0.075 and 98.6 ± 0.45 in season 2005, while these values were 99.3 ± 0.025 and 99.2 ± 0.042 when the compounds carried out in season 2006 using Triflumuron and Flufeoxuron, respectively. Generally, there is no significant

difference among the general mean percent of reduction in the rate of *S. littoralis* infestation as a result of applying the two tested IGRs in both seasons 2005 and 2006. It is obvious that the two tested IGRs induced high reduction in *S. littoralis* infestation in both seasons up to 15 days after spray. Generally, these findings associated to the reduction percentages in infestation in *S. littoralis* on clover field are in agreement with those of Desuky *et al.* (2005).

TABLE (II)

Effectiveness of insect growth regulators on the cotton leafworm, *S. littoralis* expressed as Percentage reduction of larval population and residual toxicity after spraying on Egyptian clover at Kwisna region Menofya Governorate, Egypt, seasons 2005 and 2006.

Seasons	Treatment	Rate	No of larvae Before spray	No. of larvae and reduction % in larval population at indicated days after spray						
				Initial effect after		Residual effect				Residual toxicity
				5 days		10 days		15 days		
				No.	Red %	No.	Red %	No.	Red %	
2005	Triflumuron	50ml/100 l	130.5	5.3	96.1 ± 0.54	2.2	98.3 ± 0.035	1.6	99.1 ± 0.075	97.8 ± 0.055
	Flufenoxuron	200ml/feddan	160.2	8.38	94.7 ± 0.025	3.3	97.9 ± 0.075	2.1	98.6 ± 0.45	97.06 ± 0.026
	Control		146.5	140	-	135	-	152	-	
2006	Triflumuron	50ml/100 l	200.2	6.3	96.8 ± 0.056	2.1	98.8 ± 0.027	1.3	99.3 ± 0.025	98.3 ± 0.42
	Flufenoxuron	200ml/feddan	235.4	4.2	98.2 ± 0.025	2.8	98.8 ± 0.078	1.7	99.2 ± 0.042	98.3 ± 0.052
	Control		342.9	354	-	324	-	362	-	

3. Toxicological studies

Table (3) show the susceptibility of both laboratory and field strain of *S. littoralis* to tested compounds .The data indicated that Triflumuron proved to be the most effective compound against the 4th instars of the laboratory CLW followed by Flufenoxuron , Neemazal and Soyabeai oil , where the LC_{50} values were 0.032,

0.082, 10.8, and 12.4 ppm respectively .On the other hand, the two used IGRs namely Triflumuron and Flufenoxuron recorded higher toxic effect on the 4th instars of field CLW than the two plant extracts giving LC_{50} values of 365 , 350 ,410 and 445 for Triflumuron ,Flufenoxuron, Neemazal and Soyabean oil respectively. According to the resistant ratio, the obtained data recorded in table (3) revealed that the fold of resistant varied considerably according to the nature of chemical structure of the two used groups .The high differences in LC_{50s} values were observed between the laboratory and field strains as demonstrated by resistance ratio of Neemazal and Soyabean oil gave lower resistance ratio with degree of folds 22.69 and 25, respectively. While it recorded 426 and 1140 fold for Triflumuron and Flufenoxuron, respectively .All treatments used in the present work caused considerable toxic effects against the larvae of *S. littoralis*, particularly in case of treatment with IGRs. The present result is similar to the findings of the toxicity of the IGRs against *S. littoralis* larvae and to that of the same compounds against *S. littoralis* (Abdel-Aal, 2003). Shaurub *et al.* (1999) reported that the chitin synthesis inhibitors are very toxic to insects and metabolize slowly inside the insect body, this may explain the higher toxicity of IGRs against *S. littoralis* in the present study.

TABLE (III)

Toxicity data and resistance ratios of Nemazal , Soyabean oil, Triflumuron and Flufenoxuron against 4th instar larvae of a laboratory and field strains of*Spodoptera littoralis* collected from Menofia Governorates.

Tested compounds	LC_{50}(ppm)		Resistance ratio(Fold)
	Laboratory strain	Field strain	
Nemazal	10.8	245	22.69
Soyabean	12.4	310	25
Triflumuron	0.32	365	1140.6
Flufenoxuron	0.82	350	426.8

Resistance ratio = LC_{50} of Field strain/ LC_{50} of Laboratory strain

On the other hand, the toxic effect of the tesed plant extract against 4th instar larvae of *S. littoralis* in the present study is similar to the findings on other lepidopteran insects. For example, Hashem *et al.* (1998) used neem suspension against *S. littoralis*, and Abd El-Wahab (2002) used acetone extract of *M. azedarach* seeds against *S. littoralis*. The resistance level of *S. littoralis* to both used groups fluctuated from one year to another. The emergence of resistance to urea derivatives was expected to occur (Keddis *et al.*, 1986). It was originally thought that insects would be

unable to develop resistance to molecules that mimic their own hormones, but there is already evidence of developing resistance to several IGRs .Resistance seems to result from decreasing penetration and increasing metabolism of the compound (Hoffman and Lorenz, 1998). However, it is emphasized that the resistance level cannot be considered serious, and insecticides in these classes should be used rationally to maintain their efficacy for as log as possible. It is concluded that the high levels of resistance to some compounds (IGRs) may be related to the type of pesticide and its widespread and intensive application of this class, at in last decade for the spray programme oriented to management of cotton pests in Egypt.

4- Biological studies on field stain of *S. littoralis*

The obtained data in (Table 4) show that the treatment 4th instar larvae of *S. littoralis* field strain, gave significant decrease in larval duration due to Neemazal , Triflumuron and Flufenoxuron treatments and insignificant decrease in case of using Soyabean oil . On the other hand, Pupal duration was decreased significantly according to Neemazal and Triflumuron treatments, while decreased insignificantly in case of Soyabean oils and Flufenoxuron treatments. Moreover, the data show significant decrease for all treatments in the pupal weight resulted from treating 4th instar larvae. The pupation percentage of all treatments was highly reduced in comparison to control (Table 4). This reduction was 38%, 32%, 18% and 14% for Triflumuron , Flufenoxuron , Neemazal and Soyabean oil treatments, respectively as compared to control. On the other hand, the results in (Table 4) show a reduction in the adult emergence of *S. littoralis* pretreated as 4th instars. The highest reduction in the adult emergence was obtained in case of treatment with Flufenoxuron (28%) and the lowest reduction (8%) was in case of Soyabean compared to control. The obtained data also show a significant reduction in adult longevity for all treatments for both sexes of *S. littoralis*. Haga *et al.* (1984) reported that the excellent bioefficiency of used IGR's as chitin synthesis inhibitors (Triflumuron, Flufenoxuron) seemed to be due to the slow detoxification in the insect body. IGRs used in this study significantly affected different biological parameters as compared to control. The decrease in both larval and pupal duration, percentage of pupation and of adult emergence, adult longevity of *S. littoralis* due to treatment with IGR, is similar to the data obtained by many workers using different chitin synthesis inhibitors against many lepidopterous insects, *e.g., S. littoralis* (EL-Deeb *et al.*, 1991 ; Sokar , 1995; Abdel Aal, 2003), *A .ipsilon* (El-Kady *et al.*, 1990 ; Shaurab *et al*, 1999). The decrease in the larval and pupal duration may reflect metamorphosis disruption. The decrease in percentage of adult emergence could be due to the fact

that the toxin blocks the maturation of imaginal discs which are primordial for many adult integumentary structures in endopterygote insects (Schneidermann, 1972). As the tested compounds displayed toxic effect ,therefore, the observed decrease in the adult longevity of *S. littoralis* may be explained according to the assumption of Lu *et al.* (1978) that the accumulation of toxic xenobiotics in any organism may be expected to affect longevity which is a complicated balance of such factors as absorption ,excretion intoxication and detoxication; they also added that significant difference in longevity displayed under equivalent xenobiotic stresses must be the result of the degree of protection afforded by the cytochrome P-450 of microsomal system.

TABLE (IV)

The effect of LC_{50} of plant extracts (Neemazal and Soyabean) and insect growth regulators (Triflumuron and Flufenoxuron) on larval duration, pupal duration, Pupal weight, Pupation %, Adult emergence % and Adult longevity for both male and female moth

Compounds	Larval duration (days±S.E)	Pupal duration (days±S.E)	Pupal weight (mg±S.E)	Pupation %	Adult emergence %	Adult longevity (days±S.E)	
						Male	Female
Neemazal	11.6±0.26	7.5±0.12	285±1.89	82	89	13.4±0.24	15.2±0.52
Soyabean	12.7±0.27	8.03±0.32	313±2.1	86	92	13.6±0.21	16.2±0.23
Triflumuron	9.7±0.22	7.3±0.14	234±3.21	62	75	11.3±0.84	14.3±0.26
Flufenoxuron	10.9±1.2	7.8±0.12	259±5.3	68	72	12.4±0.18	14.4±0.34
Control	12.8±0.22	8.57±0.31	415±3.4	96	100	14.8±0.25	17.4±0.23

5 - Biochemical studies

5.1. Determination of α-and *B* esterases and achetyl-cholinesteras activity

Results in table (5) indicated that flufenoxuron recorded the highest elevation in α- esterases activity where the enzyme activity was 132.21 and 146.36 μg α- Naphthol/ min/ml for both the laboratory and field strain, respectivly, compared to 61.71 and 98.24 μg α- Naphthol/ min/ml in control of laboratory and field strain, respectivly. On the other hand, the data in table (5) revealed that Triflumuron gave the highest increase in *B* – esterases for both laboratory and field strain where the enzyme activity recorded 183.5 and 179.3 μg β-- Naphthol/ min/ml for laboratory and field strain ,respectivly, while Soyabeen show the lowest effect on the treated larvae ;where the enzyme activity recorded 130.23 and 142.33 μg β– Naphthol/ min/ml of both laboratory and field strain respectivly, compared to 122.26 and 136.23 μg β-- Naphthol/ min/ml in control of both laboratory and field strain, respectivly. It is clear that both α-and *B* esterases activity were higher in field strain than in laboratory strains .The activity of AchE was increased in case of

treatment with IGRs (Triflumuron and Flufenoxuron) than plant extracts Neemazal and Soyabean; the enzyme activity recorded 130.47and 121.3 μg Acetyl-cholinebromide/min/ml for laboratory strain and 121.46 and 112 μg Acetyl-cholinebromide/min/ml for field strain in case of Triflumuron and Flufenoxuron respectivly. While in both plant extracts (Neemazal and Soyabean), the enzyme activity recorded 121.23 and118.34 μg μg Acetyl-cholinebromide/min/ml and 110.24 and 101.95 μg Acetylcholinebromide/min/ml for Neemazal and Soyabean treatment in case of laboratory and field strains, respectivly . Esterases played an important role in insecticide resistanse of the pest Abdel Megeed, *et al.* (2000) found that the increase in non specific esterases activity in the Menofia field strain of the CLW was higher than that of laboratory strain. Hamdy and Azab (2002) found that the enzyme activity of α-esterase in *S. littoralis* after treatment with IGRs was increased with Chlorfluazuron and Hexaflumuron except in El-menia field strain treated with Chlorfluazuron that was decreased .The levels of beta- esterases enzym were decreased in field strain with all tested compounds except unsuscepteble strain treated with Chlorfluazuron ,the reduction was more obvious in El-Menia field strain than BanySwif strain. Farag (2001) demonstrated that there are many changes in the activity of non–specific esterases as the result of diflubenzuron, abamectin and pyriproxyfen treatment for *S. Littoralis* larvae. Also, El-Nemaky (2000) found that the activity of AchE in whole homogenates of the pink and spiny bollworms full-grown larvae treated with M-pede and MVPII was increased.

TABLE (V)

Effect of LC_{50} of Nemazal , Soyabean and Triflumuron on α - *B* Esterases activity and Acetylecolinesteras of late 6th instars of *Spodoptera littoralis*.

Compounds	**α –esterases μg α-Naphthol/min/ml**		***B* –esterases μg β-Naphthol/min/ml**		**Acetyl- cholinesterase μg Acetyl-cholinebromide/ min/ml**	
	Laboratory strains	Field strains	Laboratory strains	Field strains	Laboratory strains	Field strains
Nemazal	75.81 ±3.65	129.25 ±3.5	175.23 ± 8.16	145.33 ±3.2	121.23 ±5.12	110.24 ±7.3
Soyabean	95.31 ±2.86	125.2 ±5.15	130.23 ± 2.26	142.35 ±6.2	118.34 ±13.12	101.95 ±8.8
Triflumuron	122.14 ±5.76	135.3 ±3.25	183.51 ±19.72	185.37 ±1.2	130.47 ±17.64	121.46 ±5.3
Flufenoxuron	132.21 ±3.5	146.36 ±1.2	170.25 ±3.6	179.31 ±2.4	121.3 ±5.2	112 ±4.21
Control	61.71 ± 4.61	98.24 ± 3.52	122.65 ± 7.30	146.23 ±4.2	117.34 ± 14.30	100.71 ±7.1

5.3 - Determination of phosphatases activity

Results in table (6) revealed that all treatments increased acid phosphatases activity for both laboratory and field strains; the enzyme activity recorded 4.51, 5.22, 3.31.and 2.96 μg phosphate /min /ml for Neemazal, Soyabean, Triflumuron and Flufenoxuron ,respectively, compared to 2.56 μg phosphate/min/ml for control in case of laboratory strain. While the enzyme activity recorded 2.84, 2.30, 4.27 and 4.65 for Neemazal, Soyabean, Triflumuron and Flufenoxuron, respectively compared to 2.11 μg phosphate/min/ml in control in case of field strain.

On contrary, the enzyme activity of alkaline phosphatases was decreased in case of both laboratory and field strains recorded 1.49, 1.21, 1.60 and 1.5 μg phosphate/min/ml for Neemazal, Soyabean, Triflumuron and Flufenoxuron respectively, in case of laboratory strain compared to 1.86 for control. While the enzyme activity recorded 2.84, 2.30, 4.27 and 4.65 for Neemazal, Soyabean, Triflumuron and Flufenoxuron, respectively compared to 2.11 μg phosphate/min/ml in control in case of field strain. 1.11, 1.2, 0.88 and 0.98 for Neemazal, Soyabean, Triflumuron and Flufenoxuron respectively compared to 1.34 μg phosphate/min/ml in control.

Acid and alkaline phosphatases have been shown to be associated with insect development, especially in relation to nutrition and egg maturation (Mathai and Nair, 1982). Acid phosphatase has received considerable attention in developmental studies because of its association with histolysis. This latter process is appreciable at the metamorphic molts of holometabolous insects. Ecdysone is responsible for increase in the number of lysosomes (Radford and Misch, 1971) and of the activity of acid phosphatase (Van Pelt-Verkuil, 1979). This indicates that the increased activity of acid phosphatase in the present study may be due to increased number of lysosomes. In the present study, acid phosphatase activity was also higher than that of alkaline phosphatase. Pant and Lacy (1969) reported that increased activity of acid phosphatase over that of alkaline phosphatase during insect development, particularly during pupal-adult transformation, and suggested that it could be related to an active mobilization of glycogen with dephosphorylation at the acid range.

5.1- Determination of soluble protein

Data in table (6) indicated that all treatments caused reduction in the total protein of both laboratory and field strain; it recorded 31.15, 32.6 , 33.45 and 5.85 mg/ml for Triflumuron, Flufenoxuron , Neemazal and Soyabean treatments respectively in case of field strain compared to 40.35 for control. While recorded 29.48, 31.1, 37.18 and 40.87 mg/ml for Triflumuron, Flufenoxuron , Neemazal and

Soyabean treatments respectively in case of laboratory strain compared to 48.46 for control. Similar results were obtained by Mostafa (1993) and Sokar (1995) for the total haemolymph protein of the same species treated with Triflubenzuron and Hexaflumron, respectively (Florkin and Jeanuiaux, 1964). Wilkinson (1976) stated that proteins help to synthesize microsomal detoxifying enzymes which assist in the detoxification of toxicants that enter into the insect body. Ahmed *et al.* (1985) added that proteins are the most important components of the biochemical milieu of insect that bind with the foreign compounds. In general, the problem of protein synthesis is intimately related to the metabolism of nucleic acids.

TABLE (VI)

Effect of LC_{50} of Nemazal, Soyabean, Triflumuron and Flufenoxuron on total protein (mg/ml) and phosphatases (μg phosphate/min/ml) activity of late 6th instars of *Spodoptera littoralis*.

Compounds	**Acid phosphatase μg phosphate/min/ml**		**Alkaline phosphatase μg phosphate/min/ml**		**Total Protein (mg/ml)**	
	Laboratory strains	Field strains	Laboratory strains	Field strains	Laboratory strains	Field strains
Nemazal	4.51 ± 0.49	2.84 ±1.3	1.49 ± 0.19	2.84 ±1.3	33.54 ±2.37	1.49 ± 0.19
Soyabean	5.22 ± 0.61	2.3 ±0.35	1.21 ± 0.12	2.3 ±0.35	35.85 ±2.77	1.21 ± 0.12
Triflumuron	3.31 ± 0.35	4.27 ±0.42	1.60 ± 0.13	4.27 ±0.42	31.15 ±4.28	1.60 ± 0.13
Flufenoxuron	2.96 ±1.9	4.65 ±0.63	1.52 ±0.15	4.65 ±0.63	32.6 ±2.3	1.52 ±0.15
Control	2.56 ± 0.19	2.11 ±0.21	1.86 ± 0.10	2.11 ±0.21	40.35 ±3.22	1.86 ± 0.10

SUMMARY

Field experiments were carried out on Egyptian clover, in Quisna region, Menofia Governorate during the two successive seasons of 2005 and 2006 .The efficiency of the Neemazal, soyabean extract and Triflumuron, Flufeoxuron compounds against *S.littoralis* was measured and the tested compounds caused reduction in percentage of the population density of insects. The used compounds affected larval and pupal duration ,pupal weight ,pupation percent and adult emergence of e field strain .Also The tested compounds affected on α-and *B*

esterases and achetylcholinesteras activity, phosphatases activity and the total protein of both laboratory and field strain.

REFERENCES

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

ABDALLA, E. F. and E. A SAMMOUR (1991-1992): Comparative study on the efficacy of neem seed extract and diflubenzuron against the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.). *(Bull. ent. Soc. Egypt, Econ. Ser., 19: 53-61).*

ABDEL-AAL, A. E. (2003): Effect of some insect growth regulators on certain biological, biochemical and histological aspects of the cotton leafworm, *Spodoptera littoralis* (Boisd.). *(Unpublished Ph. D. Thesis, Dep. of Entomo., Fac. of Sci, Cairo Univ., Egypt. 119 pp.).*

ABDEL-MEGEED, M. I., M. G. ABBAS., A. I. GADALLA and A. HANAFY (2000): Non specific esterases activities of susceptible and field strains of cotton leafworm, *Spodoptera littoralis* (Boisd.) as affected by certain chitin syntheses inhibitors. *(Ann. Agric. Sci. vol. 4, No. Special, pp. 1585 – 1595, 11 refs).*

ABDEL-WAHAB, H. H. (2002): Biological and biochemical studies on the effect of some botanical extracts on cotton leafworm, *Spodoptera littoralis* (Boisd.) *(Unpublished M. Sc. Thesis, Ain Shams University, Egypt).*

AHMED, M. S., A. F. ALI and A. R. SHAKOORI (1985): Effect of dieldrin on the whole body protein content of *Periplaneta americana. (Pak.J.Zool., 17 (1): 105-109).*

AHMED, Y. M., A. M. A. MOSTAFA and A. SHOUKRY (1990): Effect of hlorfluazuron on transaminase activities in the larvae and pupae of *Spodoptera littoralis. (Rijksuniversiteit Gent, 55 (2b): 621-627).*

AUDA, M. and H. SALEM (1997): Effect of diflubenzuron on growth, development and digestive enzymes of the beet armyworm *Spodoptera exigua* larvae. *(Bull. ent. Soc. Egypt, Econ. Ser., 24: 95-106).*

DESUKY, M. H., M.M. KHEDER., S. I. YOUSIF and S. M. A. EL-SHAKAA, (2005): Field and biochemical studies on same compounds against cotton leafworm, *Spodoptera littoralis*(Boisd). *(Egypt J.Agric .Res .,83(3), 2005).*

El-DAFRAWI, M.; A TAPPOZADA; N.MANSOUR and M. ZEID (1964): Toxicological studies on the Egyptian cotton leafworm, *Prodenia litura* .I. Susceptibility of different larval instar to insecticides. *(J.Econ. Entomol. 57(4):591-593).*

EL-DEEB, M. A., F. E. GHOUNEIM and F. I. HELMY, (1991): Potential use of the juvenile hormone pyriproxyfen for the control of *Spodoptera littoralis. (4th Arab Cong. Plant Protect., Cairo, Egypt: 7-13).*

EL-KADY, M. B., E. H. TAYAB H., A. MESBAH and A. A. SAAD, (1990): The relative efficiency of the pregnancy inhibitory hormone medroxy progesterone acetate (MPA) and the insect growth inhibitor (XRD) on the greasy cutworm, *Agrotis ipsilon* (Huf.) (Noctuidae, Lepidoptera). *(Bull. ent. Soc. Egypt, Econ. Ser., 118: 93-104).*

El-NEMAKY, I. H. I. (2000) : Studies on the effect of integrated pest management elements on physiology of bollworms. *(Unpublished M.Sc. Thesis, Al-Azhar Univ., Egypt 169p).*

El-SAIDY, M., M. AUDA and D. DEGHEELE (1989): Detoxification mechanisms of diflubenzuron and triflubenzuron in the larvae of *Spodoptera littoralis* (Boisd.). *(Pestic. Biochem. Physiol., 35: 211-222).*

FARAG, M. A. (2001): Biochemical studies on the effect of some insect growth regulator on the cotton leafworm. *(Unpublished M.Sc. Thesis, Cairo Univ., Egypt. 155P.).*

FINNEY, D. J. (1971): Probit Analysis, A statistical treatment of the sigmoid response curve. (7th Ed., *Cambridge Univ. Press, Cambridge, England. 467P.).*

FISK, T. and D. J. WRIGHT (1992): Speed of action and toxicity of acylurea insect growth regulators against *Spodoptera exempta* (Walk.) and *Spodoptera littoralis* (Boisd.) larvae: effect of intermoulting. *(Pestic. Sci., 35 (4): 331-337).*

FLORKIN, M. and C. H. JEANUIAUX (1964): Haemolymph composition. *(In "Physiology of Insecta": (Edited by Rockstein, M.). Vol. 3, pp. 109-152. Academic Press, New York & London 746 pp.).*

FRANK, R.; H. E BRAUN; B. D.RIPLEY and B. S. CLEGY (1990): Contamination of rural ponds with pesticides, 1971-1985. *(Ontario, Canada Bull. Environm. Contamin. Toxicol., 13: 771-817).*

FRANZ, J. M. (1974): Testing of side effects of pesticides on beneficial arthropods in laboratory: a review. *(Z. Pfl. Krankh.Pfl. Schutz., 18: 141-174).*

HAMDY A. M. and A. M.H. AZAB (2002): Effect of insect growth regulators and Binary mixtures on enzymes activity of Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) larvae. *(2nd International conference, Plant Protection Research Institute, Cairo, Egypt, 21 – 24 December, 2002, Vol (1), 617 – 623).*

HASHEM, M., S. M. El-MESIRI., F. A. El-MENIAWI, and I. RAWASH (1998): Potency of three plant extracts on the development stage of the cotton leafworm, *Spodoptera littoralis* (Boisd.). *(Alex. J. Agric. Res., 43 (3): 61- 79).*

HOFFMAN, K.H. and M.W. LORENZ (1998): Recent Advances in Hormones in Insect pest control. *(phytoparasitica 26 : 40 327P.)*

KIDDIS , M.E., F.A. AYAD., M.S. ABDEL-FATAH and M. A. El–GUINDY, (1986): Studies of resistance to urea derivatives and their mixtures with insecticides in field strain of the cotton leafworm, *Spodoptera littoralis* (Boisd.) during the cotton seasons 1983 , 1984 and 1985 .*(Bull. Ent. Soc. Egypt Econ. Series 15,229 – 234).*

LAUFER, H. and K. S. SCHIN (1971): Quantitative studies of hydrolytic enzymes activity in the salivary gland of *Chironomus tentans* (Diptera: Chironomidae) during metamorphosis.*(Can. Entomol., 103: 454-457).*

LOWRY, O. H., N. J. ROSEBROUGH., A. L. FARR and R. J. RANDALL (1951): Protein measurement with folin phenol reagent. *(J. Biol. Chem., 193: 265-275).*

LU P., S. Y. YONG and R. METCALF (1978): Influence of alderin, methoxychlor and parathion on longevity of *Musca domestica* and *Phormia regina. (J. Econ. Entomol., 17: 407-415).*

MATHAI, S. and V. S. K. NAIR (1982): Levels of phosphatases during pupal-adult transformation in *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). *(Entomon, 7: 311-313).*

MOSTAFA, S. A. (1993): Biochemical effects of some chemical compounds on *Spodoptera littoralis* (Boisd.). *(Unpublished Ph. D. Thesis, Al-Azhar Univ., Egypt).*

PANT, R. and P. S. LACY (1969): Phosphatase activity in *Philosamia ricini* during development. *(Ind. J. Biochem., 6: 154-156.Press, New York. World Health Organization/Vector Biology and Control/Insect Genetic and resistance. 74: 27).*

RADFORD, S. and D. MISCH (1971): Cytological effects of ecdysterone on the midgut cells of the flesh fly, *Sarcophaga bullata. (J. Cell Biol., 49: 702-711).*

RIDDIFORD, L. M. and J. W. TRUMAN (1978): Biochemistry of insect hormones and insect growth regulators. *(Biochemistry of insects. (M. Rockstien, Ed.). p. 307).*

SENHNEIDERMANN, H.A. (1972): Insect hormone and insect control. *(In: Insect Juvenile Hormone, chemistry and Action. J.J. Menn and M. Beroza (Eds.), Academic press, New York, Landon: 3-27).*

SHAURUB, E. H., S. A.EMARA., N. Z. ZOHDY and A. E. ABDEL-AAL, (1999): Effect of four insect growth regulators on the black cutworm, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae). *(The 2nd Int. Conf. of Pest Control, Mansoura, Egypt, Sept., 1999 PP: 773-776).*

SIMPSON, L.D. BULL and A. D. LIQUIST (1964): A semi-microtecnique for estemation of cholinesterase activity in boll Weevil. *(Ann. Ent. Soc. Am. 57(3) 367- 377).*

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

SOKAR, L. A., A.A. FARGALI and A.Y El-DEEP (1999): Biochemical effect the *Bacillus thuringiensis* on the 4th instars larvae of cotton leafworm *Spodoptera littoralis* (Boisd.). *(J. Agric. Sci. Mansoura Univ., 24(11):6937 – 6943).*

TABASHINK, B. E.; CUSHING, N. L. and JOHNSON, M. W. (1987): Diamond back moth (Lepidoptera : Plutellidae) resistance to insecticides in Hawaii: Intra-island variation and cross resistance. *(J. Econ. Entomol., 80: 1091-1099).*

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

VAN PELT-VERKUIL, E. (1979): Hormone mediated induction of acid phosphatase activity of the fat body of *Calliphora erythrocephala* prior to metamorphosis. *(J. Insect Physiol., 55: 965-973).*

WILKINSON, F. (1976): Insecticide biochemistry and physiology. *(Plenum Press, New York. World Health Organization/ Vector Biology and Control/Insect Genetic and Resistance 74: 27. 519).*