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

DESUKY, W. M. H[1], M. M. A. KHEDR[1], S. I. YOUSIF- KHALIL[2] AND S. M. A. EL-SHAKAA[2]

1- Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.
2- Faculty of Agriculture, Zagazig University, Egypt.

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Abstract

This study was conducted to evaluate the efficiency of three plant extracts i. e. NeemAzal, soybean and garlic (Biorepel) and five insect growth regulators (IGRs) i. e. Consult, Atabron, Cascade, Match and Mimic using the recommended field rate to control the cotton leafworm in cotton and Egyptian clover fields at Zagazig region, Sharkia Governorate in 1999 and 2000 seasons.

Plant extracts: NeemAzal recorded the highest initial and residual reduction percentages in both seasons in cotton fields, the highest initial reduction reached 30.33% whereas the highest residual reduction was 39.88%. However, in clover fields the highest initial reduction reached 32.11% in 1999 season with Biorepel, whereas NeemAzal recorded the highest residual reduction percentage in the same season (36.03%). As for the initial and residual reduction percentages in 2000 season (25.99 and 38.63%, respectively. IGRs, The initial reduction percentages did not recorded reduction percentages 92.00% in both crops for the all tested compounds except Mimic which recorded initial reduction percentages less than 77.00% in 1999 season in cotton field, while the residual reduction did not rise than 87.00% in 2000 season. As a general trend, the IGR Atabron is consider the most effective in reducing the pest population Changes in total soluble protein, transaminase enzymes (GOT and GPT) and carbohydrate hydrolyzing enzymes (amylase, invertase and trehalase) activities in the 4th instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd) as affected by plant extracts laboratory application were studied. Results appeared the following foundings,

1. All the tested compounds decreased the total soluble protein content except the lowest concentration of Biorepel after 2 days.
2. All the tested compounds exhibited high increase in GOT and GPT activities in comparison with the control at all interval times.
3. GPT activity in all tested compounds at all concentrations was higher than check samples.
4. Trehalase, invertase and amylase enzymes activities were higher than the control at all concentrations of all tested compounds.

INTRODUCTION

Cotton, *Gossypium barbadense* L. and Egyptian clover, *Trifolium alexanderinum* L. are two of the most economic agricultural crops in Egypt. Cotton due to its importance as a main cash crop for the Egyptian farmers besides it is the main row material for textile industry, as well as the main source of locally produced cotton seed oil. Meanwhile, Egyptian clover is consider as the principal animal food otherwise it became one of the most dangerous sources which refuge the first generations of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) which re-invade cotton plantations. Cotton leafworm is one of the most economic and wide spread pest not only on clover and cotton, but also on many other vegetable crops and ornamental plants in Egypt, almost all over the year. It takes a lot of government considerations from different points (such as chemical control), so it drove the scientists attention to find out another safer and lesser expensive agents to control insect pests. Plant extracts and insect growth regulators (IGRs) are gaining significant importance in crop protection on account of their favorable properties. They are vary largely selectively, spare beneficial insects and favorable technological properties, so that they especially are suitable in using in integrated pest control programs. The protein content are the most complex and others, in the same time the most characteristic component of living matter the total protein in treating larvae may reflect the decrease in the activity of various enzymes (Kung and Kim 1990). Glutamic-oxaloacetic transaminase (GOT) and Glutamic-pyruvic transaminase (GPT) in insects (Carbtree and Newsholme, 1970) stated that the most active transaminase enzymes.

Metabolism of carbohydrates is controlled mainly by invertase, amylase and trehalase enzymes which play a principle role in the digestion and utilization of carbohydrates by insects (Wigglesworth, 1972) . These enzymes have received a great deal of attention in concern with digestion and utilization of carbohydrates in insects. Trehalase is activated during moulting to generate production of glucose for chitin build up. Invertase and amylase are two important digestive enzymes, however, little is known about their physiological and biochemical contribution to the insecticidal toxicity (Meisner *et al.*, 1978).

The aim of the present study is to provide data about the efficiency of certain plant extracts and IGRs against cotton leafworm under field conditions and to find some biochemical relationships between plant extracts treatment and activities of some enzymes in *S. littoralis* larvae.

MATERIALS AND METHODS

1. Tested compounds

1.1. Plant extracts

Azadirachtin A, an active ingredient in the extract of seed kernels of neem tree, *Azadirachta indica* A. Juss, produced by Trifolio-M Gmbh Company. Lahnau, Germany. The extract is formulated as NeemAzal TS 1% containing 10000 ppm Azadirachtin A. The recommended rate is 200 ml./100 L.

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

Biorepel 10% EC (a. i Garlic juice) manufactured by JH Biotech., Inc. California, U. S. A. The recommended rate is 10 ml./L.

1.2. Insect growth regulators Flufenoxuron (Cascade 10% EC)

[4-[2-chloro 4-(trifluoromethyl phenoxy)] 2-flurophenyl 3-(2,6-difluorophenzoyl) urea. The recommended rate is 200 ml./feddan.

- Chlorfluazuron (Atabron, IKI- 7899 5% EC)

1- (2, 6, - difluorobenzoy l3 - [4 (chloro - 5 - trifluoromathyl-2-pyridyloxy)3,5,-dichlorophenyl] urea. The recommended rate is 400ml. / feddan.

Benzoylphenyl urea (Consult 10% EC)

N - (3, 5- dichloro – 4 - (1,1,2,2, - tetrafluoroethoxy)-phenylamino) carbanyl 2,6-difluorobenzamide. The recommended rate is 200 ml./feddan.

Lufenuron (Match 5% EC)

N-[[[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy]- (phenyl]amino] carbonyl] – 2,6 – difluoro – benzamide. The recommended rate is 400 ml./feddan.

Tebufenozide (Mimic 24% EC)

3,5- dimethyl benzoic acid 1- (1,1 dimethyl ethyl) 2-(4- ethylbenzoyl) hydrazide. The recommended rate is 350 ml./feddan.

2. Field experiments Field experiments were carried out on both Egyptian clover, *Trifolium alexanderinum* L. and cotton, *Gossypium barbadense* L. (variety Giza 85) cultivated in Zagazig region, Sharkia Governorate during the two successive seasons of 1999 and 2000. 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 efficiency of the tested compounds against *S. littoralis* was measured after 5 days of spray (Gomaa *et al.*, 1996, Badr, 2000). The percentage of reduction in the

population density of insects was calculated according to Henderson and Tilton (1955) equation:

2.1. Initial and residual activity of the tested plant extracts and insect growth regulators against *Spodoptera littoralis.*

2.1.1. In cotton fields An area of 2.5 feddans divided into 10 equal plots for the three plant extracts, five IGRs and one plot as control for each group was used. Each plot was divided into four replicates of 1/16 feddan. A pre-treatment count was made for each treatment so as the control. Twenty-five marked cotton plants presented in each replicate. Post treatment count were recorded after 3, 5, 7 and 9 days for plant extracts treatment (Badr 2000) and after 5, 10 and 15 days for IGRs treatment (Gomaa *et al.*, 1996 and Badr 2000). The initial effect was calculated at 3 and 5 days post treatment for plant extracts and IGRs, respectively. The general mean residual effect was calculated as the mean reduction percentages of larvae observed at days (5, 7 and 9, 10 and 15 post treatment) for plant extracts and IGRs, respectively.

2.1.2. In Egyptian clover fields An area of 1.5 feddans divided into 10 equal plots was used. Each plot divided into four parts as replicates. The area of each replicate (6 × 21 m), with untreated belt (6×21 m) between each two replicates. Pre treatment count for each treatment and control was recorded in one-meter square randomized in each replicate.

The initial and the general mean residual activity of the tested compounds were calculated using the same precedent procedures in cotton fields.

3. Rearing technique the strain of *S. littoralis* was reared on castor bean leaves according to El-Defrawi *et al.* (1964) and some modification of Khedr (2002) under laboratory conditions25 ± 2 °C and 65 ± 5 % R.H.

4. Preparation of samples for biochemical assay the preparation of samples involved the use of the 4^{th} instar larvae after 2, 5 and 7 days of application. For each concentration applied, 5 larvae were picked up and placed in clean jars, then starved for 4 hr. The starved larvae were homogenized in distilled water (5 larvae/5 ml.) using a teflon homogenizer surrounded with jacket of crushed ice for 3 minutes. The homogenate was centrifuged at 3500 r.p.m for 10 minutes at 5 °C. The supernatant was immediately assayed to determine total soluble protein and the activities of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transminase (GPT), trehalase, amylase, and invertase enzymes.

5. Determination of total soluble protein Colourimetric determination of total soluble protein in total homogenate of larvae of *S. littoralis* was carried out as described by (Gornall *et al.*, 1949).

6. Determination of enzymes activities:

6.1.Transaminase enzymes (GOT and GPT) the activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transminase (GPT) enzymes were determined colourimetically according to the method of Reiteman and Frankle (1957).

6.2. Carbohyd ate hydrolyzing enzymes (trehalase, invertase and amylase): The methods used to determine the activities of trehalase, amylase and invertase enzymes in digesting trehalose, starch and sucrose, respectively were determined according to Ishaaya and Swiriski (1976).

RESULTS AND DISCUSSION

1. Initial and residual activity of the tested plant extracts against *Spodoptera littoralis* infesting cotton and Egyptian clover fields:

1.1. Cotton fields

- **Initial effect (After three days)** the results presented in Table 1 indicate that the initial effect of the tested plant extracts, expressed as the rate of reduction in the percent infestation recorded 25.45 ± 0.32, 24.57 ± 0.25 and 25.08 ± 0.36 %, in the first season (1999) 30.33 ± 0.29, 23.70 ± 0.14 and 24.55 ± 0.19 %, in the second one (2000) for NeemAzal, soybean and Biorepel extracts, respectively .Table 1

- **Residual effect**

- **After five days** Percent reduction of *S. littoralis* infestation after five days of spray recorded 31.38 ± 0.56, 12.02 ± 0.60 and 15.06 ± 0.50 %, in 1999 season, 37.57 ± 0.18, 26.54 ± 0.14 and 25.84 ± 0.50 %, in 2000 season for NeemAzal, soybean and Biorepel, respectively .Table1

- **After seven days** Percent reduction in the rate of *S. littoralis* infested cotton fields after seven days of spray recorded 33.68 ± 0.48, 12.85 ± 0.28 and 13.42 ± 0.34 %, in the first season, 43.30 ± 0.28, 27.22 ± 0.70 and 24.40 ± 0.33 %, in the second season, for the three tested extracts, respectively Table 1.

- **After nine days** It is obvious from the results obtained and presented in Table 1 that the reduction percentages of *S. littoralis* infestation due to the treatment of the tested plant extracts recorded 32.71 ± 0.32, 17.45 ± 0.56 and 12.16 ± 0.36 %, in 1999 season, 38.76 ± 0.32, 31.07 ± 0.61 and 23.30 ± 0.40 %, in 2000 season, respectively.

Table 1. The mean number of all larval instars and reduction percentages of cotton leafworm, *Spodoptera littoralis* (Boisd.) as affected by plant extracts sprayed on cotton fields during 1999 and 2000 seasons at Zagazig region, Sharkia Governorate.

Season	Treatments	Recommen-ded rate/feddan	Mean No. of larval instars before spray	No. of insects/plant and % reduction								General mean of residual effect	
				Initial effect after:		Residual effect after:							
				3 days		5 days		7 days		9 days			
				No.	% Red.	No.	% Red.	No.	% Red.	No.	% Red.	No.	% Red.
1999	NeemAzal	400ml	40.20	36.00	25.45±0.32	40.01	31.38±0.56	34.76	37.68±0.48	31.39	32.71±0.3	35.38	33.92±0.44
	Soybean	325ml	33.00	29.90	24.57±0.25	42.11	12.02±0.60	39.90	12.85±0.28	31.61	17.45±0.56	37.87	14.11±0.32
	Biorepel	2000ml	41.00	36.90	25.08±0.36	50.51	15.06±0.50	49.25	13.42±0.34	41.79	12.16±0.36	47.18	13.55±0.43
	Control		33.30	40.00	-	48.30	-	46.20	-	38.64	-	44.38	-
	Mean °C		30.7	29.0		29.0		28.5		29.1			
	R.H. %		62.0	62.0		63.0		57.0		56.0			
2000	NeemAzal	400ml	39.80	39.10	30.33±0.29	38.10	37.57±0.18	33.10	43.30±0.28	29.90	38.76±0.32	33.70	39.88±0.11
	Soybean	325ml	35.60	38.30	23.70±0.14	40.10	26.54±0.14	38.00	27.2±0.70	30.10	31.07±0.61	36.06	28.72±0.43
	Biorepel	2000ml	42.30	45.00	24.55±0.19	48.10	25.84±0.50	46.90	24.40±0.33	39.80	23.30±0.40	44.93	24.52±0.38
	Control		30.00	42.30	-	46.00	-	44.00	-	36.80		42.26	
	Mean °C		33.1	32.4		30.7		29.8		33.0			
	R.H. %		53.0	60.0		60.0		62.0		55.0			

Table 2. The mean number of all larval instars and reduction percentages of cotton leafworm, *Spodoptera littoralis* (Boisd.) as affected by plant extracts sprayed on Egyptian clover fields during autumn season of 1999 and 2000 at Zagazig region, Sharkia Governorate.

Seasons	Treatments	Recommended rate/feddan	Mean No. of larval instars before spray	No. of insects/plant and % reduction								General mean of residual effect	
				Initial effect after:		Residual effect after:							
				3 days		5 days		7 days		9 days			
				No.	% Red.	No.	% Red.	No.	% Red.	No.	% Red.	No.	% Red.
1999	NeemAzal	400ml.	34.00	31.10	26.73±0.53	30.30	27.90±0.63	23.20	35.38±0.59	15.50	44.81±0.47	23.00	36.03±0.42
	Soybean	325ml.	37.00	36.10	21.85±0.74	34.50	24.56±0.36	28.00	28.33±0.60	26.80	12.32±0.98	28.93	21.74±0.35
	Biorepel	2000ml.	36.40	32.11	29.36±0.57	35.10	21.98±0.71	29.80	22.47±0.63	24.30	19.19±0.64	29.73	21.22±0.60
	Control		32.20	40.22	-	39.80		34.00	-	26.60	-	33.46	-
	Mean °C		32.9	29.5		25.8		25.8		26.9			
	R.H. %		46.0	56.0		54.0		54.0		55.0			
2000	NeemAzal	400ml.	44.50	39.60	25.99±0.83	35.20	30.16±0.71	26.60	40.94±0.52	22.50	44.78±0.51	28.10	38.63±0.51
	Soybean	325ml.	43.00	39.00	24.57±1.46	35.10	27.92±0.51	30.80	29.22±0.21	27.30	30.66±0.64	31.06	29.27±0.43
	Biorepel	2000 ml.	46.30	40.20	27.79±0.37	41.20	21.43±0.88	36.10	22.96±0.43	33.00	22.16±0.77	36.76	22.19±0.60
	Control		41.50	49.90	-	47.00	-	42.00	-	38.00	0	42.33	-
	Mean °C		26.4	28.2		27.2		29.4		27.5			
	R.H. %		62.0	65.0		62.0		60.0		64.0			

The general mean rate of reduction in the infestation percentage of the cotton leafworm as a result to plant extracts applied recorded 33.92 ± 0.44, 14.11 ± 0.32 and 13.55 ± 0.43 %, in the first season, 39.88 ± 0.11, 28.77 ± 0.43 and 24.52 ± 0.38 %, in the second one for NeemAzal, soybean and Biorepel, respectively .Table 1

1.2. Egyptian clover fields

- Initial effect (After three days) Obtained results in Table 2 indicated that the initial effect of the tested plant extracts recorded 26.78 ± 0.53, 21.85 ± 0.74 and 29.36 ± 0.57 %, in the first season, 25.99 ± 0.83, 24.57 ± 1.46 and 27.79 ± 0.37%, in the second one for NeemAzal, soybean and Biorepel, respectively.

- Residual effect

- After five days the rate of the percent of reduction in *S. littoralis* infestation after five days of spray recorded 26.73 ± 0.53, 21.85 ± 0.74 and 29.36 ± 0.57, in 1999 season, 25.99 ± 0.83, 24.57 ± 1.46 and 27.79 ± 0.37 %, in 2000 season for the three extracts, respectively .Table 2

- After seven days the reduction percentages of the tested botanical extracts recorded 35.38 ± 0.59, 28.33 ± 0.60 and 22.47 ± 0.63 %, in the first season, 40.94 ± 0.52, 29.22 ± 0.21 and 22.96 ± 0.43 %, in the second one for NeemAzal, soybean and Biorepel, respectively.

- After nine days percent reduction in the rate of *S. littoralis* infestation after nine days of spray recorded 44.81 ± 0.47, 12.32 ± 0.98 and 19.19 ± 0.64 %, in the first season, 44.78 ± 0.51, 30.66 ± 0.64 and 22.16 ± 0.77 %, in the second one for NeemAzal, soybean and Biorepel, respectively.

The general mean of residual effect of the tested botanical extracts recorded 36.03 ± 0.42, 21.74 ± 0.35 and 21.22 ± 0.60 % during the first season, 38.63 ± 0.51, 29.27 ± 0.43 and 22.91 ± 0.60 % in the second one for NeemAzal, soybean and Biorepel, respectively. Biorepel was the least effective extract induced the highest reduction (25.08 ± 0.36 – 25.84 ± 0.50 %) between 3rd – 5th day after spray on cotton and (27.79 ± 0.37 – 29.36 ± 0.57 %) on the 3rd day on clover during the two seasons of study. Therefor, its efficiency decreased irregularly. This phenomenon could be attributed to the higher speed of volatility of this extract. The reverse is true in case of the other two extracts, especially NeemAzal. It could be concluded that, NeemAzal extract proved to be the most potent plant extract tested against *S. littoralis* larvae infesting both cotton and Egyptian clover plants. The higher toxicity of NeemAzal could be attributed to its toxic effect against nearly all the developmental stages of the pest.

For instance, EL-Sayed (1983 a) and EL-Ghareeb *et al.* (2001) stated that the plant extracts showed significant ovicidal effect against *S. littoralis*. In addition, Dimetry *et al.* (1998) stated that neem seed extract is considered as an effective larvicide against *S. littoralis*. Moreover, EL-Sayed (1983 a) recorded 10 % larval mortality at concentrations of 0.2 – 0.5 % of neem seeds. More ever, Salem and Ahmed (1997) realised 100 % larval mortality at 50 ppm when added methanol extracts of chinaberry to the diet of *S. littoralis*.

The highest effect of NeemAzal was detected after seven and nine days of spray on cotton and Egyptian clover fields, respectively, being slightly more effective against the target insect pest on Egyptian clover cultivations.

This variation may be related to one or more factors, the whether factor (day length, temperature and RH %) prevailing during the two seasons which are likely and more suitable for the target insect development i. e. the lower temperature and higher R. H. % during cultivation period of Egyptian clover (October) may increase the activity of the extracts than the relatively higher°C and lower R.H. % prevailing during the cotton season (June). In addition, the biological activity of *S. littoralis* tended to be ceased during the cold weather seasons (autumn). Moussa *et al.* (1960), Nasr (1962), Nasr *et al.* (1973) and Hassanein *et al.* (1995) studied the effect of climatic factors on *S. littoralis* activity, indicating that cold and humid weather during autumn prolonged the longevity of adult moth.

2. Initial and residual activity of the tested Insect growth regulators against *Spodoptera littoralis* infesting cotton and Egyptian clover fields:

2.1. Cotton fields

-Initial effect (After five days) The results obtained in Table 3 indicate that the initial effect of the tested IGRs induced significant reduction in the rate of *S. littoralis* infestation, as it recorded 95.82 ± 0.23, 96.16 ± 0.20, 95.73 ± 0.23, 93.51 ± 0.39 and 77.03 ± 1.36 % reduction, in 1999 season, 95.88 ± 0.38, 96.37 ± 0.31, 95.56 ± 0.32, 95.94 ± 0.28 and 72.75 ± 0.95 %, during 2000 for Cascade, Atabron, Consult, Match and Mimic, respectively.

- Residual effect

- After ten days Percent reduction in the rate of *S. littoralis* infestation after ten days of spry recorded 99.11 ± 0.09, 99.29 ± 0.12, 99.30 ± 0.08, 98.46 ± 0.23 and 86.69 ± 0.21 %, in 1999 season, 99.47 ± 0.10, 99.12 ± 0.11, 99.53 ± 0.08, 99.28 ± 0.10 and 87.42 ± 0.39 % in 2000 season for the five tested IGRs, respectively Table, 3.

Table 3. The mean number of all larval instars and reduction percentages of cotton leafworm, *Spodoptera littoralis* (Boisd.) as affected by insect growth regulators sprayed on cotton fields during 1999 and 2000 seasons at Zagazig region, Sharkia Governorate.

Seasons	Treatments	Recommended rate/feddan	Mean No. of larval instars before spray	No. of insects/plant and % reduction						General mean of residual effect	
				Initial effect after:		Residual effect after:					
				5 days		10 days		15 days			
				No.	% Red.	No.	% Red.	No.	% Red.	No.	% Red.
1999	Cascade	200 ml.	28.80	1.70	95.82±0.23	0.37	99.11±0.09	0.2 0	99.16±0.14	0.28	99.14±0.10
	Atabron	400 ml.	29.50	1.60	96.16±0.20	0.30	99.29±0.12	0.18	99.26±0.30	0.24	99.28±0.17
	Consult	200 ml.	34.80	2.10	95.73±0.23	0.35	99.30±0.08	0.22	99.24±0.21	0.28	99.27±0.07
	Match	400 ml.	18.00	1.65	93.51±0.39	0.40	98.46±0.23	0.00	100.00±0.00	0.20	99.23±0.11
	Mimic	350 ml.	37.00	12.0	77.03±1.36	7.10	86.690.21	4.40	85.66±0.69	5.75	86.18±0.44
	control	-	17.00	24.0	-	24.50	-	14.10	-	19.30	-
	Mean °C		30.7	29.00		28.8		32.20			
	R.H. %		62.0	63.0		63.0		68.0			
2000	Cascade	200 ml.	32.50	1.95	95.88±0.38	0.30	99.47±0.10	0.10	99.74±0.07	0.20	99.60±0.04
	Atabron	400 ml.	33.30	1.76	96.37±0.31	0.51	99.12±0.11	0.34	99.13±0.12	0.42	99.12±0.08
	Consult	200 ml.	31.00	2.00	95.56±0.32	0.25	99.530.08	0.12	99.67±0.09	0.18	99.60±0.08
	Match	400 ml.	26.40	1.56	95.94±0.28	0.33	99.280.10	0.00	100.00±0.00	0.16	99.64±0.05
	Mimic	350 ml.	31.50	12.50	72.75±0.95	6.86	87.420.39	4.93	86.63±0.46	5.89	87.20±0.42
	control	-	18.20	26.50	-	31.50	-	21.30	-	2640	-
	Mean °C				30.7		32.60		31.30		
	R.H. %				60.0		60.0		62.0		

- After fifteen days Percent reduction in the rate of *S. littoralis* infestation after fifteen days of spray recorded 99.16 ± 0.14, 99.26 ± 0.30, 99.24 ± 0.21, 100.00 ± 0.00 and 85.66 ± 0.69 %, in 1999 season, 99.74 ± 0.07, 99.13 ± 0.12, 99.67 ± 0.09, 100.00 ± 0.00 and 86.63 ± 0.46 %, in 2000 season for Cascade, Atabron, Consult, Match and Mimic, respectively.

The general mean percent reduction in the rate of *S. littoralis* infestation as a result to the tested IGRs application recorded 99.14 ± 0.10, 99.28 ± 0.17, 99.27 ± 0.07, 99.23 ± 0.11 and 86.18 ± 0.44 % in 1999 season, 99.60 ± 0.04, 99.12 ± 0.08, 99.60 ± 0.08, 99.64 ± 0.05 and 87.20 ± 0.42 %, in 2000 one for Cascade, Atabron, Consult, Match and Mimic Table 3.

2.2. Egyptian clover fields

-Initial effect (After five days) Obtained results indicate that the initial effect of the tested IGRs expressed as the rate of reduction in the percent infestation recorded 93.12 ± 0.39, 93.61 ± 0.30, 92.49 ± 0.27, 92.55 ± 0.30 and 72.56 ± 1.05 %, in the first season, 93.16 ± 0.24, 94.08 ± 0.15, 93.70 ± 0.09, 93.52 ± 0.29 and 74.03 ± 0.25%, in the second one for Cascade, Atabron, Consult, Match and Mimic, respectively .Table 4

- Residual effect

- After ten days Percent reduction in the rate of *S. littoralis* infestation after ten days of spray with Cascade, Atabron, Consult, Match and Mimic, recorded 98.51 ± 0.15, 98.64 ± 016, 98.44 ± 0.19 and 83.82 ± 0.61 %, in the first season, 98.55 ± 0.12, 98.59 ± 0.16, 98.79 ± 0.18, 98.96 ± 0.07 and 85.58 ± 0.18% in the second one, respectively.

- After fifteen days Percent reduction in the rate of *S. littoralis* infestation after fifteen days of spray recorded 98.32 ± 0.18, 98.55 ± 0.26, 98.65 ± 0.26, 100.00 ± 0.00 and 80.55 ± 0.93 % in the first season, 98.92 ± 0.08, 99.08 ± 0.12, 98.73 ± 0.15, 100.00 ± 0.00 and 82.55 ± 0.57%, in the second one for the tested IGRs, respectively. Table 4

The general mean percent reduction in the rate of *S. littoralis* infestation as a result to the tested IGRs application recorded 98.42 ± 0.14, 98.59 ± 0.12, 98.63 ± 0.17, 99.22 ± 0.09 and 82.76 ± 0.15, 99.48 ± 0.04 and 84.07 ± 0.34%, in 2000 for Cascade, Atabron, Consult, Mimic and Match, respectively .Table 4

It is obvious that all the tested IGRs induced highly reduction in the rate of cotton leafworm infestation on both cotton and Egyptian clover cultivation up to 15 days after spray. However the reduction was slightly more pronounced on cotton fields.

All the tested IGRs caused over 93% reduction in the rate of infestation in both seasons on cotton cultivation with exception that of Mimic which ranged between 27.75 ± 0.95 – 87.47 ± 0.39% in both seasons. The same trend was also noticed on Egyptian clover cultivation.

Generally, the detected reduction percentages in the rate of cotton leafworm infestation on both crops are in agreement with those of Gomaa *et al.* (1996) and Badr (2000).

3. The effect of the plant extracts on the total soluble protein As shown in Table 5 the three applied concentrations of the each tested plant extracts decreased the total soluble protein in the supernatant of the homogenated 4th instar larvae of *S. littoralis* as compared to control at the inspected times 2, 5 and 7 days, except least concentration of Biorepel after two days of treatment. The highest decrease in the total soluble protein recorded 20.33 and 20 % after five days of treatment with NeemAzal at 20 and 10 ppm, respectively. While no effect was recorded after two days of Biorepel treatment at the least concentration. The same trend was also reported by Mostafa (1993) who recorded a decrease in the total soluble protein in 4th and 6th instar larvae of *S. littoralis* treated with Margosan-O (neem extract). On the other hand, EL-Sheakh *et al.* (1990 a) noticed an increase in total soluble protein in the 4th instar larvae of *S. littoralis* treated with Soybean phytoalexins (plant extract).

4. The effect of the plant extracts on the enzyme activities

4.1. Glutamic oxaloacetic transaminase (GOT) Results in Table 6 clear that the activity of GOT enzyme in the supernatant of the homogenated 4th instar larvae of *S. littoralis* was generally increased at different time intervals as affected by all the concentrations of the plant extracts. The highest increase in the activity of GOT as 133.67 % as control was recorded after two days of treatment with the highest concentration of NeemAzal, whereas the least as 105 % as the control for NeemAzal and Biorepel at the least concentration after seven days and soybean at concentration 39.38 ppm after five days considered the control as 100 %. Other concentrations caused intermediate increase in GOT activity and ranged between as 107 – 130.67 % as the control.

Table 4. The mean number of all larval instars and reduction percentages of cotton leafworm, *Spodoptera littoralis* (Boisd.) as affected by insect growth regulators sprayed on Egyptian clover fields during 1999 and 2000 seasons at Zagazig region, Sharkia Governorate.

Seasons	Treatments	Recommended rate/feddan	Mean No. of larval instars before spray	No. of insects/plant and % reduction						General mean of residual effect	
				Initial effect after:		Residual effect after:					
				5 days		10 days		15 days			
				No.	% Red.	No.	% Red.	No.	% Red.	No.	% Red.
1999	Cascade	200 ml.	18.00	2.00	93.12±0.39	0.45	98.51±0.15	0.26	98.32±0.18	0.03	98.42±0.14
	Atabron	400 ml.	18.40	1.90	93.61±0.30	0.42	98.64±0.16	0.23	98.55±0.26	0.32	98.59±0.12
	Consult	200 ml.	20.60	2.50	92.49±0.27	0.48	98.61±0.21	0.24	98.65±0.26	0.36	98.63±0.17
	Match	400 ml.	19.10	2.30	92.55±0.30	0.50	98.44±0.19	0.00	100.00±0.00	0.25	99.22±0.09
	Mimic	350 ml.	18.50	8.20	72.56±1.05	5.02	83.82±0.61	3.10	80.55±0.93	4.06	82.19±0.73
	control	-	13.00	21.00	-	21.80	-	11.20	-	16.50	-
	Mean °C		32.9	25.8		27.2		26.5			
	R.H. %		46.0	54.0		60.0		53.0			
2000	Cascade	200 ml.	24.00	2.80	93.16±0.24	0.62	98.55±0.12	0.25	98.92±0.08	0.43	98.74±0.03
	Atabron	400 ml.	20.30	2.05	94.08±0.15	0.51	98.59±0.16	0.18	99.08±0.12	0.43	98.83±0.07
	Consult	200 ml.	22.80	2.45	93.70±0.09	0.49	98.79±0.18	0.28	98.73±0.15	0.38	98.76±0.15
	Match	400 ml.	21.70	2.40	93.52±0.29	0.40	98.96±0.07	0.00	100.00±0.00	0.20	99.48±0.04
	Mimic	350 ml.	23.70	10.50	74.03±0.25	6.10	85.58±0.18	4.00	82.55±0.57	5.05	84.07±0.34
	control	-	15.30	26.10	-	27.30	-	14.80	-	21.05	-
	Mean °C		26.4	27.2		27.2		27.3			
	R.H. %		62.0	62.0		61.0		63.0			

Table 5. Total soluble protein content % in the supernatant of the homogenated *S. littoralis* 4th instar larvae as affected by plant extracts treatment.

Plant extracts	Conc. ppm	Total soluble protein %		
		2 days	5 days	7 days
NeemAzal	40	80	40	54.67
	20	83.67	20.33	50
	10	95	20	50.33
Soybean	78.75	83.33	50	60
	39.38	93.67	45	65
	19.69	98	40.67	73.33
Biorepel	2000	80	67.67	45.33
	1000	67.67	63.33	50
	500	100	70	60.33
Control	-	100	100	100

Table 6. Transaminase enzymes % in the supernatant of the homogenated *S. littoralis* 4th instar larvae as affected by plant extracts treatment.

Plant extracts	Conc. ppm	Transaminase enzymes					
		GOT %			GPT %		
		2 days	5 days	7 days	2 days	5 days	7 days
NeemAzal	40	133.67	106.67	130.67	140.33	125	180
	20	12.33	110.33	126.67	135.67	150	200
	10	120	115.67	105	160	160.67	220.33
Soybean	78.75	110.67	108.33	120	220	250	280.33
	39.38	107.33	105	110	190.67	235.33	260
	19.69	112.67	115	107	180	200.67	220
Biorepel	2000	109	120	112.33	175	245.67	275
	1000	115.67	118	116	160	220.33	245
	500	119	115.67	105	140.33	195.67	211
Control	-	100	100	100	100	100	100

4.2. Glutamic pyruvic transamiase (GPT) Data in Table 6 reveal that all the concentrations applied of the tested plant extracts caused increase in GPT activity in the supernatant of the homogenated larvae at all examined times. This increase ranged between 135.67and 280.33 % as the control for NeemAzal at 20 ppm after two days and soybean at the highest concentration after seven days, respectively.

It is obvious that the highest enzyme activity was detected at seven days after spray. In addition, the higher the concentration of soybean and Biorepel extracts the higher the enzyme activity, meanwhile, the inverse is true in case of NeemAzal extract.

Generally, the tested plant extracts increased the activity of GOT and GPT enzymes in the 4th instar larvae as compared to the control at different time intervals. These results are in agreement with those of EL-Sheakh *et al.* (1990 b) who recorded an increase in GOT and GPT activity of *S. littoralis* (4th instar larvae) after treatment with LC_{50} of plant extracts.

4.3. Carbohydrate hydrolyzing enzymes

4.3.1. Trehalase Data given in Table 7 show the changes in trehalase activity in the supernatant of the homogenated 4th instar larvae of *S. littoralis*. It is clear that the tested plant extracts caused considerable increase in the activity of the enzyme. The highest activity was recorded with NeemAzal at 40, 20 and 10 ppm after seven days of treatment being as 390, 340 and 380 % as that of the control, respectively. A noticeable drop in trehalase activity was noticed after five days of treatment with the tested extracts.

Similar results were obtained by Abo-EL-Ghar *et al.* (1996) who found that a considerable increase in the activities of trehalase after feeding the 6th instar of *Agrotis ipsilon* larvae on most selected plant extracts.

4.3.2. Invertase Results in Table 7 show that the tested plant extracts induced a marked increase in invertase activity after 2, 5 and 7 days of application. However, the highest enzyme activity was recorded with NeemAzal seed extracts as it reached as 163.33, 220 and 235 % as that recorded for control larvae at the three intervals, respectively. Meanwhile, the least enzyme activity was noticed in Biorepel-treated larvae.

Generally, the severe effect was noticed at 7 days after application for the three tested extracts. In addition, the increase in invertase activity is directly proportional with the concentrations.

Table 7. Trehalase, invertase and amylase enzymes % in the supernatant of the homogenated *S. littoralis* 4th instar larvae as affected by plant extracts treatment.

Plant extracts	Conc. ppm	Trehalase %			Invertase %			Amylase %		
		2 days	5 days	7 days	2 days	5 days	7 days	2 days	5 days	7 days
NeemAzal	40	220	120.33	390	163.33	220	235	150.33	160	240.33
	20	212.50	133.33	340	150	190	210	144	170.67	220.67
	10	195.50	160	380	140.75	133.33	195.67	106	120.33	180
Soybean	78.75	205	150.33	320.75	160	145	220	165	225	195
	39.38	175.25	170	296	140.50	130.33	205	185.67	215	200
	19.69	190	135.87	310.25	120	125	190.67	120	160.67	210
Biorepel	2000	200	115	290	135.25	140	175	133.33	185	170
	1000	192	120	300.50	120	130.67	160	150	148.33	195
	500	170	111.33	275	109.25	125	133.33	109	160.67	185
Control	-	100	100	100	100	100	100	100	100	100

4.3.3. Amylase As shown in Table 7 all the tested extracts increased the activity of amylase enzyme in the supernatant of the homogenated 4th instar larvae. NeemAzal recorded the highest increase in amylase activity after 7 days of treatment that recorded as 240.33, 220.67 and 180 % as that of the control at 40, 20 and 10 ppm, respectively. Soybean caused the highest increase in the activity after 5 days of treatment, recording as 225, 215 and 160.67 % as that of the control at 78.75, 39.38 and 19.69 ppm, respectively. Biorepel recorded the least increase in the amylase activity.

In conclusion, all the tested extracts caused noticeable fluctuations in the total soluble protein, transaminase enzymes and carbohydrate hydrolyzing enzymes. Also, NeemAzal was the most potent extract, while Biorepel was the least effective one.

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دراسات حقلية ومعملية على بعض المركبات ضد دودة ورق القطن

وحيد محمود حسين دسوقى[١]، محمد محمد احمد خضر[١]، سعد إبراهيم يوسف خليل[٢]، سعد محمد على الشكعة[٢]

١- *معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقى – الجيزة – مصر .*
٢- *كلية الزراعة – جامعة الزقازيق – مصر .*

أجريت هذه الدراسة لتقييم فعالية ثلاثة مستخلصات نباتية هى نيمازال ،فول الصويا و بيوريبل ، وخمسة منظمات نمو حشرية هى كونسلت، أتابرون، كاسكيد، ماتش ومميك باستخدام المعدل الحقلي الموصى به فى مكافحة دودة ورق القطن وذلك فى حقول القطن والبرسيم المصري فى منطقة الزقازيق بمحافظة الشرقية خلال موسمى١٩٩٩، ٢٠٠٠.

بالنسبة للمستخلصات النباتية: حقق مركب نيمازال أعلى معدلات الخفض الأولى والمتبقي فى تعداد اليرقات فى موسمي الدراسة فى حقول القطن، وبلغت أعلى نسبة خفض أولى للتعداد ٣٠,٣٣٪، فيما بلغت أعلى نسبة خفض متبقي للتعداد٣٩,٨٨٪، أما فى حقول البرسيم فقد حقق مركب بيوريبل أعلى نسبة خفض أولى فى التعداد فى موسم ١٩٩٩ وبلغت ٣٢,١١٪، فيما تفوق مركب نيمازال فى الخفض المتبقي للتعداد فى نفس الموسم وبلغت ٣٦,٠٣٪، وكذلك الخفض الأولى والمتبقي فى موسم ٢٠٠٠ (٢٥,٩٩ و ٣٨,٦٣٪، على الترتيب).

بالنسبة لمنظمات النمو الحشرية: لم تقل نسبة الخفض فى التعداد عن ٩٢,٠٠٪ فى كلا المحصولين لجميع المركبات ما عدا مميك الذى لم يزد الخفض الأولى للتعداد عن ٧٧,٠٠٪ فى موسم ١٩٩٩ فى حقول القطن، والخفض المتبقى عن ٨٧,٠٠٪ فى موسم ٢٠٠٠ فى حقول القطن، وبصفة عامة يعتبر مركب أتابرون الأكثر فعالية وخفضا لتعداد الآفة.

تم دراسة التغيرات لكل من البروتين الكلى الذائب ، للإنزيمات الناقلة لمجموعة الأمين (GOT,GPT) والإنزيمات المحللة للكربوهيدرات (الاميليز ، الانفرتيز ، التريهاليز) فى يرقات العمر الرابع لدودة ورق القطن نتيجة المعاملة المعملية بالمستخلصات النباتية وكانت النتائج كالتالي:

١- ظهر ان كل المركبات المختبرة أنقصت محتوى البروتين الكلى الذائب باستثناء أقل تركيزات مركب بيوريبيل بعد يومين من المعاملة.
٢- أحدثت كل المركبات زيادة ملحوظة فى إنزيم GOT مقارنة بالكنترول عند مختلف فترات الفحص.
٣- إنزيم GPT فى كل المركبات عند مختلف التركيزات كان أعلى من عينات المقارنة.
٤- زاد نشاط انزيمات التريهاليز والانفرتيز والاميليز عن الكنترول عند كل التركيزات لجميع المركبات المختبرة.