

## TOXICOLOGICAL AND BIOCHEMICAL STUDIES ON THE EFFECT OF SOME INSECT GROWTH REGULATORS ON *SPODOPTERA LITTORALIS* (BOISD.) LARVAE

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### Abstract

The toxicological and biochemical effects of five insect growth regulators (IGRs) namely Cascade, Atabron, Consult, Match and Mimic against 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *Spodoptera littoralis* (Boisd.) were studied under laboratory conditions. Larvae were fed on castor bean leaves treated with 9 successive concentrations. The obtained results indicated that: Atabron proved to be the most potent insect growth regulator, whereas Mimic was the least toxic one among the tested IGRs against both 2<sup>nd</sup> and 4<sup>th</sup> instar larvae. The 4<sup>th</sup> instar larvae proved to be more sensitive to all the tested IGRs than the 2<sup>nd</sup> one at all tested concentrations.

In concerning the biochemical effects the obtained results indicated that:

The tested IGRs increased the total soluble protein after 2 days of treating 2<sup>nd</sup> and 4<sup>th</sup> *S. littoralis* larvae except Cascade at the two lower concentrations. However, a marked decrease was detected after 5 days of treatment both 2<sup>nd</sup> and 4<sup>th</sup> instars with Consult, Match and Cascade.

The tested IGRs decreased the activity of glutamic oxaloacetic transaminase (GOT), while it increased (glutamic pyruvic transaminase (GPT) activity at 2 days post treatment the 2<sup>nd</sup> instar larvae. The inverse was true at 5 days post treatment. In case of the 4<sup>th</sup> instar, the tested IGRs increased the activity of the two enzymes after 2 and 5 days of treatment.

Exposing the 2<sup>nd</sup> and 4<sup>th</sup> instar larvae to the tested IGRs increased markedly the activity of trehalase, invertase and amylase in both 2<sup>nd</sup> and 4<sup>th</sup> instars after 2 and 5 days except Consult and Mimic in case of invertase of the 4<sup>th</sup> instar.

### INTRODUCTION

The toxicological effects of some IGRs have been thoroughly investigated against *S. littoralis*, Bayoumi *et al.* (1998) and Badr (2000). Using synthetic IGRs which interfere with cuticle deposition by inhibition of chitin synthesis, may become important in controlling insect pest populations that are resistant to conventional insecticides, due to the action of enzymes which are either insensitive to the

insecticide or able to degrade it to non-toxic metabolites. The reduced level of chitin in the cuticle seems to result from inhibition of biochemical process leading to chitin formations. Carbohydrates are contributed to the structures and functions of all insect tissues. Metabolism of carbohydrates are controlled mainly by invertase, amylase and trehalase enzymes which play a principal role in the digestion and utilization of carbohydrates by insects (Salem *et al.* 1995). Trehalase is estimated by ...

## RESULTS AND DISCUSSION

### Effects of complete and supplementary feeding on water quality

Data collected on water quality are presented in Table 2. Surface water temperature increased gradually up to the end of August, then, decreased. The range of recorded temperature ( 22.1 – 29.9 °c) was suitable for tilapia ( Boyd, 1992 ). The present results agree with those obtained by Abd – ElMageed ( 1997 ) who found that temperature in Fount Jo Nile, tilapia ponds, was 19 to 27 C, from June to November ).

*S. littoralis*.

## MATERIALS AND METHODS

### 1. Tested compounds:

- Flufenoxuron (Cascade 10 % EC)

[4-[2-chloro 4-(trifluoromethyl phenoxy)] 2-fluorophenyl 3-(2,6-difluorobenzoyl) urea.

The recommended rate is 200 ml/feddan.

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

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

- Benzoylphenyl urea (Consult 10 % EC)

N - (3, 5- dichloro - 4 - (1,1,2,2, - tetrafluoroethoxy)-phenylamino) carbonyl 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.

Rearing technique: Susceptible 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 conditions  $25 \pm 2$  °C and  $65 \pm 5$  % R. H.

## **2. Oral toxicity of the tested insect growth regulators against the second and the fourth instars of *Spodoptera littoralis* larvae:**

The leaf dipping technique was applied to evaluate the insecticidal activity of tested insect growth regulators against the 2<sup>nd</sup> and 4<sup>th</sup> instar larvae. Successive concentrations, i.e. 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ppm for Cascade, Atabron, Consult and Match were prepared. However, the tested concentrations of Mimic were 420, 210, 105, 52.5, 26.25, 13.13, 6.56, 3.28 and 1.64 ppm.

Mortality counts were made after 1, 2, 3, 4 and 5 days of treatment. Mortality data were corrected according to Abbott's formula (1925).

The dosage mortality regression lines were statistically analyzed according Finney (1952).

**3. Preparation of samples for biochemical assay** Samples of tested 4<sup>th</sup> instar larvae were prepared after 2 and 5 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 rpm 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 transaminase (GPT), trehalase, amylase, and invertase enzymes.

**4. 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).

## **5. Determination of enzymes activities:**

**5.1. Transaminase enzymes (GOT and GPT)** The activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzymes were determined colourimetrically according to Reitman and Frankle (1957).

## 5.2. Carbohydrate hydrolyzing enzymes (trehalase, invertase and amylase)

Determination of trehalase, amylase and invertase enzymes activities in digesting trehalose, starch and sucrose, respectively were determined according to Ishaaya and Swirski (1976).

## RESULTS AND DISCUSSION

### Oral toxicity of the tested insect growth regulators against the second and fourth instars of *Spodoptera littoralis* larvae:

#### i. The 2<sup>nd</sup> instar larvae:

**Comparison on the basis of LC<sub>50</sub> and LC<sub>90</sub> values** The tested toxicants could be arranged discerningly according to their potency against 2<sup>nd</sup> instar larvae of *S. littoralis* at the LC<sub>50</sub> level as follows: Atabron, Cascade, Consult, Match and Mimic. The corresponding concentrations (LC<sub>50</sub>) are: 0.18, 0.22, 0.26, 0.34 and 1.60 ppm. (Table 1) whereas the toxicity lines are drawn in Figures 1 and 2.

The descending order of the tested toxicants at LC<sub>50</sub> level is Atabron, Cascade, Match, Consult and Mimic. The respective LC<sub>90</sub> values were 5.40, 5.80, 7.00, 80.00 and 105 ppm (Table 1). It is clear that Consult and Mimic changed their places at the LC<sub>90</sub> level. This allocation of steepness of Mimic being less steeper than the line of the other compound.

However, Atabron proved to be the most potent insecticide, whereas Mimic was the least toxic one among the tested compounds against 2<sup>nd</sup> instar larvae.

**-Toxicity index** Obtained results show that the toxicity index at LC<sub>50</sub> level is as follows: Cascade (81.82 %), Consult (69.23 %), Match (52.94 %) and Mimic (11.25 %) as toxic as Atabron.

Basing on the LC<sub>90</sub>, the tested insect growth regulators could be divided into two groups according to their toxicity to 2<sup>nd</sup> instar larvae as follows:

- 1- The first and the most toxic group includes, Atabron, Cascade and Match.
- 2- The second and the least toxic group include Consult and Mimic (Table 1).

Similar data were also reported by EL-Ghareeb (1992) they stated that Atabron proved to be highly effective against *S. littoralis*.

Table 1. Toxicity data of the tested insect growth regulators applied orally to 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* under laboratory conditions.

Treatments	Field rate ppm	LC <sub>50</sub> 2 <sup>nd</sup> instar	LC <sub>90</sub>		Slope		Toxicity index *		
			2 <sup>nd</sup> instar	4 <sup>th</sup> instar	2 <sup>nd</sup> instar	4 <sup>th</sup> instar	LC <sub>50</sub> 2 <sup>nd</sup> instar	LC <sub>90</sub>	
								2 <sup>nd</sup> instar	4 <sup>th</sup> instar
Atabron	100	0.18	0.80	5.40	1.6579	1.8549	100	100	100
Cascade	100	0.22	1.40	5.80	1.7063	1.9359	81.82	93.10	57.14
Consult	100	0.26	1.70	80	0.6589	2.0287	69.23	6.75	47.05
Match	100	0.34	2.60	7.0	1.7382	1.4285	52.94	77.14	30.76
Mimic	420	1.60	30	1050	0.4477	0.4802	11.25	5.142	2.67

\* =Toxicity index (Sun, 1950) =  $\frac{\text{LC}_{50} \text{ or } \text{LC}_{90} \text{ of the efficient compound}}{\text{LC}_{50} \text{ or } \text{LC}_{90} \text{ of the other compound}} \times 100$

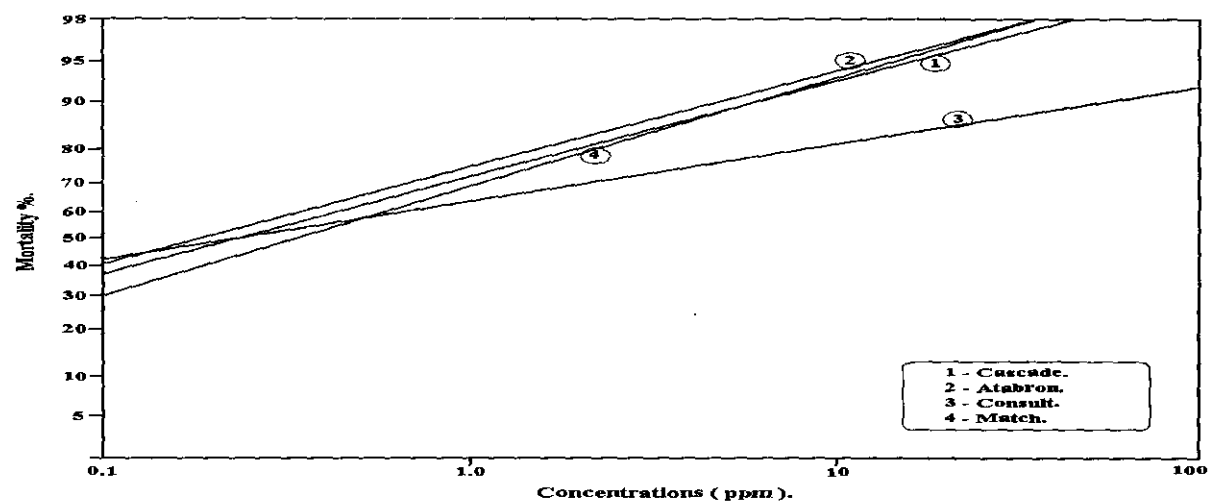


Figure 1. Concentration-mortality regression lines for IGRs applied against 2<sup>nd</sup> instar larvae of *S. littoralis*.

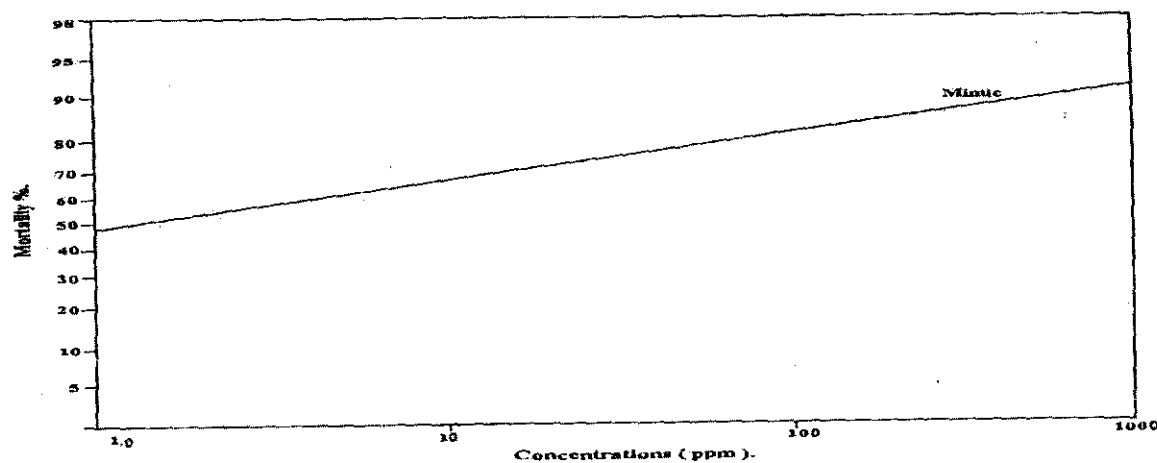


Figure 2. Concentration-mortality regression lines for IGR (Mimic) applied against 2<sup>nd</sup> instar larvae of *S. littoralis*.

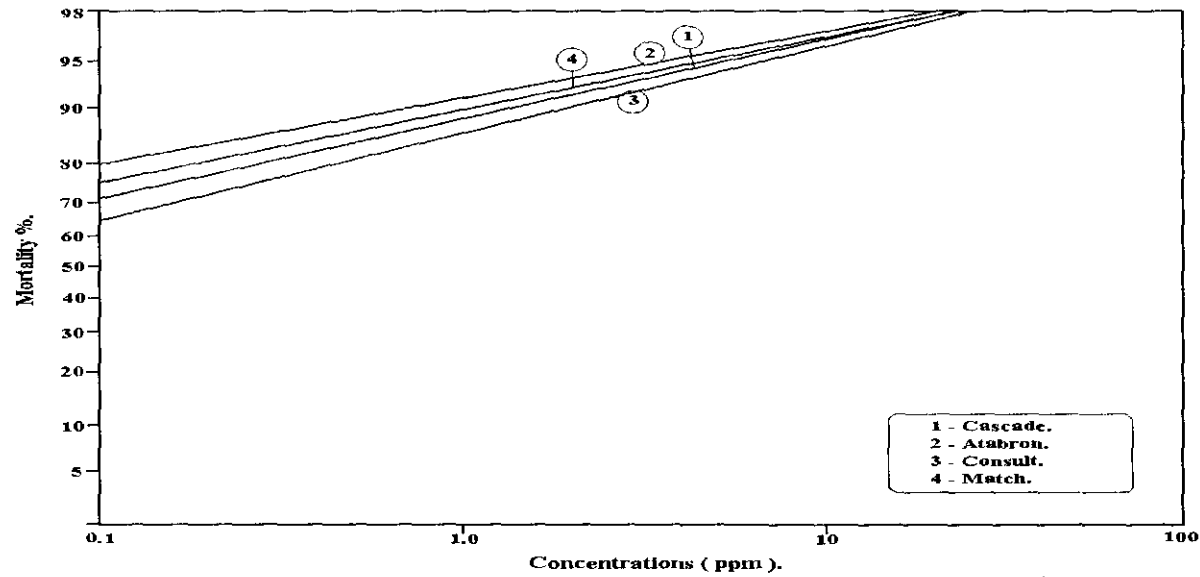


Figure 3. Concentration-mortality regression lines for IGRs applied against 4<sup>th</sup> instar larvae of *S. littoralis*.

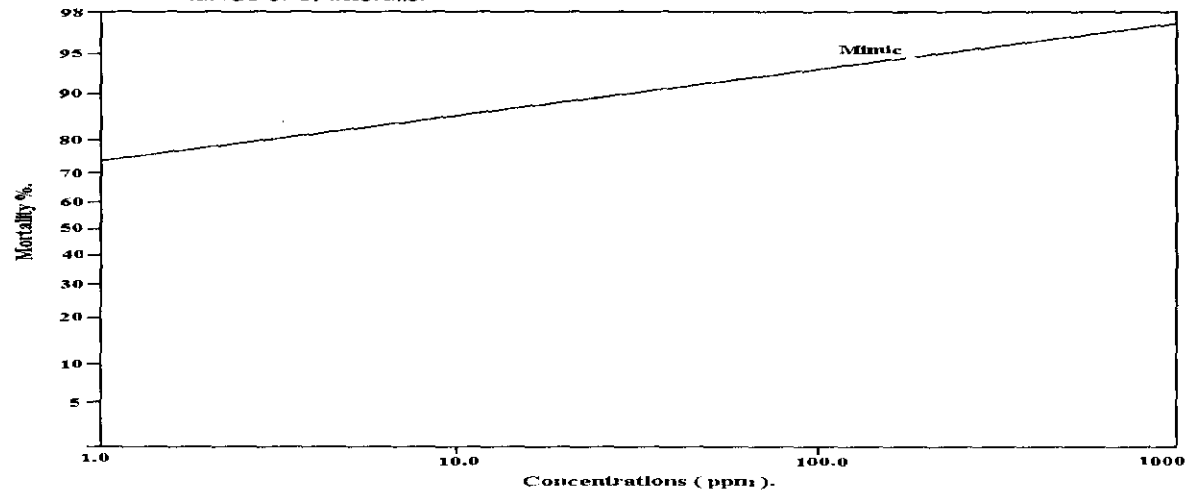


Figure 4. Concentration-mortality regression lines for IGR (Mimic) applied against 4<sup>th</sup> instar larvae of *S. littoralis*.

## ii. The 4<sup>th</sup> instar larvae:

**-Comparison on the basis of LC<sub>90</sub> values** The tested toxicants could be arranged discerningly according to their potency against 4<sup>th</sup> instar larvae of *S. littoralis* as follows: Atabron, Match, Cascade, Consult and Mimic. The corresponding lethal concentrations (LC<sub>90</sub>) are 0.80, 1.40, 1.70, 2.60 and 30.0 ppm (Table 1), whereas the toxicity lines are shown in Figures 3 and 4. Atabron proved to be the most potent insect growth regulators, meanwhile Mimic was the least toxic one to 4<sup>th</sup> instar larvae at LC<sub>90</sub> level.

**-Toxicity index** Data of the toxicity index at LC<sub>90</sub> levels are presented in Table 1. It is clear that the toxicity index is as follow: Match (57.14 %), Cascade (47.05 %), Consult (30.76 %) and Mimic (2.67 %) as toxic as Atabron.

Generally, the tested insect growth regulators could be divided into three groups according to their toxicity to 4<sup>th</sup> instar larvae. The first and the more toxic group include Atabron. The second group includes Match, Cascade and Consult, whereas the third and the least toxic one include Mimic.

Generally, the 4<sup>th</sup> instar larvae proved to be more sensitive to all the tested IGRs than the 2<sup>nd</sup> one at all concentrations.

One of the explanations of this phenomenon is that the food uptake of the 4<sup>th</sup> instar larvae is greater than the 2<sup>nd</sup> one that means greater uptake of the IGRs.

On the contrary, Badr (2000) reported that the 2<sup>nd</sup> instar larvae of *S. littoralis* are more sensitive than the 4<sup>th</sup> instar ones when fed on cotton leaves sprayed with Consult at 5 and 10 days after spray.

Due to the IGRs are chitin synthesis inhibitors, therefore their effect would appear after suppressing moulting process. As shown in Table 1, LC<sub>90</sub> of the tested IGRs is greatly reduced as compared to the applied field rate. Subsequently, the malformations in the following stages (pupa and adult) would not exist as all the treated larvae died. Therefore Figure 5 a – c manifested the symptoms of death due to the exposure of larvae to the tested IGRs. The symptoms of death usually being at three days post treatment with different IGRs at different concentrations, larvae became inactive, alive without feeding, appeared to be wet, completely paralyzed, dark colour spread all over the body and viscous excretion comes out the larval body, then it became unable to ecdyse (Figure 5 a). In addition, (in case of Mimic at different concentrations) a partially exit of the posterior part of the alimentary canal



from the anus Figure 5. (b and c). Similarly Abd El-hakim (1996) showed some malformation effects in cotton bollworms treated with IGRs.

**Biochemical effects insect growth regulators on *Spodoptera littoralis* larvae:**

**a- Total soluble protein** Data in Table 2 show the level in the total soluble protein detected in the supernatant homogenate of 2<sup>nd</sup> and 4<sup>th</sup> instar larvae treated with different concentrations of the tested IGRs. It is obvious that all the tested soluble protein in both 2<sup>nd</sup> and 4<sup>th</sup> *S. littoralis* larvae after two days of treatment with the exception of Cascade, especially at lower concentrations.

The 4<sup>th</sup> instar larvae proved to be more sensitive to the tested IGRs than the 2<sup>nd</sup> instar ones. For instance, the total soluble protein level ranged between 150 – 450 % in 4<sup>th</sup> instar larvae while it ranged between 120 – 300 % in the 2<sup>nd</sup> instar. Atabron was the most effective IGR.

The total soluble in the treated larvae after five days of treatment was greatly reduced in general with all the tested IGRs, except Atabron down to a concentration equivalent to 1/128 as that of field rate on 4<sup>th</sup> instar.

In addition, Mimic at higher concentration caused slight increase in this parameter especially on the 4<sup>th</sup> instar larvae.

In this connection, Abdel-Hafez *et al.* (1983) found that the IGRs, diflubenzuron and triflumuron caused reduction in the levels of protein and free amino acids of the treated *S. littoralis* larvae. Similarly, Mostafa (1993) showed significant decrease in the level of total soluble protein as affected by IGRs. Both flufenoxuron and teflubenzuron were more effective on the 6<sup>th</sup> instar larvae than 4<sup>th</sup> instar ones of *S. littoralis*.

Generally, the tested IGRs increased markedly the total soluble protein in the treated larvae in both instars after two days of exposure. On the contrary, the level of total soluble protein in the treated larvae was severely decreased after five days of application with the exception of Atabron against only 4<sup>th</sup> instar larvae and the higher concentrations of Mimic on 2<sup>nd</sup> and 4<sup>th</sup> instars.

Fourth instar larvae was more susceptible to the IGRs application than the 2<sup>nd</sup> instar ones.

**b- Transaminase enzymes:**

**i. GOT** Data in Table 3. indicate that the activity of GOT increased after two days as affected by Cascade (300 – 150 %) as control and Consult (350 – 175 %) and the

higher 2-3 concentrations of Match and Mimic induced, slight and the other concentrations had no effect or became under the control level.

On the contrary, all concentrations of Atabron at all concentrations and the lower concentration of Match and Mimic decreased the activity of GOT severely (80 - 205 %) as control. After five days of treatment the picture was completely inverse for all the tested IGRs. For instance, Cascade and Consult at all concentrations and Match and Mimic at the lower concentrations showed severe reduction in the activity for GOT enzyme (as 25 – 83.3 % as the control).

On the contrary, all concentrations of Atabron and Match induced marked increase in GOT activity recording between as 150 - 366.67 %, 116.67 – 316.67 %, and 133.33 – 200 % as the control, respectively.

In case of 4<sup>th</sup> instar larvae treatment, data in Table 3 indicate that most concentrations of the tested IGRs increased activities of GOT in 4<sup>th</sup> instar larvae at two

المعاملة الرابعة أجريت بدون إضافة أي غذاء (كونترول) مجموعة ضابطة. بلغ متوسط الوزن النهائي للأسماك في نهاية التجربة [ ١٩٣,٤ - ١٣٨,٠٢ - ١٦٧,٣ - ١١٠,٧ ] جم في معاملات التغذية الكاملة فقط والتغذية الإضافية فقط والتغذية الكاملة بالإضافة إلى التغذية الإضافية وأخيراً الأحواض الغير مغذاة على التوالي . بلغ متوسط الإنتاج الصافي للأسماك في نهاية التجربة [ ٥٣٠,١ - ٣٧٦,٥ - ٤٥٧,٧ - ٢٩٨,٨ كجم / حوض ، أ ف م ، هذه النظم علم ، التوالي . أوضحت النتائج نمو

decreased the enzyme activity, recording as 80, 50, 66.67 and 66.67 % as control for 1/32 f.r., 1/64 f.r. 1/128 f.r. and 1/256 f.r., respectively. The same trend was nearly noticed after five days, all IGRs at different concentrations increased the activity of GOT with the exception of Cascade at 1/256 f.r. and Match at 1/16 and 1/3 f.r. that recorded as 66.67, 50 and 66.67 % as the control, respectively. However, the higher two concentrations of Mimic did not cause any change in the enzyme activity.

**ii. GPT** Data in Table 4. indicate that all IGRs increased GPT activity as compared to control after two days of treating 2<sup>nd</sup> instar larvae, except Mimic at the least concentration that recorded 50 %. Atabron recorded the highest increase in GPT activity that ranged between 150.0 – 366.67 %.

Meanwhile, five days post treatment, Consult and Atabron caused decreasing activity that ranged between 20 – 75% and 25 - 83.33 %, respectively. Other IGRs increased GPT activity at the higher concentrations and decrease it at the lower ones.

Table 2. Total soluble protein content % in the supernatant of the homogenated *S. littoralis* larvae as affected by insect growth regulators treatment.

2 <sup>nd</sup> instar																		
Time	After 2 days									After 5 days								
Conc.	f.r.*	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.	f.r.	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.
Treat.																		
Cascade	180	220	140	160	200	180	200	100	100	-	-	80	80	50	60	40	40	50
Atabron	280	300	280	260	300	240	250	200	150	-	-	90	60	50	50	80	40	20
Consult	200	200	150	160	180	200	200	140	120	50	80	70	100	80	90	50	50	50
Match	260	280	300	250	240	200	180	160	120	-	-	50	40	80	60	100	80	100
Mimic	200	180	140	220	240	200	250	140	140	100	120	80	50	60	60	40	40	50
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4 <sup>th</sup> instar																		
	After 2 days									After 5 days								
Cascade	350	250	300	300	250	250	200	150	100	-	-	-	-	-	80	40	80	60
Atabron	400	400	450	300	250	300	150	200	250	-	-	-	-	-	220	160	140	80
Consult	200	200	180	150	120	130	150	200	120	-	-	-	80	40	40	50	40	20
Match	200	150	200	200	200	250	250	350	300	-	-	-	-	-	20	20	20	20
Mimic	300	300	300	300	300	150	150	150	150	-	120	100	100	120	80	80	60	40
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

\* f.r. = recommended field rate

Table 3. GOT enzyme % in the supernatant of the homogenated *S. littoralis* larvae as affected by insect growth regulators treatment.

2 <sup>nd</sup> instar																		
Time	After 2 days									After 5 days								
Conc. Treat.	f.r.*	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.	f.r.	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.
Cascade	200	300	237.50	262.50	300	237.50	275	175	150	-	-	83.33	75	66.67	83.33	33.33	33.33	33.33
Atabron	80	75	50	40	50	35	25	25	20	-	-	200	166.67	150	350	333.33	366.67	366.67
Consult	175	350	237.50	300	300	250	212.50	212.50	200	100	33.33	75	66.67	50	40	50	33.33	25
Match	175	112.50	100	75	62.50	50	25	25	20	-	-	250	233.33	316.67	200	116.67	266.67	250
Mimic	100	112.50	125	75	100	100	37.50	50	25	50	50	100	133.33	150	200	166.67	133.33	166.67
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4 <sup>th</sup> instar																		
	After 2 days									After 5 days								
Cascade	200	166.67	100	266.67	166.67	200	166.67	120	200	-	-	-	-	200	166.67	120	109.33	66.67
Atabron	333.33	300	300	250	240	266.67	220	166.67	180	-	-	-	-	350	333.33	300	250	166.67
Consult	100	266.67	66.67	66.67	109.33	120	120	140	133.33	-	-	300	300	266.33	250	180	140	109.33
Match	66.67	60	40.33	100	266.67	166.67	266.67	250	200	-	-	-	-	50	66.67	120	160.67	200
Mimic	200	233.33	166.67	220	230	80	50	66.67	66.67	-	100	100	200	266.67	300	320	320	333.33
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

\* f.r. = recommended field rate

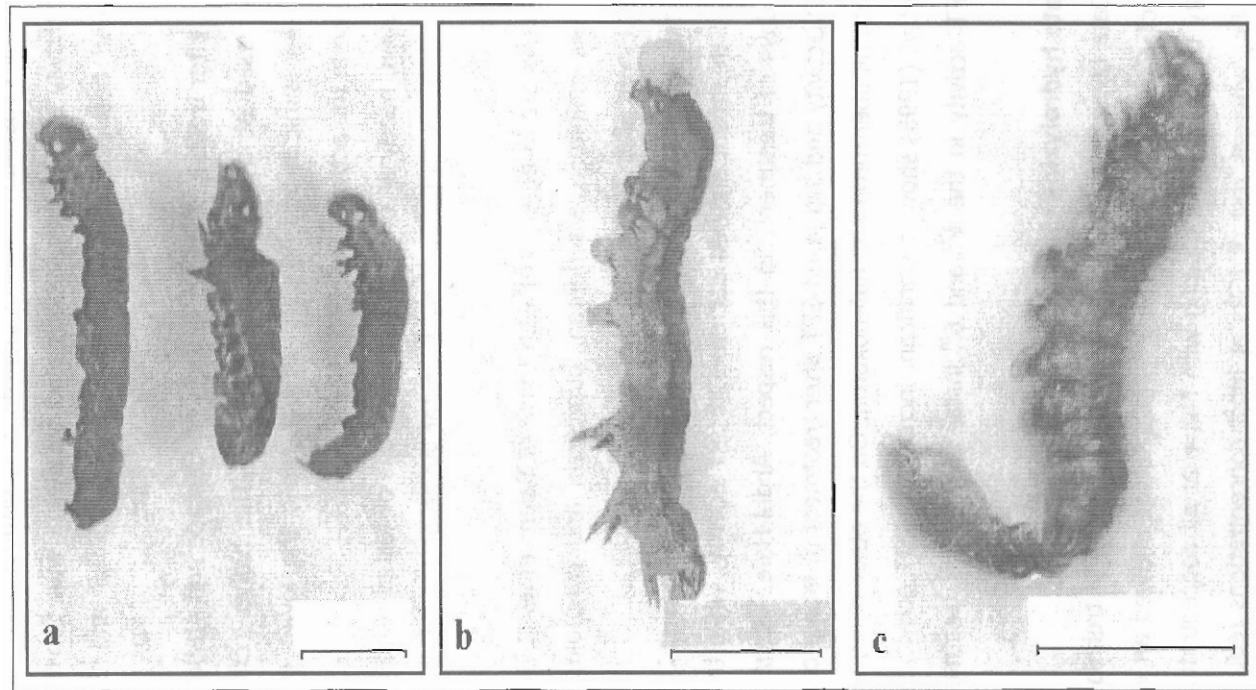


Figure 5. Symptoms of death of cotton leafworm larvae due to the exposure to the tested IGRs.

In case of 4<sup>th</sup> instar, an increase in the activity of GPT that observed after two days of treatment with Cascade and Atabron at all concentrations. However, a gradual decrease in the activity of GPT was noticed after treatment with Consult, Match and Mimic, that ranged between 220 – 50 %, 266.67 – 75 % and 350 – 75 %, respectively.

Five days after treating 4<sup>th</sup> instar larvae with Cascade and Atabron the activity of GPT was increased at all tested concentrations. The higher concentrations of Consult, Match and Mimic increased GPT activity, while the lower ones caused a noticeable decrease in the activity.

In conclusion, nearly all or most concentrations of tested IGRs increased GOT and GPT enzymes activities in the treated 4<sup>th</sup> instar larvae of *S. littoralis*. Atabron was the most effective IGR.

These results are in agreement with those of Salem *et al.* (1995) working on the chitin synthesis inhibitor diafenthiuron, buprofezin and pyriproxyfen against *S. littoralis* 4<sup>th</sup> instar larvae after 24, 48 and 72 hours.

Most of the tested concentrations applied for all IGRs against 2<sup>nd</sup> instar larvae after two days of treatment showed decrease in GPT activity, while the inverse was true after five days of treatment. In this respect, Abdel-Hafez *et al.* (1993) found reduction in GOT activity and increased GPT after treatment of laboratory strain larvae of *S. littoralis* with diflubenzuron and flufenoxuron. On the other hand, Mostafa (1993) and EL-Kordy *et al.* (1995) showed significant increase in GOT activity and significant reduction in GPT activity in the 4<sup>th</sup> and 6<sup>th</sup> instar larvae after treatment with three IGRs.

### **c- Carbohydrate hydrolyzing enzymes:**

**i. Trehalase** Data given in Table 5. summarize the changes of trehalase in both 2<sup>nd</sup> and 4<sup>th</sup> instars of *S. littoralis* larvae. The 2<sup>nd</sup> instar larvae showed an increase in the trehalase activity two days after treating with Mimic at all concentrations nearly and Match till 1/16 f.r., whereas rest of IGRs at most concentrations reduce the enzyme activity. The highest activity was recorded at f.r. for Mimic, whereas the least value (40 %) was recorded at 1/256 and 1/128 f.r. consult and Match, respectively.

After five days of treatment the 2<sup>nd</sup> instar larvae treated with the different concentrations of tested IGRs increased trehalase activity. The highest value (375 %) was recorded at 1/16 f.r. for Atabron.

Table 4. GPT enzyme % in the supernatant of the homogenated *S. littoralis* larvae as affected by insect growth regulators treatment.

2 <sup>nd</sup> instar																		
Time	After 2 days									After 5 days								
Conc.	f.r.*	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.	f.r.	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.
Treat.																		
Cascade	250	200	220	100	100	180	175	200	150	-	-	175	150	125	75	50	50	25
Atabron	366.67	350	350	333.33	300	250	200	150	150	-	-	83.33	66.67	75	50	33.33	50	25
Consult	150	200	300	350	120	175	150	220	100	100	100	75	66.67	50	66.67	33.33	40	20
Match	200	150	100	100	220	175	180	200	250	-	-	225	200	125	100	75	50	50
Mimic	150	250	300	200	175	150	100	100	50	150	250	225	175	166.67	120	100	100	50
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4 <sup>th</sup> instar																		
	After 2 days									After 5 days								
Cascade	366.66	350	350	333.33	300	250	280	266.67	250	-	-	-	-	283.33	266.67	200	166.67	166.67
Atabron	300	280	266.67	266.67	250	233.33	220	200	175	-	-	-	-	316.67	350	333.33	250	266.67
Consult	220	200.33	180	166.67	120	109.33	75	66.67	50	-	-	250	233.33	133.33	116.67	66.67	50	33.33
Match	266.67	233.33	200	180	166.67	150	100	100	75	-	-	-	-	216	150	266.67	133.33	66.67
Mimic	350	333.33	300	275	250	250	233.33	75	75	-	150	166.67	100	133.33	120	100	83.33	66.67
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

\* f.r. = recommended field rate

Table 5. Trehalase enzyme % in the supernatant of the homogenated *S. littoralis* larvae as affected by insect growth regulators treatment.

2 <sup>nd</sup> instar																		
Time	After 2 days									After 5 days								
Conc.	f.r.*	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.	f.r.	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.
Treat.																		
Cascade	75	105	66.67	109.33	150	80	150	60	50	-	-	120	266.67	105	109.33	105	100	100
Atabron	91.66	80	60	66.67	200	250	75	50	66.67	-	-	275	200	375	166.67	233.33	200	175
Consult	175	90	75	66.67	80	72.33	33.33	50	40	200	225	270	150	109.33	105	120	100	100
Match	150	200	66.67	180	175	90	56	40	60	-	-	250	270	280	266.67	233.33	109.33	100
Mimic	333.33	300	350	266.67	250	266.67	109.33	105	50	266.67	290	300	270	300	270	320	250	200
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4 <sup>th</sup> instar																		
	After 2 days									After 5 days								
Cascade	200	133.33	166.67	105	109.33	109.33	100	100	100	-	-	-	-	166.67	233.33	100	250	200
Atabron	366.67	333.33	350	275	250	150	109.33	80	66.67	-	-	-	-	50	75	80	66.67	60
Consult	200	100	100	75	50	66.67	50	33.33	50	-	-	250	266.67	333.33	300	275	200	150
Match	220	200	166.67	200	106.67	90	66.67	75	50	-	-	-	-	275	200	330	300	333.33
Mimic	250	200	230	175	150	166.67	60	50	40	-	275	220	266.67	250	175	150	100	50
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

\* f.r. = recommended field rate



Table 6. Invertase enzyme % in the supernatant of the homogenated *S. littoralis* larvae as affected by insect growth regulators treatment.

2 <sup>nd</sup> instar																		
Time	After 2 days									After 5 days								
Conc.	f.r.*	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.	f.r.	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.
Treat.																		
Cascade	200	166.67	150	120	200	109.33	100	100	100	-	-	105	109.33	120	100	100	100	100
Atabron	109.33	150	175	200	133.33	100	100	100	100	-	-	109.33	118	112	109.33	100	100	100
Consult	100	100	100	100	233.33	220	100	300	200	100	120	100	100	133.33	105	109.33	109.33	100
Match	109.33	109.33	100	100	100	100	100	100	100	-	-	120	133.33	105	109.33	100	100	100
Mimic	333.33	333.33	275	200	220	100	100	166.67	175	300	200	100	166.67	120	120	133.33	100	100
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4 <sup>th</sup> instar																		
	After 2 days									After 5 days								
Cascade	275	300	250	300	200	175	150	120	100	-	-	-	-	200	150	33.33	133.33	33.33
Atabron	400	375	300	350	200	200	250	150	100	-	-	-	-	266.67	200	233.33	150	166.67
Consult	350	300	300	300	200	250	275	200	100	-	-	233.33	166.67	150	50	100	66.67	33.33
Match	300	275	200	200	250	150	175	175	100	-	-	-	-	75	133.33	150	200	166.67
Mimic	300	150	100	225	200	250	150	200	100	-	233.33	200	100	66.67	100	50	33.33	33.33
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

\* f.r. = recommended field rate

It is clear that the 4<sup>th</sup> larvae showed increased trehalase activity after two days of treatment with the higher concentration followed by decreased activity with lower concentration. Atabron recorded the highest values (366.67 to 109.33 %), meanwhile Consult caused the lowest values (33.33 to 75.0 %). All the tested IGRs showed increased activity of trehalase except Atabron at all the tested concentrations and Mimic at the least concentration.

**ii. Invertase** Data in Table 6. clear that at two days after application of the 2<sup>nd</sup> instar larvae, the tested IGRs affected the enzyme in three patterns, i.e. Cascade, Atabron and Mimic induced greatly the enzyme activity at the higher concentrations being ineffective at lower ones. Meanwhile, Consult manifested an inverse effect. On the other hand, Match seemed to be ineffective.

After five days of treatment, Mimic at higher concentrations induced noticeable increase in invertase activity then it decreased with decreasing the concentrations to be normal at 1/128 f.r. Other IGRs caused slight increase with the higher concentrations and no effect with lower concentrations.

In case of 4<sup>th</sup> instar larvae, all the applied concentrations, but not the least tested IGRs increased greatly the activity of invertase enzyme two days post treatment for 4<sup>th</sup> instar larvae, Atabron followed closely by Consult recorded the highest increase of activity ranged between as 400 to 150 % and as 350 to 200 % as the control, respectively.

After five days of treatment, irregular enzyme activity patterns were noticed due to the IGRs application. However, Atabron increased the enzyme activity that ranged between as 150 – 266.67 % as control.

**iii. Amylase** Data in Table 7 show the changes in activity of amylase enzyme after two days of treating 2<sup>nd</sup> instar larvae with the tested IGRs. It is clear that Match at the higher five concentrations caused noticeable increase in the enzyme activity (as 166.67 – 266.67 % as the control). The other four IGRs induced slight or no effect on the amylase activity.

Similarly, the amylase enzyme after five days of treatment showed slight or no response to the IGRs application. Regarding the effect of the tested IGRs to 4<sup>th</sup> larval instar on the activity of amylase after two days of treatment, it is clear that there was noticeable increase in the enzyme activity especially at higher concentrations of all tested IGRs.

On the other hand, the lower concentrations of Cascade, Consult and Match and all the concentrations of Mimic except f.r. caused sever reduction in amylase activity.

The alive larvae of the 4<sup>th</sup> instar larvae after five days of treatment showed increased amylase activity with the higher concentrations of Match, Consult and Cascade and nearly all the concentrations of Atabron and Mimic.

Discussing the data concerning, the activity of carbohydrate hydrolyzing enzymes (trehalase, invertase and amylase) revealed the following:

- 1- The 4<sup>th</sup> larval instar proved to more sensitive than the 2<sup>nd</sup> instar.
- 2- Amylase activity seemed to be more tolerant to the IGRs application whereas trehalase was the most sensitive.
- 3- Atabron proved to be the most potent IGR.

It is obvious that there are some factors affected the efficiency of the tested IGRs on carbohydrate hydrolyzing enzymes. For instance, the tested IGRs them selves has its specific effect therefore some compounds were found to be highly effective, others were less effective. Moreover, some IGRs compounds affected severely the activity of one enzyme and others less effective another enzyme such as Match.

In addition, the higher concentration used in Match, Mimic and Consult caused the highest effect, while the lower concentration caused the lowest effect in case of trehalase. Moreover, the treated instar as the 4<sup>th</sup> proved more susceptible than the 2<sup>nd</sup> instar larvae. Also, the time of evaluation affected the efficiency of the tested IGRs, for instance after two days of treatment most the IGRs increased the enzymes activity while after 5 days the activity was decreased. The role of these factors could explain the varied data obtained and those documented in the literature where some authors reported an increase and/or decrease others reported no effect. For instance, Abdel-Fattah *et al.* (1986) found that diflubenzuron and triflumuron decreased greatly the activity of trehalase, invertase and amylase enzymes.

On contrary, AL-Elimi and Eid (1998) found an increase in carbohydrate hydrolyzing enzymes activity after treating larvae of *S. littoralis* with different concentrations of the IGRs diflubenzuron and buprofezin.

Table 7. Amylase enzyme % in the supernatant of the homogenated *S. littoralis* larvae as affected by insect growth regulators treatment.

2 <sup>nd</sup> instar																		
Time	After 2 days									After 5 days								
Conc.	f.r.*	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.	f.r.	1/2 f.r.	1/4 f.r.	1/8 f.r.	1/16 f.r.	1/32 f.r.	1/64 f.r.	1/128 f.r.	1/256 f.r.
Treat.																		
Cascade	100	109.33	105	100	103.33	111.67	100	100	100	-	-	111.33	103	112	105	109.33	100	100
Atabron	103.33	105	120	109.33	109	100	100	100	100	-	-	105	106.67	103	100	100	103	100
Consult	106.33	111.66	100	100	112	109.33	100	120	100	112	100	106.67	109	111.33	100	105	100	100
Match	266.67	250	200	175	166.67	105	100	100	100	105	-	109.33	133.33	105	100	166.67	100	100
Mimic	100	111.33	100	112	105	100	109.33	100	100	-	112	100	109.33	109.33	120	112	100	100
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4 <sup>th</sup> instar																		
	After 2 days									After 5 days								
Cascade	133.33	120	80	66.67	75	60	50	50	33.33	-	-	-	-	150	133.33	100	100	100
Atabron	333.33	300	250	333.33	330	50	150	200	50	-	-	-	-	120	166.67	100	100	100
Consult	200	109.33	133.33	90	75	66.67	80	50	25	-	-	330	333.33	350	266.67	266.67	133.33	109.33
Match	150	133.33	166.67	60	50	75	66.67	33.33	50	-	-	-	-	275	330	250	100	100
Mimic	100	60	66.67	80	50	40	33.33	50	25	-	225	200	166.67	100	100	300	275	330
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

\* f.r. = recommended field rate

## REFERENCES

1. Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Ent., 18 : 265 - 267.
2. Abdel-Fattah, M. S., M. A. EL-Malla, and M. N. Shaaban. 1986. Effect of diflubenzuron and triflumeroun on the activity of esterases in susceptible and profenofos-resistant strains of *S. littoralis* (Boisd.). Bull. Ent. Soc. Egypt. Econ. Ser., 15 : 221 - 227.
3. Abdel-Hafez, M. M., A. Mohanna, A. EL-Sheakh and M. Farag. 1993. Effect of IGR / insecticide mixtures on transamines and phosphatase activities of *Spodoptera littoralis* (Boisd.) larvae. J. Product. & Dev., 1 (2) : 178 -193.
4. Abdel-Hafez, M. M., M. A. EL-Malla, W. S. Saleh, and I. E. Salem. 1983. Changes in amylase, invertase and trehalase enzyme activities during different development stages of *Spodoptera littoralis* (Boisd.). Med. Fac Lanbow. Risjksuniv. Gent, 48 (2): 375 - 384.
5. Abd EL-Hakim, M. 1996. Some biological and physiological studies on the effect of some IGRs and plant extracts on *Pectinophora gossypiella* (Saund.) and *Earias insulana* (Boisd.). Ph. D. Thesis, Fac. Agric., Al-Azhar Univ., Egypt.
6. AL-Elimi, M. H. and A. M. H. Eid. 1998. Effect of some insect growth regulators on the activities of non-specific esterases, phosphatase and carbohydrate hydrolyzing enzymes in *Spodoptera littoralis* (Boisd.). Egypt. J. Appl. Sci, 13 (10): 286 - 294.
7. Badr, N. A. 2000. Efficiency of some natural products and insect growth regulator, Consult against the cotton leafworm, *Spodoptera littoralis* (Boisd.). Egypt. J. Appl. Sci., 15 (9): 316 - 327.
8. Bayoumi, A. E., R. Balana-Fouce, A. k. Sobeiha and E. M. K. Hussein. 1998. The biological activity of some chitin synthesis inhibitors against the cotton leafworm *Spodoptera littoralis* (Boisduval). (Lepidoptera: Noctuidae). Boletin de Sanidad Vegetal, Plagas, 24 (3): 499 - 506.
9. Bursell, E. 1963. Aspects of metabolism of amino acids in the tsetse fly, *Glossina* sp. (Diptera) J. Insect physio., 439 - 542.
10. El-Defrawi, M., A. A. Toppozada, A. E. Salama and S. A. El-Khishen. 1964. Toxilogical studies on the Egyptian cotton leafworm, *Prodenia litura* F. II

- Reversion of Toxaphane resistance in Egyptian cotton leafworm. J. Econ Entomol., 57 : 595 - 597.
11. EL-Ghareeb, A. M. 1992. Comparative toxicity of some benzoylphenyl urea molt-inhibiting insecticides to cotton leafworm *Spodoptera littoralis* (Boisd.). Indian, J. Ent., 54 (4) : 388 - 393.
  12. EL-Kordy, M. W., A. I. Gadallah, M. G. Abbas and S. A. Mostafa. 1995. Effect of pyriproxyfen, flufenoxuron and teflubenzorn on some biochemical aspects of *S. littoralis*. Al-Azhar J. Agric. Res., 2: 223 - 238
  13. Finney, D. J. 1952. Probit analysis a statistical treatment of the sigmoid response curve. Cambridge Univ. Press.
  14. Gornall, A. G., C. D. Bardawil, and M. M. David. 1949. Determination of serum protein by means of bruit reduction. J. Biochemistry, 177 : 751 - 766.
  15. Ishaaya, I. and E. Swirski. 1976. Trehalase, invertase and amylase activities in the black scale, *Saissetia oleae* and their relation to host adaptability. J. Ins. Physiol., 16 : 1025 - 1029.
  16. Katunuma, N., M. Okada, T. Katunuma, A. Fujino and T. matsuzawa. 1968. Different metabolic rates of transaminase isozymes. In "Pyridoxal Catalysis: Enzymes and Model Systems". Ed. By Snell, E.E., A. E. Braunstein, E. S. Sevrin and M. Y. Torchinsky. New York.
  17. Khedr, M. M. A. 2002. Effect of some plant extracts and insect growth regulators applied to control cotton leafworm on honeybees, *Apis mellifera* L. M. Sc. Thesis, Fac. Agric., Zagazig Univ., Egypt.
  18. Mostafa, S. A. 1993. Biochemical effect of some chemical compounds on *Spodoptera littoralis* (Boisd.). Ph. D. Thesis, Fac. Agric., AL-Azhar Univ., Egypt.
  19. Reitman, S. and F. Frankel. 1957. Colourmetric method for aspartate and alanine transaminase. Amer. J. Clin. Pathol., 28-56.
  20. Salem, I. E. M., A. A. EL-Sheakh, A. A. Khidr, W. M. H. Desuky and S. A. A. Raslan. 1995. Biochemical effect of some IGRs on phosphatases and treansaminases of the cotton leafworm, *S. littoralis* (Boisd.) (Lepidoptera). Zagazig J. Agric. Res., 22 (3) : 895 – 899.

## دراسات سمية وبيوكيميائية على تأثير بعض منظمات النمو الحشرية على دودة ورق القطن

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درس تأثير خمسة منظمات نمو حشرية هي كاسكيد، أتابرون، كونسلت، ماتش ومميك على السمية وبعض القياسات (الأنشطة) البيوكيميائية للعمرين الثاني والرابع لدودة ورق القطن على فترات مختلفة، وقد غذيت اليرقات الحديثة الانسلاخ على أوراق خروج معاملة بتسعة تركيزات مخففة من التركيز الحقلية وقد أشارت النتائج إلى:

ظهر مركب أتابرون كأكثر منظمات النمو الحشرية فعالية بينما كان مركب مميك أقلها تأثيراً ضد كل من العمر اليرقى الثاني والرابع، وقد أظهر العمر اليرقى الرابع حساسية أكبر لكل المركبات المختبرة عن العمر اليرقى الثاني عند كل التركيزات المختلفة.

- زادت منظمات النمو الحشرية محتوى البروتين الكلى الذائب بعد يومين من معاملة العمرين اليرقيين الثاني والرابع عدا كاسكيد عند التركيزات الأقل، وقد سجل انخفاضاً ملحوظاً بعد خمسة أيام من معاملة العمرين اليرقيين الثاني والرابع بكل من كونسلت، ماتش وكاسكيد.
- خفضت المركبات المختبرة نشاط إنزيم GOT بينما زاد نشاط إنزيم GPT بعد يومين من معاملة العمر اليرقى الثاني وكان العكس صحيحاً بعد خمسة أيام من المعاملة، وفي حالة العمر الرابع زادت منظمات النمو نشاط كلا الإنزيمين بعد ٢ و ٥ يوم من المعاملة.
- زيادة نشاط إنزيمات تريهاليز، انفرتيز وأميليز في العمرين اليرقيين الثاني والرابع بعد ٢ و ٥ يوم من المعاملة ما عدا مركبي كونسلت ومميك في حالة إنزيم الانفرتيز في يرقات العمر الرابع.