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SOME BIOLOGICAL AND BIOCHEMICAL EFFECTS OF CHITIN SYNTHESIS INHIBITORS ON PINK BOLLWORM *Pectinophora gossypiella* (SAUNDERS).

BY

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ABSTRACT

The effectiveness of two compounds diflubenzuron (Dimilin) and chlorfluazuron (Atabron 5% EC) belonging to insect growth regulators (IGR) groups, against newly hatched larvae of pink bollworm (PBW), *Pectinophora gossypiella* (Saund.) was tested under laboratory condition of 27° ± 1° C and 75-80% R. H. LC_{50} was determined (0.0423) for Dimilin and (0.1961) for Atabron. Also, the effect of the two (IGR) compounds on some biological aspects of PBW was studied.

Insect growth regulators prolonged the larval duration by 21.62& 23.76 for Dimilin & Atabron, respectively, than control. Also, increased the pupal period and duration of immature stages.

Two IGR caused shortened the oviposition period, where, this period estimated by 12.06 and 10.8 days for Dimilin & Atabron, than control. Also, effect on some other biological characters, such as total deposited eggs / female, hatchability, fecundity and longevity of emerged female. Results indicated that the two tested IGR compound (Dimilin & Atabron) increased pre-oviposition period, but caused shortness of female oviposition period and adult longevity. Also, they decreased the average number of deposited eggs /female.

Also some biochemical studies were carried out on larvae treated by LC_{50} Dimilin & Atabron to determination activities of enzymes GOT & GPT, carbohydrate hydrolyzing enzymes and total nutrient content, i.e. proteins, lipids and carbohydrates. Data revealed that treated larvae of PBW by Dimilin & Atabron caused increased total lipids, transaminase enzymes GOT & GPT, carbohydrate hydrolyzing enzymes (Invertase & Trehalase). While, cause decreased in total soluble proteins and the activity of carbohydrate hydrolyzing enzymes Amylase.

INTRODUCTION

Pink bollworm, *Pectinophora gossypiella* (Saund.) is considered the most destructive pest infesting cotton plants in Egypt; it cause serious damage to cotton bolls resulting in high loss in both quantity and quality of cotton yield (El-Feel *et al.*, 1991; El-Feshawi *et al.*, 1991 and Lohag & Nahyoon, 1995)

The effect of chitin – inhibitors, diflubenzuron (Dimilin) and chlorfluazuron (Atabron 5%EC) on the cotton bollworms and other insects was studied by several authors, Flint & smith (1977), Hussein *et al.*, (1984), Abd El-Megeed *et al.*, (1987) and Simwat & Dahawan (1992). The mode of action of both IGR is specific for control certain major pests in cotton, Soya bean, Citrus, tea plants and some vegetable crops –non systemic insect growth regulator with contact and stomach action, acts at time of insects molting, or at hatching of eggs. The present work aimed to evaluate the biological and biochemical effects of the two chitin inhibitors potency of (Dimilin) and (Atabron) on the newly hatched larvae of pink bollworm *P. gossypiella* in laboratory under controlled conditions.

MATERIALS AND METHODS

Rearing technique of the PBW *P. gossypiella*

The newly hatched larvae of pink bollworm *Pectinophora gossypiella* used in the present study were obtained from a laboratory culture reared for about 20 generations on an artificial diet that previously described by Rashad and Ammar (1985).

Materials used:

Insect growth regulators(IGR)

In this study, two antichitin compounds, (Dimilin and Atabron). were tested against PBW, *P. gossypiella.*

1- Diflubenzuron (Dimilin)

Empirical formula: C_{14} Hg Cl F_2 N_2 O_2

Chemical name: N- [(4-chlorophenyl) amino carbaryl]- 2,6- difluorobenzamide (IUPAC)

2- Chlorfluazuron (Atabron)

Empirical formula: C_{20} Hg Cl_3 F_6 N_2 O_3

Chemical name: 1- [3,5- dichloro- 4- (3- chloro- 5- trifluromethyl-2- pyridyloxy) phenyl] -3 (2,6- diflurobenzoyl) urea (IUPAC).

Pilot experiment was conducted to evaluate LC_{50} for each compound. Serial concentration dilutions, ranged from (0.0047 to 0.5) for Dimilin and from (0.033 to 1.0) for Atabron, were freshly prepared from the stock solution of each compound (2 ml/ 1 liter water).

An artificial diet was poured into glass tubes (2 x 7 cm.) at rate of 2 g diet / tube. Four replicates of 50 tubes /concentration of each compound were used in addition to 25 other tubes containing untreated diet.

The tested concentrations were spread (0.02 ml / tube) using micropipette on the upper surface of the diet all tubes were held uncapped for an hour to allow dryness and then individual of neonate larvae of PBW were placed into each tube using fine hair brush and capped by cotton wool, then incubated at 27 ± 1° C and 75-80 % R. H. for 24 hours, LC_{50} and slope values of both compounds were calculated according to Finney (1971).

Biological studies and biochemical evaluations.

Two groups of tubes, each group consists of 4 replicates were used for each tested compound, each tube contain an artificial diet. Each replicate (50 tubes) were used for each treatment. LC_{50} of the two IGR were applied on the upper surface of the diet / tube, as mentioned before. Also, 4 replicates, each replicate (25 tubes) was used for control.

Newly hatched larvae of *P. gossypiella* were placed individually into each tube treated and untreated using a fine hair brush and then capped by a piece of cotton wool and incubated at 27° ± 1° C and 75-80 % R.H. The tubes were examined after 1, 3, 7, and 15 to 24 days, then daily examined until adult emergence to estimate mortality percentage of larvae, pupae and adults malformation of larvae and pupae and duration of larvae, pupae and total immature stage, each treatment. Also, to determine the pre – oviposition, oviposition, post – oviposition periods, number of deposited eggs and number of hatching larvae from the eggs for both treated compounds.

Statistical analysis

One – way analysis of variance (ANOVA) and Duncan's multiple range test of means were used (Duncan's, 1955).

For the biochemical studies, ten replicates of 20 tubes prepared by the previous manner with LC_{50} / each compound. Another group of newly hatched larvae were reared on untreated diet (control). Larvae were sampled after 10 days of treatment, collected and placed in clean jars for biochemical analysis.

Determination of enzyme activities:

a-Transaminase enzymes (GOT & GPT):

These activities were carried out in association of Insect Physiology Department in Plant Protection Research Institute

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities were determined colourimetrically according to the method of Reitman and Frankle (1957).

b- Carbohydrate analysis enzymes:

The methods used to determine the digestion of trehalose, starch and suerose by trehalase, amylase and invertase enzymes, respectively were similar to those described by Ishaaya and Swiriski (1976). The free aldehydic groups of glucose formed after trehalose, starch and sucrose digestion were determined using 3.5 dinitro salicylic acid reagent.

Total Protein:

The content of total proteins was determined colorimetrically with the biuret method according to Henry (1964). The optical density (O D)of the samples was measured with specto photometer, at 546 nm. and expressed in mg / ml of haemolymph using the following equation.

Total protein (mg / ml) = OD sample / OD standard x 6

The total content of haemolymph carbohydrates was determined colorimetrically at the wave length of 620 nm. using the atthron method according to Singh and Sinha (1977)

The total content of haemolymph lipids was determine colorimetrically at the wave length of 525 nm using the method of Knight *et al.* (1972).

RESULTS AND DISCUSSION

To reveal the LC_{50} of the two chitin inhibitor tested compounds (Dimilin & Atabron) which can be expressed as their latent effects on some biological aspects of the pesticidal survived larvae, pupae and subsequent developmental stages from them were determined. The results could be discussed as follow.

Duration of larval stage:

Analysis of variance of the data given in Table (1) indicates highly significant effects of both insecticides on larval duration in comparison with control. Larval period was significantly prolonged when neonate treated with Dimilin and Atabron than control. These periods were 21.625±0.236 23.76± 0.50 15.41±0.208 days for Dimilin, Atabron and control, respectively. This period prolonged by 1.4 time when newly hatched larvae fed on treated diet by Dimilin and 1.54 time when fed on Atabron.

-Larval weight:

Larval weight was greatly reduced when the *P. gossypiella* newly hatched larvae were fed on treated diet with (Dimilin or Atabron). Untreated larvae were about 2.2 times and 4.64 times as large as those fed on treated diet Dimilin and Atabron, respectively. Such reduction, in larval feeding induced by Dimilin and Atabron treatment, consequently resulted in a significant decrease in larval weight gain compared to that of control.

Welland *et al.* (1997), Found that (diflubenzuron) Dimilin alone and combination with Bt. Cotton was more affective reducing larval feeding and increasing larval mortality for cotton boll worm *Helicoverpa zea*.

- Morphological deformation in larvae:

Data presented in Table (1) and Fig. (1A&B) show the morphogenetic action of malformation of diflubenzuron and chlorfluazuron. Percent of Malformation was (6.9%) & (10.6%) with Dimilin and Atabron, respectively.

Percent of pupation:

As clearly shown from the results in Table (1) the two tested compounds was significant effect on the percent of pupation. The percent of high pupation (94%) was recorded for diflubenzuron and the lowest pupation (89%) was recorded for chlorfluazuron as compared with 100% pupation in cheek experiment.

-Pupul period:

Data in Table (1) demonstrate that diflubenzuron and chlorfluazuron did not influence on the developmental period of pupae resulted from the larvae fed on the diet treated with LC_{50} of diflubenzuron and chlorfluazuron this period was 10.3±0.29 and 10.0±0.248 days. While, this period was differ significantly in the control (7.58±0.14 days) where as prolonged pupa period by 1.3 time than the control.

-Morphological deformation in pupa:

According to the results in Table (1) and Fig. (2) the highest percent morphological deformed pupae was 32.35% when the larvae fed on treated diet by chlorfluazuron and the lowest percent 4% when the larvae fed on treated diet by chlorfluazuron, while, it was (1%dead) for control.

-Total immature stage:

Statistical analysis of data indicate that the effects of both tested compounds were highly significant. The total developmental period of immature stages was longer than control. Total duration of immature stages did not significantly differ between the two CSI (chitin synthesis inhibitors). Rizk and Rradwan (1975) and Flint and Smith (1977); found that the higher role of diflubenzuron caused death to the larvae and later deformation to pupal stage of pink bollworm.

-Percent of adult emergence and malformation:

Data in Table (1) show that in untreated, all formed pupae succeeded to develop into adults. Adult emergence resulted from treated larvae were 98% and 83.4% when larvae treated by diflubenzuron and chlorfluazuron, respectively. About 21% and 5% of survived adults show morphological malformations Fig. (3 A&B) compared with 0% for control. Flint and Smith (1977) recorded that diflubenzuron caused 64% reduction in adult emergence.

- Pre-Ovipostion.

Data in Table (2) indicate that diflubenzuron gave pre-ovipostion period significantly longer than control (the period was 4.1 ± 0.16 days). While, there was no difference, between chlorfluazuron and control, the period was 2.93±0.21 days. This result indicated that the diflubenzuron increased the pre– ovipostion period by 1.8 times than control.

-Ovipostion period:-

Table (2) show that the ovipostion period was high significantly influenced by both compounds. The ovipostion period was 12.06±1.08 and 10.8±1.96 days, when the newly hatched larvae fed on diet treated by diflubenzuron and chlorfluazuron, respectively compared with 18.1 ± 0.3 days for control.

That means that the two IGR caused shortness in female ovipostion period from (1.5 to 1.8 time) than control.

Table (1): Effect of Dimilin and Atabron on some biological aspects of *P. gossypiella* under laboratory condition 27±1°C and 75-80% R. H..

Treatment	LC_{50}	Initial number of larvae	Larval stag				Pupal stag				Duration of immature stage (days) (±(SE)	Adults			
			% accumulative mortality larvae of	Duration (days) ±(SE)	weight of larva	Malformation	% pupation	% Malformation	No of normal pupae	Duration (days) ±(SE)		% Adult emergence	% Total	Malformation ♀	Malformation ♂
Dimilin	0.0423	200	64	21.62±0.23 (19-24)b	0.0118	6.9%	94	32.35	67.6	10.3±0.29 (8-12)a	31.937±0.31 (32-36)a	98	21	40	60
Atabron	0.1961	200	70	23.76±0.50 (18-27)a	0.0065	10.6%	89	4	96	10.0±0.248 (8-11)a	33.0±0.61 (28-37)a	83.4	5	0	5
Control		100	12	15.41±0.28 (13-17)C	0.026	0	100	1	99	7.58±0.14 (7-9)b	23.08±0.253 (21-25)b	100	-	-	-
F value			253.5				16.035				132.62				
Pro.			0.000**				0.01**				0.000**				
LSD at 5%			0.952				0.976				1.42				

Table (2): Effect of treated neonate Larvae of *P. gossypiella* by Dimilin and Atabron on fecundity and longevity its adult stage.

Treatment	Duration			Total no of eggs / ♀	% hatch-ability of eggs	Adult longevity (days)	
	Pre-ovipostion (Days)±(SE)	Ovipostion (Days) ±(SE)	Post-ovipostion (days)±(SE)			♀	♂
Dimilin	4.1± 0.16 (3-5)a	12.06±1.08 (5-19)b	2.26±0.25 (0-3)a	126.5 ± 0.95b	45.92 %	18.46±1.39 (9-26)a	15.4 ± 0.34 (11-16)a
Atabron	2.93 ±0.21 (2-4)b	10.8±1.46 (5-21)b	1.6±0.21 (0 – 3)a	123.3 ± 0.54b	59 %	15.3±1.49 (8 – 26)a	14.5± 0.57 (10-16)a
Control	2.7 ± 0.01 (2-3) b	18.1±0. 3 (11-21)a	2.3±0.11 (1– 3)a	177.1± 6.3a	94.3 %	21.9±1.3 (18– 36)b	16.1 ± 0.3 (13 – 18)a
F value	10. 182	43.55	2.333			8.4	1.47
Pro.	0.012 *	0.000 **	0.178			0.018 *	0.301
LSD at 5%	0.79	2.165	ns			3.65	ns

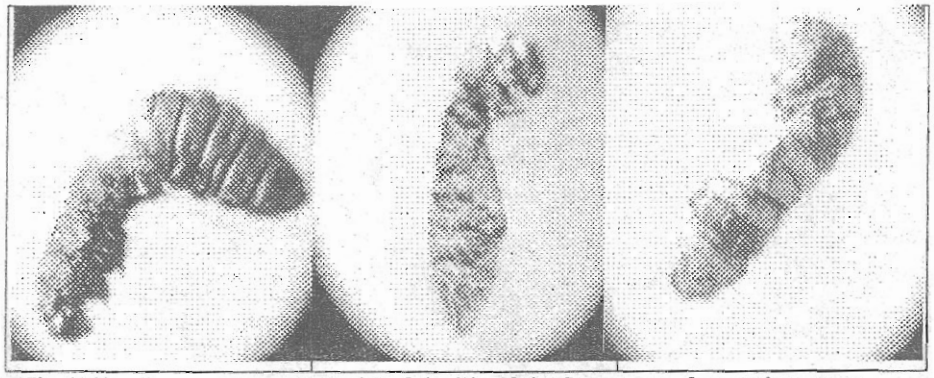

Fig.(1A): Larvae treated with Dimilin failed to transformation to pupa (shape intermediate between larvae and pupa).

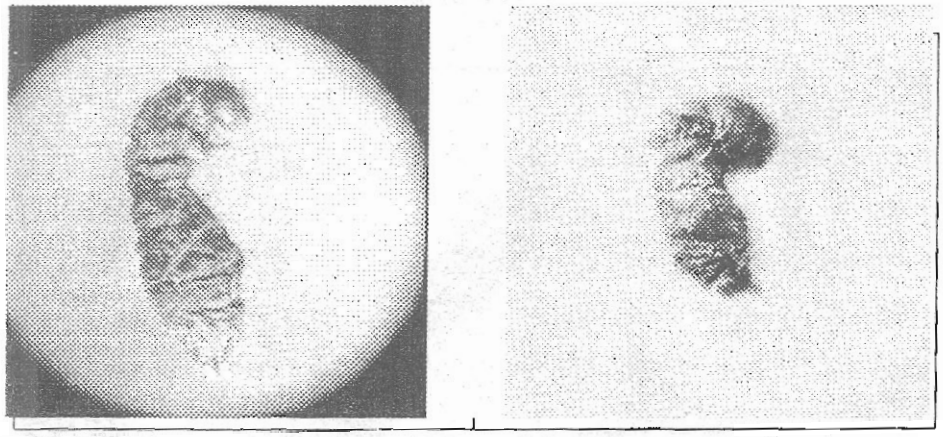

Fig.(1B): larvae treated with Atabron failed to transformation to pupa (shape intermediate between larvae and pupa).

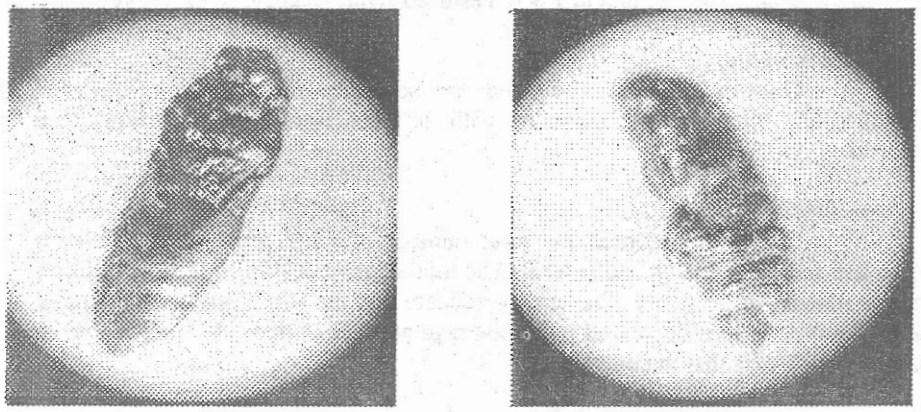

Fig. (2): Pupal- larval intermediates of PBW resulted from Atabron treatment

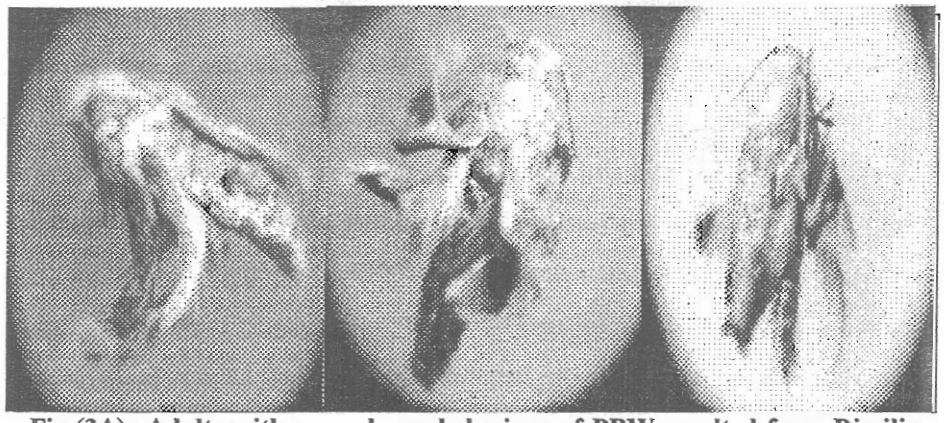

Fig.(3A): Adults with severely curled wings of PBW resulted from Dimilin treatment.

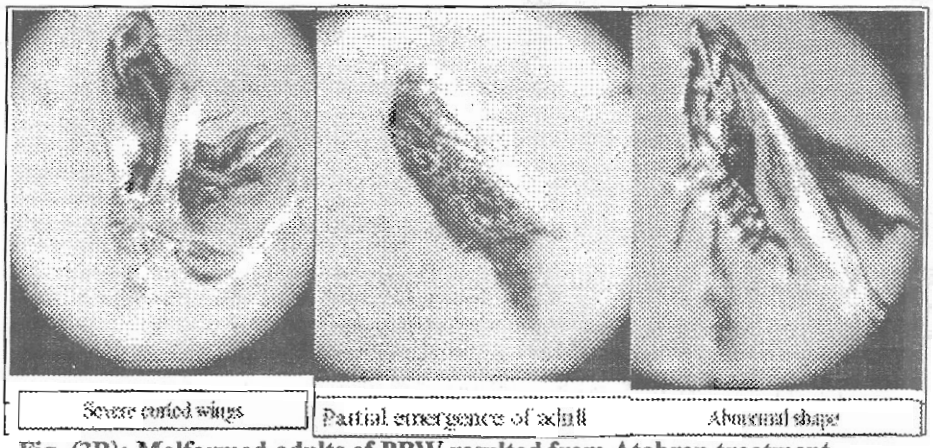

Fig. (3B): Malformed adults of PBW resulted from Atabron treatment

- Post-Ovipostion.

Data in Table (2) show that the post-ovipostion period of emerged females was affected by treatment with diflubenzuron, chlorfluazuron than control.

- Fecundity:-

Table (2) recorded the total number of eggs deposited by females emerged from treatments and control. The total eggs were 126.5, 123.3 and 177.1 eggs / female, respectively. The results indicate that the two compounds (Dimilin and Atabron) caused decreased in the average number of deposited eggs / female as compared with that in control.

Fertility of eggs:-

Data in Table (2) indicate that the percent of egg hatching was high significant affected by treatments. The percent of hatchability of eggs were

45.92% and 59 % of eggs for females emerged from treated larvae with diflubenzuron and chlorfluazuron compared with 94.3% eggs / female of control. This data indicate that the two tested compounds caused failure of the insect embryonic development. showing the lowest percentages egg hatching compared with of control.

- Adult longevity:-

females:-

Statistical analysis of data in Table (2) show that female longevity was highly significant affected by IGR treatment than control, where adult longevity was 18.46 and 15.3 days / female for diflubenzuron and chlorfluazuron, respectively, compared with 21.9 days / female for control.

Males:-

Data in Table (2) show that no significant affected between the two treated compounds and untreated this period was 15.4, 14.5 days /male when adult males emergence from newly hatched larvae treated by diflubenzuron and chlorfluazuron, respectively than 16.1 days for control.

-Biochemical effects of the two compounds on larvae.

- Total soluble proteins:

Data in Table (3) reveal that the LC_{50} of both Dimilin and Atabron caused decrease in total soluble proteins in larvae compared with control. The high decrease in total soluble proteins recorded with Dimilin (-33.162). While, decrease of total soluble proteins was noticed in Atabron- treated larvae (-25.31) than control.

- Total Lipids:

Data in Table (3) show that larvae treated by diflubenzuron and chlorfluazuron caused increased total lipid activity to 103.653 and 202.587, respectively. The two tested compounds increased total lipids in treated larvae by 1-2 times than control.

-Carbohydrate hydrolyzing enzymes.

-Invertase.

Results in Table (3) clear that the two tested compounds caused increase in invertase activity in the supernatant of homogenated larvae of pink bollworm as compared to control. Data revealed that diflubenzuron caused increase in invertase by 2 times and by 1.45 times when larvae treated with chlorfluazuron.

-Amylase

Data in Table (3) indicate that the activity of amylase enzyme in the supernatant of homogenated larvae of pink bollworm was generally decrease as affected by the two compounds. The decrease in amylas activity recorded -19.63 & -63.27 with diflubenzuron and chlorfluazuron, respectively.

-Trehalase

Data in Table (3) reveal that the tested compounds increased the activity of trehalase,.(81.137 and 158.397) when the newly hatched larvae fed on diet treated by diflubenzuron and chlorfluazuron respectively. Kheder (2002) reported that the activity of trehalase, invertase and amylase was increased after treated *S. littoralis* larvae by Biorple.

- Transaminase enzymes (GOT & GPT):

Data in Table (3) indicate that the activity of (GOT and GPT) in larvae of pink bollworm was increased when treated by diflubenzuron, and chlorfluazuron. Also, the compounds caused high increase in (GOT and GPT) than control.

Table (3): Biochemical Effects of diflubenzuron (Dimilin) and chlorfluazuron (Atabron) on larvae of Pink bollworm

Treatment	Total soluble protein	Total lipid	% Transamenase enzymes		Carbohydrate Enzymes %		
			GOT	GPT	Amylase	Inverts	Trehalase
Diflu-benzuron	--33.162	103.653	85.768	85.768	-19.63	44.655	81.137
Chlorf-luazuron	-25.31	202.587	19.85	19.85	-63.27	11.89	158.397

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دراسات بيولوجية وبيوكميائية لبعض مانعات الإنسلاخ على دودة اللوز القرنفلية

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** قسم وقاية النبات ــ كلية الزراعة بمشتهر – جامعة بنها

تم دراسة تأثير مركبين من مانعات الإنسلاخ تنتمي لمجموعة (IGR) على بعض الصفات البيولوجية لحشرة دودة اللوز القرنفلية تحت ظروف المعمل من درجة حرارة ٢٧± 1° م ، رطوبة نسبية من ٧٥- ٨٠ % وأوضحت النتائج أن:-

استخدام التركيز النصف مميت LC_{50} لمركبي الديميلين والأتابرون سبب نسبة موت عالية مع إطالة معنوية في مدة طور اليرقة والعذراء عن الكنترول. كما أظهرت النتائج أن نسبة التعذير انخفضت في اليرقات المعاملة عن الكنترول وكذلك نسبة خروج الفراشات. كما انخفضت أيضا فترة وضع البيض ونسبة وضع البيض والنسبة المئوية للفقس وكذلك قصرت مدة حياة الحشرة الكاملة من إناث وذكور.

أما بالنسبة للدراسات البيوكيميائية فقد أوضحت النتائج أن: عند معاملة يرقات الفقس الحديث لدودة اللوز القرنفلية بواسطة مركبي الديميلين والأتابرون سبب زيادة فى الدهون و كلا من أنزيمات GOT & GPT وكذلك بعض أنزيمات المواد الكربوهيداتية مثل (Trehalase & Invertase). بينما المعاملة بهذة المركبات تسببت فى خفض نسبة البروتينات الذائبة ونشاط أنزيم الأميليز (أحد أنزيمات المواد الكربوهيدراتية).