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Bioinsecticidal Activity of some Compounds on the Cotton Leafworm, *Spodoptera littoralis* (Boisd.) under Laboratory Conditions.

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ABSTRACT

In a laboratory study investigated the changes in the metabolic products namely, total carbohydrates, total proteins and total lipids in the fourth larval instar of *Spodopter alittoralis*(Boisd.) tested with the LC_{50} values. Compounds were one of plant growth regulator (Ethephon), emamectin benzoate (Highlex), chitin synthesis inhibitor (Novo), *Bacillus thuringiensis*(Protecto) and chlorpyrifos (Pestban) under laboratory conditions. Results indicated that, protecto showed the highest total carbohydrate content in the treated larvae (16.90 mg/g.b.wt.) than the other products followed by highlex, pestban, novo, and ethephon. The percentage of the change in the total carbohydrate content in the fourth larval instar treated with the LC_{50} values recorded the highest decreasing percentage when treated larvae by plant growth regulator ethephon and represented by (-48.98%). Larvae exhibited the highest total protein content were treated with highlex (38.40mg/g.b.wt.) followed by pestban, protecto, novo then ethephon. Highest decreasing percentage of total protein content found in larvae treated with ethephon and represented by (-30.8%).Whereas the highest total lipids content was found in larvae treated with highlex (7.94 mg/g.b.wt.) followed by pestban, protecto, ethephon then novo.Highest decreasing percentage when treated larvae chitin synthesis inhibitor, (novo) and represented by (-18.60%).

Keywords: plant growth regulators, Total carbohydrates, *Spodoptera littoralis*.

INTRODUCTION

Cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is an extremely dangerous insect pest, the larvae of which can attack several economically important crops cutting across over 40 families or 112 plants belonging to 44 families over the world . *S. littoralis* inflicts excessive damage when it occurs in masses during certain years. In Egypt, it is destructive phytophagous lepidopterous pest causing various ravages not only for cotton plants but also for other field crops, vegetables and fruit trees all over the year. This insect attacking plant includes 73 species recorded at Egypt (Ahmad, 1988; Amin and Salam, 2003 and Hatem *et al*., 2009).

Control of the cotton leafworm, *S.littoralis* by chemical insecticides is the main tool for combating such pests but repeated applications of insecticides on cotton and other crops induced many problems, including toxic and persistent environmental residue, development of pesticide resistant strains of pests and destruction of non-target organisms particularly beneficial natural enemies (El-Naggar, 2013). Also (Khoja *et al.,* 2006) in Egypt, who found that the *B. thuringiensis* causes the mortality of *S.littoralis* 4th larval instar when treated by Dipel 2X and Protecto. One of the latest approaches for pest control is use of the plant growth regulator such as ethephon, gibberellic acid (GA3) against *S. littoralis* larvae. Insect growth regulators (IGRs) are produced naturally by insects to regulate the processes of moulting and development of immature stages to the adult's stage (Uckan *et al*., 2015).

Carbohydrates, protein and lipids content have an important role in biological and physiological activities of insects such as body size, growth rate, fecundity and at higher levels of organization has been linked to population dynamics and life histories (Fagan *et al* ,2002). Total protein, total lipid and total carbohydrate contents at most of the doses decreased in host larvae when treated by plant growth regulator GA3. (Uckan *et al*., 2011). The purpose of this work was conducted to investigate effect of some plant growth regulators, insect growth regulators and bio insecticides on the changes in the metabolic products namely, total carbohydrates, total proteins and total lipids in the fourth larval instar of *S.littoralis* under laboratory conditions.

MATERIAIS AND METHODS

This study was carried out under laboratory conditions at plant protection Research Institute, Sakha Agriculture Research Station, Agricultural Research Center (ARC). For studying the efficiency of some plant growth regulators, insect growth regulators, bio-insecticides and chemical insecticides on the fourth larval instar of the cotton leaf worm, *Spodopter alittoralis* (Boisd.).

1. Rearing technique of cotton leafworm:

A stock culture of cotton leaf worm, *Spodoptea littoralis* (Boisd.),laboratory strain, was obtained from cotton pest research department, Plant Protection Research Institute, Agricultural Research Center, , Sakha; Egypt.

2. Tested compounds:

1. Plant growth regulator:

Trade name: Xphone 48%

Common name: Ethephon (2-chloroethylphosphonic acid). The active ingredient concentration is 480G/L. Manufactured by Hockley International LTD, UK.Test insects: the used concentrations were 50000, 100000, 150000, 200000 and 250000 ppm.

2. Insect growth regulators:

1. Trade name: Highlex 5%SG

Common name: Emamectin benzoate (4"R)-4"deoxy-4" (methylamino) avermectin B1 benzoate (1-1)

Group: Avermectin

The active ingredient concentration is 5% SG and the application is 60 g/feddan.. The used concentrations were 1,2,3,4 and 5ppm.

2. Trade name: Novo 1o%EC

Common name: Lufenuron

Group: Benzoylurea

The active ingredient concentration is 100G/L% and the application is 500ml /feddan. The used concentrations were 200,250,300,350 and 400ppm. Supplied by Singynta Company.

3. *Bacillus thuriengensis*(Protecto10 % W.P.):

A commercial formulation of *Bacillus thuringiensis* var. *kurstaki* and it is a product of the special unit for producing Bio-insecticides, with 3200 International toxicity Unit (IU). The active ingredient was 9.4% and. The used concentrations were250, 500, 1000, 1500 and 2000 ppm.

4.Chlorpyriphos (pestban 48%E.C.):

One pesticide was used with the recommended rate and four lower concentrations.

Trade name: (pestban 48%E.C.)

Common name: Chlorpyrifos (O,O-Diethyl O-3,5,6-trichloropyridin-2-yl) phosphorothioate

Group: Organophosphorus.

Recommended rate 1liter/feddan.

The used concentrations were 50,100,150,200 and 250 ppm

3. Treatment procedure:

Four replicates each contain 30 newly molted 4th larval instar for each concentration of each tested product were fed on Fresh and clean castor oil leaves treated by dipping technique. Leaves were offered to the newly molted 4thinstarlarvae that left to feed on treated leaves for 48 hours. The same numbers of larvae were used for check experiments in which larvae were offered fresh clean castor leaves dipped in water. Mortality was recorded daily and corrected by Abbott formula (Abbott, 1925) based on death in control. Probit analysis for mortality data at 24 h (Finney, 1971) was adopted to obtain the LC50 value Treatment procedure:

4. Biochemical analysis:

- Total carbohydrates

Total carbohydrates were evaluated in acid extract of treated samples by the phenol-sulphuric acid reaction of Dubois *et al.* (1956). Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967).

% change in total carbohydrates

$$= \frac{\text{Total carbohydrates in treated larvae- total carbohydrates in control}}{\text{Total carbohydrates in control}} \times 100$$

- Total protein

Total proteins were calculated by the method of Bradford (1976).

% change in total proteins

$$= \frac{\text{Total proteins in treated larvae- total proteins in control}}{\text{Total proteins in control}} \times 100$$

- Total lipids

Total lipids were determined by the method of Knight *et al.* (1972).

% change in total lipids

$$= \frac{\text{Total lipids in treated larvae- total lipids in control}}{\text{Total lipids in control}} \times 100$$

RESULTS AND DISCUSSION

Metabolism pathway many biochemical changes have been reported to occur the different changes in the metabolic products or compounds of the total body tissues of the cotton leafworm, *S. littoralis* when treated the fourth larval instar with LC_{50} values of the different tested compounds. Values of homogenate contents of total carbohydrate, total protein and total lipids were estimated in the 4th larval instar treated with LC_{50} concentrations of Ethephon, Emamectin benzoate (high lex), novo5%, protecto10%and pestban48%EC after 48 hours of treatment.

Data arranged in Table (1) showed the effect of the tested compounds on the total carbohydrates content in the fourth larval instar of the cotton leafworm, *S. littoralis.* Results indicated that, *Bacillus thuriengensis* (Protecto 10% w.p.) showed the highest total carbohydrates content after treatment with different compounds with LC_{50} values (16.90 mg./g. b.wt.), followed by the insect growth regulator, (Emamectin benzoate 5%SG), with (15.97 mg./g. b.wt.),chlorpyrifos (Pestban 48 E.c.)with (14.97 mg./g. b.wt),chitin synthesis inhibitor,(Novo 5%EC) with (10.2 mg./g. b.wt.).On the other hand,the plant growth regulator (Ethephone)showed the lowest total carbohydrates content in the fourth larval instars treated with lC_{50} values and represented by(8.74 mg./g. b.wt.).Moreover, the percentage of the change in the total carbohydrates content in the fourth larval instar treated with the LC_{50} values recorded the highest decreasing percentage when treated larvae by plant growth regulator (Ethephone 48%) and represented by (-48.98%).

Table 1. Total carbohydrates in the 4th larval instar homogenate of *S.littoralis* treated with LC_{50} values of different compounds.

Compounds	Total carbohydrate content mg/g.b.wt.	change in the total carbohydrate content%
Plant growth regulator:(Ethephon 48%)	8.74	-48.98
Emamaectin benzoate: (high lex)	15.97	-6.78
Chitin synthesis inhibitor: (novo)	10.20	-40.3
Bacillus thuringiensis: (Protecto 10% w.p.)	16.90	-1.2
Chlorpyrifos: (Pestban)	14.97	-12.86
Control	17.13	-

Data represented in Table (2) indicated that the effect of the tested compounds on the total protein content in the fourth larval instar of the cotton leafworm,*S. littoralis.* Results indicated that Emamectin benzoate showed the highest total protein content after treatment with different compounds with LC_{50} values (38.4mg./g. b.wt.)followed by chlorpyriphos with (36.3 mg./g. b.wt.),Protecto with (32.1 mg./g. b.wt.,Novo with(27.5 mg./g. b.wt.On the other hand,the plant growth regulator ethephon showed the lowest total content in the fourth larval instars treated with LC_{50} values and represented by (26.7 mg./g. b.wt.).Moreover,the percentage of the change in the total content in the fourth larval instar treated with the LC_{50} values recorded the highest decreasing percentage when treated larvae by ethephon and represented by(-30.8 %).

Data presented in Table (3) showed the effect of the tested compounds on the total lipids content in the fourth larval instar of the cotton leafworm,*S. littoralis.* Results indicated that emamectin benzoate showed the highest total content after treatment with different compounds with LC_{50} values (7.94mg./g. b.wt.), followed by chlorpyrifos (7.85 mg./g. b.wt.), Protecto (7.72 mg./g. b.wt.), ethephon

(7.22mg./g. b.wt.). On the other hand , Novo showed the lowest total protein content in the fourth larval instar treated with LC_{50} values and represented by (6.74 mg./g. b.wt.). Moreover, the percentage of the change in the total protein content in the fourth larval instar treated with the LC_{50} values recorded the highest decreasing percentage when treated larvae by novo and represented by(-18.6%).

Table 2. Total protein content in the 4th larval instar homogenate of *S.littoralis* treated with LC_{50} values of different compounds.

Compounds	Total protein content (mg/g.b.wt.)	change in the total protein content(%)
Plant growth regulator:(Ethephone)	26.70	-30.8
Emamaectin benzoate:(Highlex)	38.40	-0.51
Chitin synthesis inhibitor: (Novo)	27.50	-28.7
Bacillus thuringiensis:(Protecto)	32.10	- 16.8
Chlorpyrifos: (Pestban)	36.30	-5.9
Control	38.6	-

Table 3. Total lipids content in the 4th larval instar homogenate of *S.littoralis* treated with LC_{50} values of different compounds.

Compounds	Total lipid content (mg/g.b.wt.)	Change in total lipid content (%)
Plant growth regulator:(Ethephone)	7.22	-12.80
Emamaectin benzoate: (high lex)	7.94	-4.11
Chitin synthesis inhibitor: (novo)	6.74	-18.60
Bacillus thuringiensis: (Protecto)	7.72	-6.76
Chlorpyrifos: (Pestban)	7.85	-5.19
Control	8.28	-

These results are in agreement with those of (El-barky *et al.,* 2008) in Egypt, who observed that significant decrease in carbohydrates content at *S. littoralis*, 5th larval instar, after treatment by radiant (Spinetoram) with LC_{50}. (El-sheikh *et al.*, 2013) in Egypt, evaluated two insect growth regulators and *B. thuringiensis* (used at LC_{50}) were used for treatment of 2nd larval instar of cotton leaf worm, *S. littoralis*. Treatment caused significant decreases in total carbohydrates during the pupal stage and the sequential combined effect treatments had more decreasing effect than the individual treatments. (El-gabaly, 2015) in Egypt, indicated that the chlorpyrifos, lufenuron and protecto at their LC_{50} values caused adecrease in total proteion content of 4th larval instar of *S. littoralis* may by arrangement in descedisly as 35,35.1,35.9,36.6 and 37.8my/g.bwt which recorded of post treatment spinetoram relative to control. (Assar *et al*., 2016) in Egypt, indicated that the total proteins, total carbohydrates and total lipids content were decreased when treated 4th larval instar of *S.littoralis* with emamectin and teflubenzuron as insect growth regulators.

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النشاط الحيوي لبعض المركبات على دودة ورق القطن تحت الظروف المعملية

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يهدف البحث الى فحص التغيرات في محتوى الكربوهيدرات الكلية والبروتينات الكلية والدهون الكلية في العمر اليرقى الرابع لدودة ورق القطن (Boisd.) *Spodoptera littoralis* على أساس (LC_{50}) من منظمات النمو النباتية (أثيفون ٤٨٪) وإيمامكتين بنزوات (هاى ليكس) ومانع الانسلاخ (نوفو) وبكتريا *Bacillus thuringiensis*(بروتكتو١٠٪) والكلوربيروفوس (بستبان ٤٨٪) تحت الظروف المعملية .أوضحت النتائج ما يلي :-بروتكتو ١٠% أحدث أعلى نسبة في محتوى الكربوهيدرات مع يرقات العمراليرقي الرابع عن المعاملات الأربعة الأخرى(١٦.٩مليجرام/جرام من وزن الجسم)يليه إيمامكتين بنزوات ثم بستبان ثم نوفو وأخيراً الإثيفون. كانت أعلى نسبة تغيير في المحتوى الكلى للكربوهيدرات فى يرقات العمر الرابع المعاملة بالتركيز النصف مميت (LC_{50})كانت المعاملة بالإثيفون (-٤٨.٩٨٪). سجلت اليرقات المعاملة بإيمامكتين بنزوات أعلى محتوى للبروتينات الكلية (٣٨.٤ مليجرام/جرام من وزن الجسم) يليه البستبان ثم بروتكتو ثم نوفو وأخيراً الإثيفون. كانت أعلى نسبة تغيير في المحتوى الكلى للبروتينات في اليرقات المعاملة بالإثيفون (-٣٠.٨٪) .أعلى محتوى للدهون الكلية سجلته اليرقات التي تم معاملتها بإيمامكتين بنزوات (٧.٩٤ مليجرام/جرام) من وزن اليرقة يليها تلك المعاملة بالبستبان ثم بروتكتو ثم أثيفون وأخيراً نوفو. أعلى نسبة تغيير في المحتوى الكلى للدهون وجد في اليرقات المعاملة بمانع الانسلاخ نوفو (-١٨.٦٪).