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HISTOCHEMICAL EFFECTS OF SOME BOTANICAL EXTRACT S ON THE MIDGUT OF THE COTTON LEAFWORM *Spodoptera littoralis* (Boisd) (LEPIDOPTERA : NOCTUIDAE).

Assar, A.A.

Zoology Department, Faculty of Science, Minufiya University, Shebin El-Kom, Egypt.

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Abstract

The histochemical effects of acetone and water extracts of Artemisia monosperma, Zygophyllum coccineum, Lupinus termis and Brassica tournefortii were investigated on the midgut cells of the 4th larval instar of Spodoptera littoralis. All the tested plant extracts induced several histochemical changes on the carbohydrate and protein content of the midgut cells. The decreased level of carbohydrates (polysaccharides) was more pronounced than that of protein. L. termis extracts were the most effective, followed by B. tournefortii extracts, while the extracts of A. monosperma and Z. coccineum gave a moderate reduction.

Introduction

The cotton leafworm *S. littoralis* (Boisd) is considered one of the most destructive cotton pest in Egypt and many other countries in the world. It is polyphagous, occurs throughout the year, feeds on a great variety of crops and attacks more than 112 host plants including 73 plant species in Egypt, of which 45 are preferred for feeding, 16 are preferred hosts for oviposition, while 12 species are used for both (Abdel- Hafez, 1978). The botanical extracts, as alternatives or adjurants to chemical insecticides, are ideal for controlling this pest, having repellent, insecticidal and antifeedant effects.

Histochemical studies on insects are considered one of the most specific and interesting types of investigation. In certain Lepidopteran insects, cytochemical or histochemical studies have been performed by many authors: (Ashhurst, 1964) on the blood cells of the wax moth *Galleria mellonella* L.; (Ashhurst and Richards, 1964), on the connective tissue associated with the central nervous system of *G. mellonella* L.; (Ramadan *et al.*, 1985), on the accessory genital glands of female *S. littoralis*; (Sorour and Osman, 1991) and on the salivary glands secretion of the last larval instar of *S. littoralis*. With regard to biochemical studies, Prasada and Ramamurty (1983) conducted biochemical studies on DNA, RNA and protein contents of the labial glands during postembryonic development in *S. litura*.

The histochemistry of the insect midgut has received very little attention. Sundman and King (1964) made histochemical studies on the alimentary canal of the adult boll weevil, *Anthonomus grandis*. Houk *et al.* (1986) conducted histochemical staining of the complex carbohydrates of the midgut of the mosquito, *Culex tarsalis*. However, the histochemical effects of insecticides and botanical extracts on insects very few investigations have been carried out. Hamed *et al.* (1974) studied the histochemical effects of DDT on the larvae of *Anopheles pharoensis*. Saleem and Shakoori (1985) studied the effect of permethrin and malathion on DNA, RNA and total protein content in *Tribolium castaneum* larvae. Assar and Emara (1996) studied the histochemical effects of Dimilin on the midgut of the cotton leafworm *S. exigua*. The histopathological effects of the present plant extracts under study were carried out on the midgut of *S. littoralis* larvae (Younes *et al.*, 1999).

The present study was carried out to investigate the histochemical effects of acetone and water extract of some plants on the midgut of the 4th larval instar of the cotton leafworm *S. littoralis*; with reference to carbohydrates (Polysaccharides), and proteins. The tested plants were *Artemisia monosperma, Zygophyllum coccineum, Brassica tournefortii* and *Lupinus termis*.

Materials And Methods

The cotton leafworm *S. littoralis* (Boisd) was reared at constant room temperature (27 ± 1ºC), 65 ± ±5% relative humidity, in a 12-hour photophase and on castor bean leaves.

The tested plants were as follows:-

Common name	Scientific name	Family	Tested parts
1- Aader-Lel-Lel	*Artemisia monosperma*	Compositae	Aerial
2- Rotreyt	*Zygophyllum coccineum*	Zygophylaceae	Aerial
3- Termis	*Lupinus termis*	Leguminosae	Seeds
4- Kabar	*Brassica tournefortii*	Cruciferae	Leaves

The 1st and 4th plants were collected from Sadat city (Minufiya province), the 2nd plant was collected from Wadi El-Natroun (El-Beheira province), while the 3rd plant was bought from the market.

The tested parts were washed, air dried and ground. Extraction was carried out using acetone and water as solvents. The solvents were evaporated by the Rotary evaporator to get the crude extract.

Experiments were carried out on the 4th larval instar of *S. littoralis*. The crude extracts were weighed and redissolved in the corresponding solvent to attain the standard concentration (8%) which was sprayed on the upper surface of castor bean leaves using a hand atomizer. Following evaporation of the solvents, the larvae were introduced and left for 48 hrs. A control experiment was performed using solvents only. After 48 hours of treatment, samples of the treated and untreated larvae were anaesthetized, dissected and parts of the midgut were quickly removed and stored in appropriate fixatives. After fixation, the materials were dehydrated, cleared and embedded in Paraffin. Sections of 5 μm thick were cut and stained to observe different histochemical reactions.

Polysaccharide material was observed following the application of periodic acid Schiff's technique (PAS) (Hotchkiss, 1948). Material was fixed in carnoy's fluid. The PAS-positive material appeared pink or red violet. Carnoy's fluid was suitable for the fixation of proteins. Total proteins were detected according to mercury bromphenol blue method (Bonhag, 1955).

Results

1 - The carbohydrates (polysaccharides):

A large amount of polysaccharide material was observed in the midgut cells of control larvae of *S. littoralis*, as indicated by the strong (marked) PAS-positive reaction given by these cells (Fig. 1 A,B).

Following 48 hours of treatment, a noticeable reduction in polysaccharide content was observed in the midgut cells, as compared to the control. The acetone and water extracts of both *A. monosperma* and *Z. coccineum*, induced a moderate reaction with PAS (Figs. 2 A,B & 3 A,B). The acetone and water extracts of *L. termis* (Fig. 4 A,B) produced a marked decrease in polysaccharides. The acetone extract of *B. tournefortii* induced a moderate reaction (Fig. 5 A), while the water extract induced a marked decrease (Fig. 5 B).

2 - The total proteins:

The total proteins in the midgut cells of *S. littoralis* were reflected by the appearance of a positive affinity to mercury bromphenol blue visualised by the appearance of a bluish colouration. This was illustrated in the normal (control) midgut cells (marked reaction) (Fig. 6). As apparent in this figure, the midgut cells contain bluish colour. Total proteins appeared in the midgut cells as a great amount of dense blue particles.

The acetone and water extracts of *A. monosperma* (Fig. 7 A,B); *Z.* coccineum (Fig. 8 A,B) and *B.* tournefortii (Fig. 10 A,B) gave moderate reactions with mercury bromphenol blue. A marked reduction in total protein was noticed in the midgut cells of *S. littoralis* larvae treated with acetone and water extracts of *L. termis* (Fig. 9 A,B).

Discussion

The positive PAS reaction could be due to the presence of a wide variety of carbohydrate complexes, polysaccharides, mucoproteins, glycoproteins and glycolipids (McManus, 1946 and Lillile, 1954).

Histochemical investigations on the midgut of untreated larvae demonstrate the presence of carbohydrates (Polysaccharides), proteins and lipids. Protein substances are essential constituents of the general structure of animal cells and also in the maintenance of different vital activities. Runham (1961) reported that the alimentary canal of the boll weevil gave a positive reaction with PAS and Alcian blue. However, chitinous structures in the alimentary canal also gave a positive PAS reaction. Sundman and King (1964) mentioned that the ventriculus of the adult boll weevil, *A. grandis*, gave a positive reaction with PAS. Houk *et al.* (1986) found complex carbohydrates in the epithelial cells of the midgut of the mosquito, *C. tarsalis*.

Histochemical studies showed that the midgut cells of *S. littoralis* larvae lost most of their carbohydrate, and protein contents after treatment with the tested plant extracts. These observations are in agreement with the findings of Chadbourne and Rainwater (1953). Hamed *et al.* (1974) showed that the gut cells of *A. pharoensis* larvae lost most of their carbohydrate content following dieldrin and DDT treatment. Assar and Emara (1996) reported that the insecticide, Dimilin induced several histochemical changes in the midgut cells of *S. exigua* larvae. The carbohydrates, proteins and lipids markedly decreased in the midgut cells.

Finally, it can be said that the tested plant extracts induced several histochemical changes in the midgut of *S. littoralis* larvae. The effect observed on carbohydrate content is greater than that on proteins. The acetone and water extracts of *L. termis* produced the greatest effect on carbohydrate, and protein content, followed by *B. tournefortii*, while the extract of *A. monosperma* and *Z. coccineum* induced moderate effects.

Abdel-Mogib *et al.* (1990) identified capillin in *A. monosperma*; Ouf *et al.* (1994) and El-Gamal *et al.* (1995) identified tritrepenoid saponins in *Z. coccineum*; Mansour *et al.* (1982) identified coumarin in L. *termis*; and Vioque *et al.* (1990) identified glucosinolates in *B. tournefortii*. The above histochemical effects may be attributed to these reported active ingredients.

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Explanation OF Figures

Fig. (1): Midgut section of control larvae stained by PAS showing polysaccharide particles.

Fig. 2 A: Midgut section of larvae treated with acetone extract of *A. monosperma* showing a moderate reaction with PAS.

B: Midgut section of larvae treated with water extract of *A. monosperma* showing a moderate reaction with PAS.

Fig. 3 A: Midgut section of larvae treated with acetone extract of *Z. coccineum* showing a moderate reaction with PAS.

B: Midgut section of larvae treated with water extract of *Z. coccineum* showing a moderate reaction with PAS.

Fig. 4 A: Midgut section of larvae treated with acetone extract of *L. termis* showing a marked decrease in polysaccharides.

B: Midgut section of larvae treated with water extract of *L. termis* showing a marked decrease in polysaccharides.

Fig. 5 A: Midgut section of larvae treated with acetone extract of *B. tournefortii* showing a moderate decrease in polysaccharides.

B: Midgut section of larvae treated with water extract of *B. tournefortii* showing a marked decrease in polysaccharides.

Fig. 6 : Midgut section of control larvae showing normal pattern and localization of total proteins.

Fig. 7 A: Midgut section of larvae treated with acetone extract of *A. monosperma* showing a moderate reaction with mercury bromphenol blue.

B: Midgut section of larvae treated with water extract of *A. monosperma* showing a moderate reaction with mercury bromphenol blue.

Fig. 8 A: Midgut section of larvae treated with acetone extract of *Z. coccineum* showing a moderate reaction with mercury bromphenol blue.

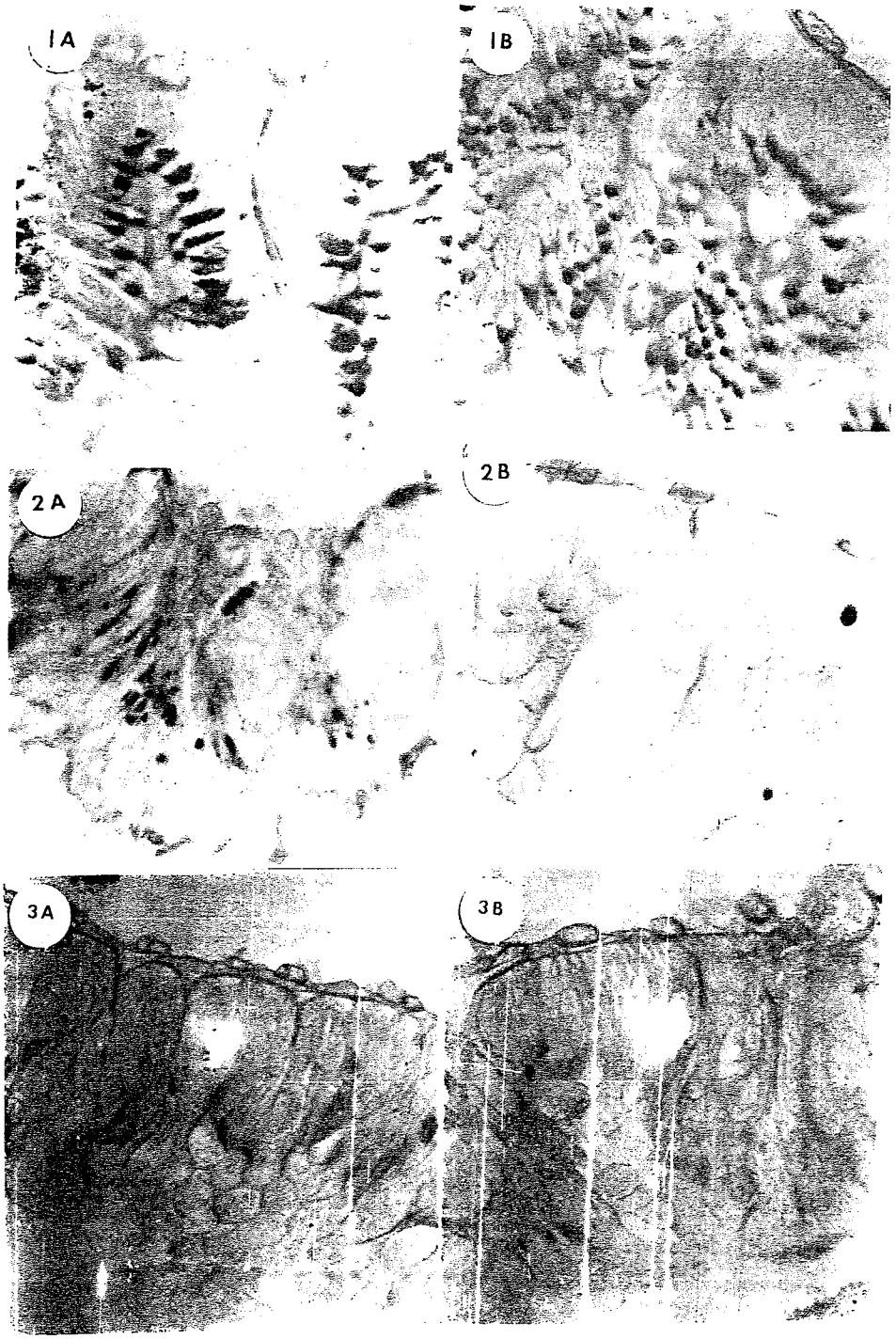
B: Midgut section of larvae treated with water extract of *Z. coccineum* showing a moderate reduction in protein content.

Fig. 9 A: Midgut section of larvae treated with acetone extract of *L. termis* showing a marked decrease in protein content.

B: Midgut section of larvae treated with water extract of *L. termis* showing a marked reduction in protein content.

Fig. 10A: Midgut section of larvae treated with acetone extract of *B. tournefortii* showing a moderate reaction with mercury bromphenol blue.

B: Midgut section of larvae treated with water extract of *B. tournefortii* showing a moderate reaction with mercury bromphenol blue.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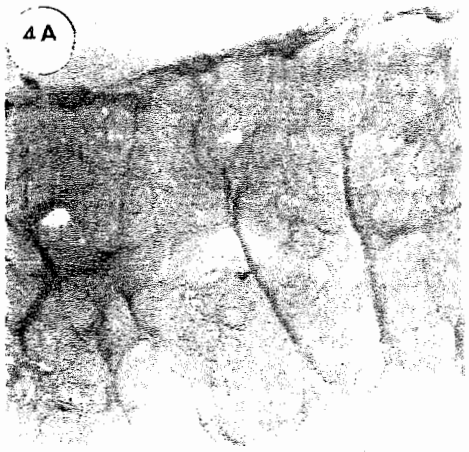

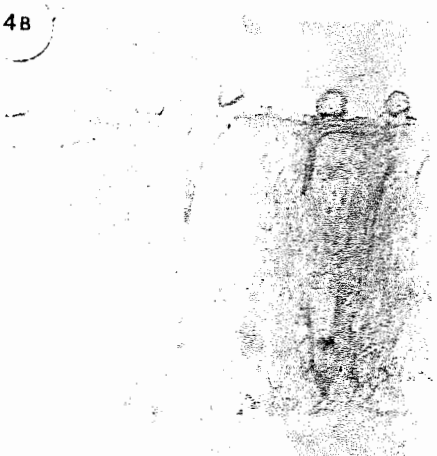

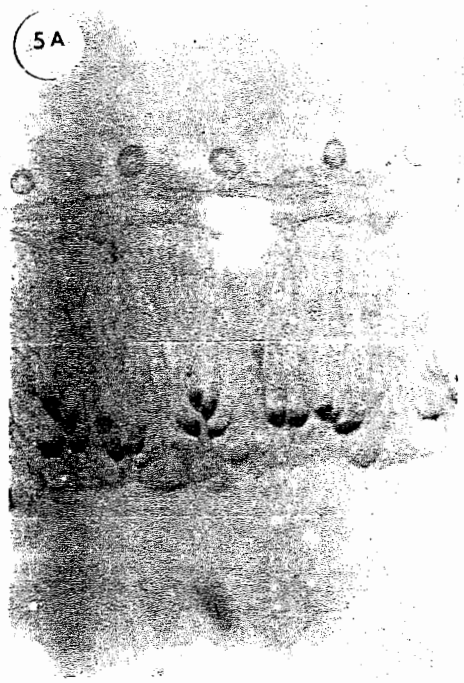

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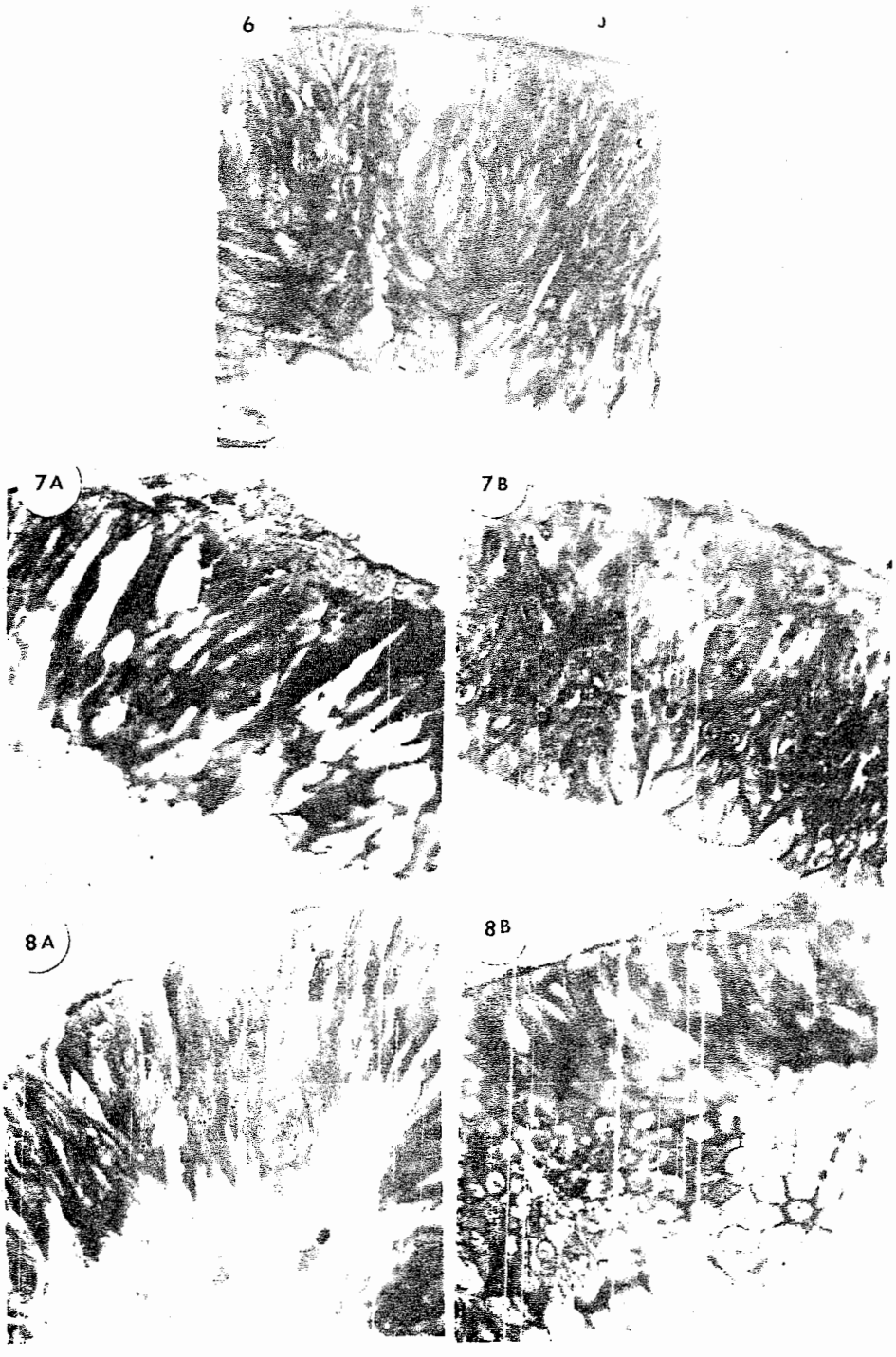
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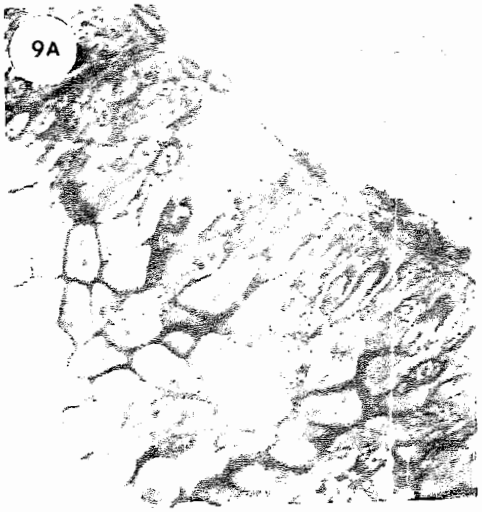

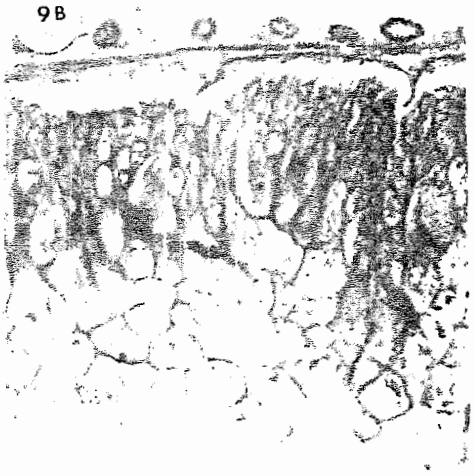

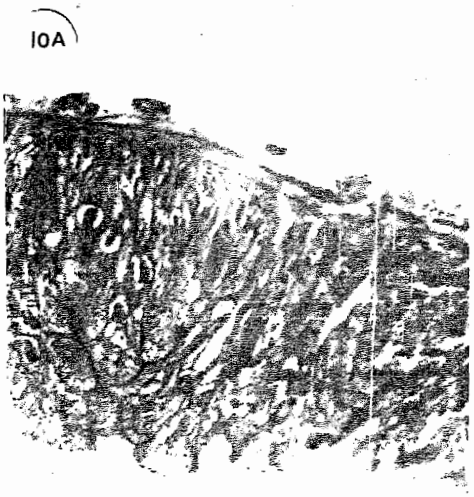

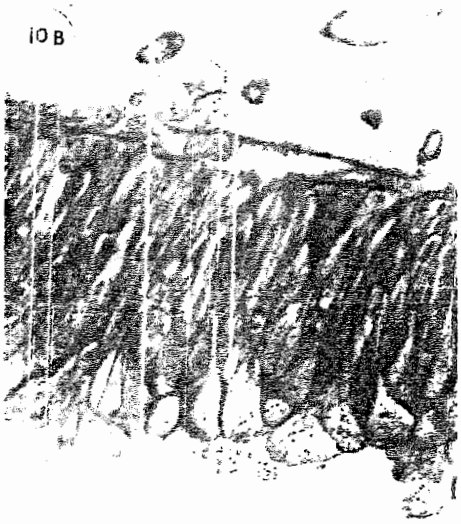

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الملخص العربى

التأثيرات الهستوكيميائية لبعض المستخلصات النباتية على المعى المتوسط لدودة ورق القطن الكبرى *سبودوبترا ليتورالس* (حرشفية الأجنحة – نوكتويدى)

عبادة أبو ذكرى عصر

قسم علم الحيوان – كلية العلوم – جامعة المنوفية – شبين الكوم – مصر

تم فى هذا البحث دراسة التأثيرات الهستوكيميائية للمستخلص الأسيتونى والمائى للعادر (ليليل) والرطريط والترمس والكبر على خلايا المعى المتوسط للعمر اليرقى الرابع لدودة ورق القطن الكبرى. أحدثت مستخلصات كل النباتات المختبرة تغيرات عديدة فى المحتوى الكربوهيدرائى والبروتينى فى خلايا المعى المتوسط. وكانت التأثيرات على الكربوهيدرات (عديدة التسكر) أكثر منها على البروتينات. كما أن مستخلصات الترمس أعطت أقوى تاثير، يليها مستخلصات الكبر بينما مستخلصات كل من نبات العادر والرطريط أحدثت نقصاً (إختزالاً) معتدلاً.