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Histopathological effects of a mixture of two bioagents on the larval midgut of the cotton leaf worm, *Spodoptera littoralis* (Boisd.)

El-Banna, A. A.[1]; Abd El-Kareem, S. M. I.[2]; El-Akad, A. S.[1]; Hussein, M. A.[1]; Fahmy, A. R.[1]; and Bekheit, H. K.[2]

1- Dept. of Entomology, Faculty of Science, Ain Shams University, Cairo.
2- Plant Protection Research Institute, Dokki, Giza.

ABSTRACT

Histopathological studies were conducted on the larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) using the commercial product Profect® (a mixture of *Bacillus thuringiensis* var. *kurstaki* and *S. littoralis* NPV). Treatment of the 4th instar larvae with the bioinsecticide revealed many ultrastructural alterations in the midgut of the 6th instar larva. This mixture formula proved to be effective against the midgut epithelial cells and induced the marker of cell death. Accordingly, utilization of this biocontrol agent for controlling the cotton leafworm provides a promising alternative to conventional insecticides.

Key words: Bioinsecticides, Cotton leafworm, Ultrastructure, Histopathology.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Boisd.), is a major cotton pest having a high reproductive capacity that averages 1000 eggs/female. In Egypt, It has three generations during the cotton season (Abul-Nasr and El-Sherif; 1973 a & b) and is considered a limiting factor affecting crop and vegetable production. In general, *S. littoralis* is one of the most destructive agricultural lepidopterous pests within its subtropical and tropical range (Hosny *et al*., 1986). The search for alternative methods of control of this pest other than chemical control is of utmost importance due to resistance development to many chemical pesticides, resurgence, and residues of chemical pesticides in the environment (Forgash, 1984 and Georghiou, 1986). Synthetic pyrethroids, insect growth regulators and other nonconventional insecticides have been used, with many reports of resistance and cross resistance development in many cases (Issa *et al*., 1984a; Issa *et al*., 1984b; and Abo-El-Ghar *et al*., 1986).

More attention should be paid to the use of bioinsecticides such as compounds based on bacteria, fungi, and viruses (Rao *et al*., 1990). These groups have unique modes of action (Asher, 1993 and Thompson *et al*., 1999) and their properties may differ considerably from the conventional agents.

This research is designed to study the histopathological effects of the commercial product Profect® (a mixture of *Bacillus thuringiensis* var. *kurstaki* and *Spodoptera littoralis* NPV), on the larvae of the cotton leaf worm with the aim of minimizing the use of chemical insecticides in controlling this destructive pest.

MATERIALS AND METHODS

Rearing Technique:

A laboratory susceptible strain of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) reared in the laboratory for more than 10 generations (without any exposure to chemicals), was obtained as egg masses from the Research Division of the cotton leaf worm, Plant Protection Research Institute.

Insects were reared under controlled conditions in an incubator at 26 ± 2^{o}C and 65 ± 10% R. H., with 8:16 L:D photoperiod (El-Sawaf, 1971) at the Plant Protection Research Institute, Dokki-Giza, Egypt. Larval jars were supplied daily with fresh Castor leaves, *Ricinus communis* L., as a source of food.

The Tested Bioagent:

The commercial bioinsecticide Profect® was used in this investigation. It was obtained as a wettable powder produced by the Plant Protection Research Institute, Biopesticide Production Unit, Dokki- Giza, Egypt.

Profect® WP (*Btk* + *Spl*iNPV) is a mixture of 5% of *Bacillus thuringiensis* var. *kurstaki* and 2% of *Spodoptera littoralis* NPV.

Specimen Preparation:

Larvae were treated as fourth instars with LC_{50} of Profect® at a concentration of $9.4X10^{-5}$ gm/ml according to Abd El-Kareem *et al.* (2010). Treated larvae were dissected in the late 6th instar and prepared for transmission electron michrograph. Preparation and ultrascan micrograph were carried out at the Military Medical Research Unit, Abassia, Cairo, Egypt.

Midguts from the larvae were dissected and immediately fixed in 2.5% glutaraldehyde at 4°C, for 3days. The midguts were then washed in 0.1M buffer, fixed in 2% osmium tetroxide in 0.2 M buffer solution for 1 hour then rinsed in 0.2 M buffer. The specimens were dehydrated by ethanol series dehydration. They were then added to Propylene oxide and transferred to eponate epoxy. Finally the specimens were embedded in labeled capsules with freshly prepared resin and polymerized at 60°C for 48hours. The pH was kept within the range 7.2 - 7.4.

Ultrathin section preparation:

Ultrathin sections of the resin embedded specimens were obtained using an ultracut E microtome. Sections for TEM analysis were collected on carbon coated formvar supports, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in a SEM electron microscope equipped with a ProScan slow scan CCD camera.

RESULTS AND DISCUSSION

Normal midgut ultrastructure of untreated larvae of the cotton leafworm:

The ultrastructure of midgut epithelial cells of untreated (normal) larvae of *Spodoptera littoralis* is presented in figures (1 – 5). The lining epithelium of the midgut consists of columnar cells resting on a basement membrane with a more or less oval centrally located nucleus bound by a well defined nuclear envelope. The nuclear chromatin is clumped into patches of varying densities (Fig. 1). The luminal surface of the epithelial cells has a striated border constituted of microvilli projecting inwards into the luminal cavity (Figs. 2 & 3). The outer surface of the cell rests on the basement membrane. The ground cytoplasm of these cells contains fine granulations dispersed in a less dense matrix. Within the cytoplasm lie the mitochondria, which are conspicuously rather elongated or spherical in shape. There is also an abundance of lamellated rough and smooth endoplasmic reticulum. The majority of elements are lamellar structure or flattened cisternal vesicles usually containing accumulations of intracisternal inclusions. In case of rough endoplasmic reticulum, there are numerous ribosomes bordering the outer surface of the membranes of the reticulum (Fig. 5). The Golgi's appear as flattened curved sacs with cluster bodies at their edges (Fig. 4).

Midgut ultrastructure of larvae of the cotton leafworm treated with *B. thuringiensis kurstaki* and *Spli*NPV mixture:

Histopathological effects of the mixture of *B. thuringiensis* var. *kurstaki* and *Spli*NPV on the late 6th instar larvae midgut are shown in figures (6 – 9).

Numerous transporter vesicles containing multicapsid polyhedra are observed. The nucleus has lost its characteristic oval shape and has become elongated. The chromatin material is severely condensed and scattered. An increased number of transporter vesicles containing virus occluded bodies in the gut lumen is visible. (Figs. 6 & 7). It is also apparent that the epithelial cells are completely separated (Fig. 8).

Fig. 9 shows that the chromatin material is scattered and the nucleus is not centrally located. The cell organelles have disappeared and the nucleus has become more cubical in shape. The nucleolus has lost its centred position. Lipid vesicle is also observed.The Microvilli appear to be swollen and have become separated into the lumen in addition to, loss of their order and destruction.It is also clear from fig. 9 that the epithelial cells are separated and the brush border is totally absent.

From the results, it is evident that many histopathological changes in the midgut of the 6^{th} instar larva have occurred due to treatment with the mixture formula of *B. thuringiensis kurstaki and Spli*NPV reflecting the action of both the bacterial infection and the viral infection.

As a target effect, this mixture has proven to be most effective against the midgut epithelial cells causing increased cytoplasmic vacuolization.The viral infection has affected the nucleus and the chromatin material, since the nucleus is the target site of the viral replication while secretory and lipid vesicles were observed as a result of the bacterial infection.

The cell organelles have become malformed and lost their integrity due to the bacterial infection, while the viral infection has caused many cell organelles to disappear and has induced the marker of cell death.

These histopathological changes were reported in details in Moser *et al.* (2001); Gomez *et al*. (2007); Sakr and Hassab El-Nabi (2007); Abdel-Aziz (2007); De Melo *et al*. (2009); Knaak *et al.* (2010); Abd-El Wahed *et al*. (2011); and Da Cunha *et al*. (2012).

Accordingly, it could be concluded that utilization of biocontrol agents and their combinations for controlling the cotton leaf worm, *Spodoptera littoralis* and other pests in general, could provide an excellent alternative to conventional insecticides or at least minimize their use.

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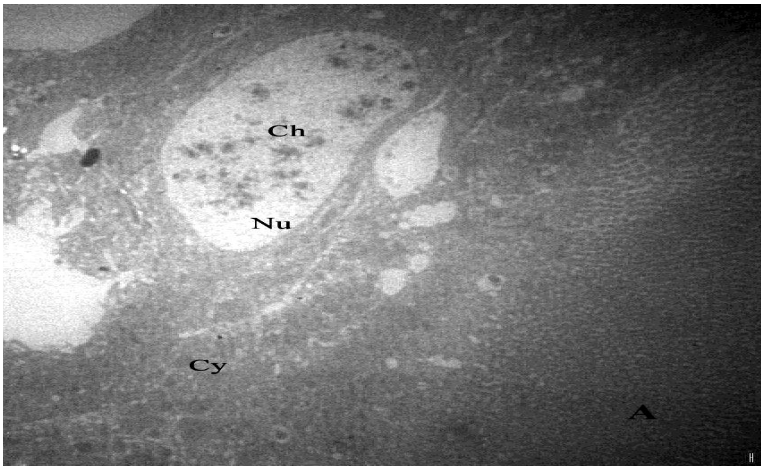

Fig. 1: Transmission electron micrograph of the untreated midgut of the late 6th instar larva of *Spodoptera littoralis* showing the normal epithelial cells. Clear cytoplasm (Cy), oval centred nucleus (Nu) containing the chromatin material (Ch) (x1500)

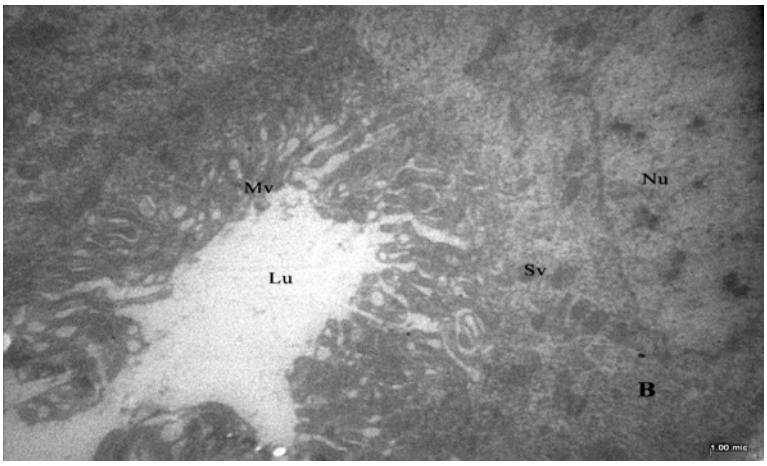

Fig. 2: Transmission Electron micrograph of the untreated midgut of the late 6th instar larva of *Spodoptera littoralis* showing the normal epithelial cell. The continuous integrated brush border (arrow) characterizes the midgut epithelium of the normal larval midgut. Lumen (Lu) and microvilli (Mv) are observed. The presence of some secretory vesicle (Sv) is recognized (x3000).

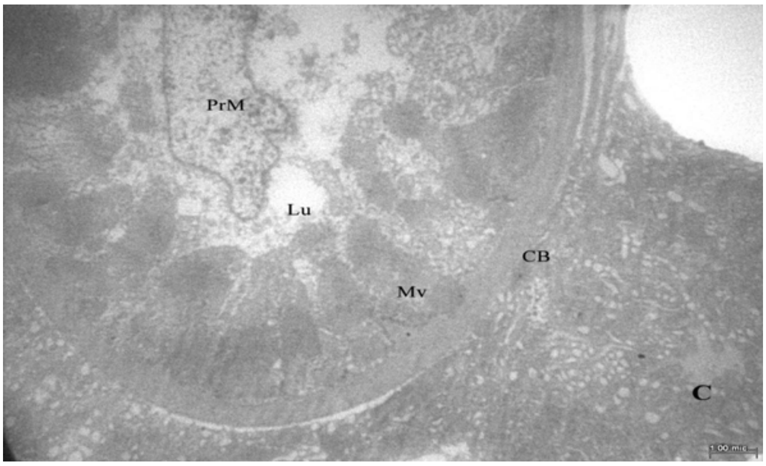

Fig. 3: Transmission Electron micrograph of the midgut of the late 6th instar larva of *Spodoptera littoralis* showing cell border (CB) attached tightly to the cell. Microvilli (Mv) have finger-like structure. Peritrophic membrane (PrM) is present (x3000).

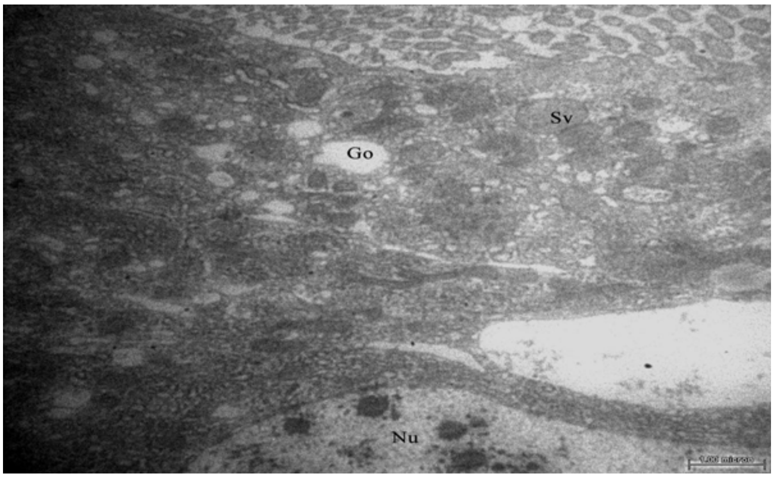

Fig. 4: Transmission electron micrograph of normal epithelial cell of the midgut of the late 6th instar larva of *S. littoralis*. Secretory vesicle (Sv) and Golgi apparatus (Go) are present (x6000).

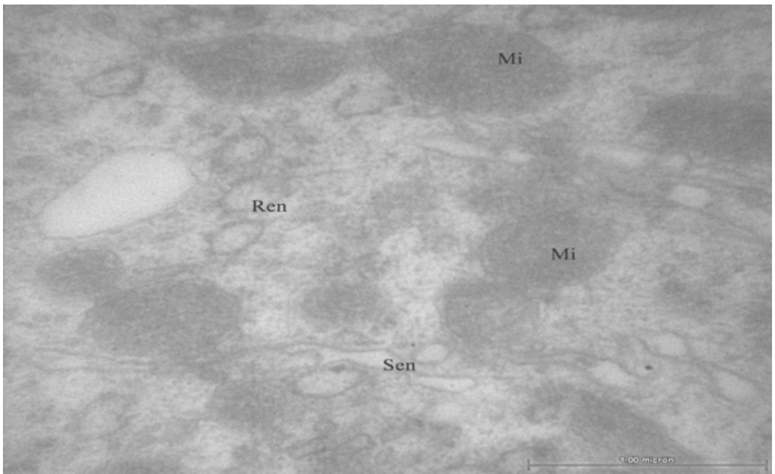

Fig. 5: Transmission electron micrograph of the midgut of the late 6th instar larva of *S. littoralis* showing the presence of many normal mitochondria (Mi). Rough endoplasmic reticulum (Ren) with ribosomes attached to its surface. Smooth endoplasmic reticulum (Sen) is also observed (x20, 000).

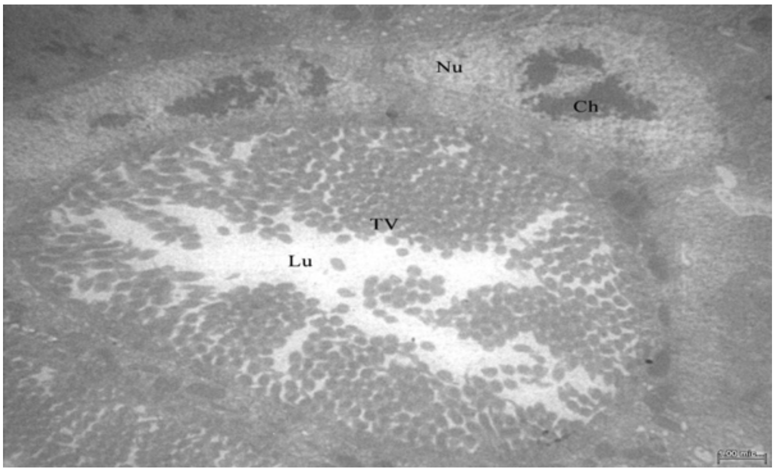

Fig. 6: Transmission electron micrograph of the midgut of the late 6th instar larva treated with *B. thuringiensis kurstaki* and *Spli*NPV. (x3000)

Nucleus (Nu) - Chromatin material (Ch)- Transporter vesicles (TV) - lumen (Lu)

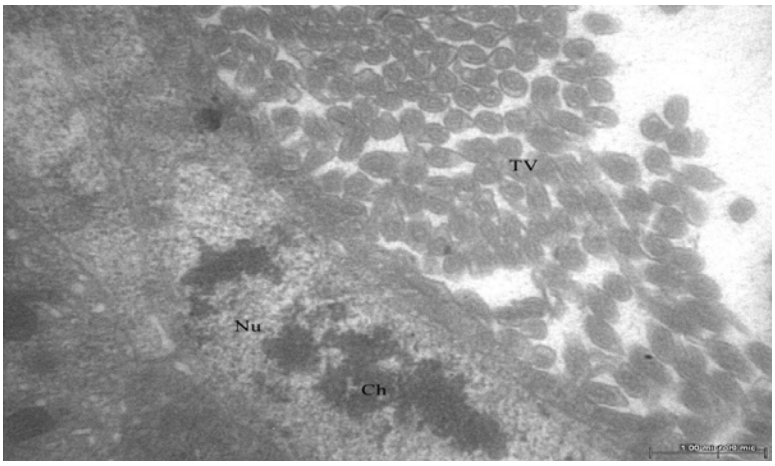

Fig. 7: Transmission electron micrograph of the midgut of the late 6th instar larva treated with *B. thuringiensis kurstaki* and *Spli*NPV. (x10000)
Nucleus (Nu) - Chromatin material (Ch) - Transporter vesicles (TV)

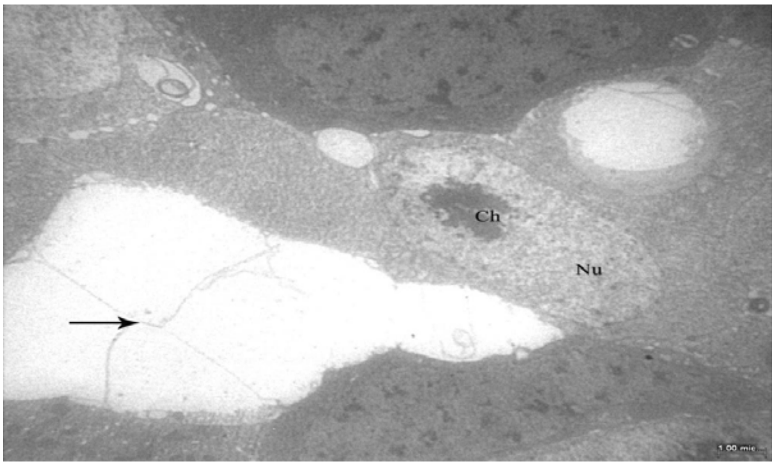

Fig. 8: Transmission electron micrograph of the midgut of the late 6th instar larva treated with *B. thuringiensis kurstaki* and *Spli*NPV. (x3000) Separated epithelial cells (arrow)

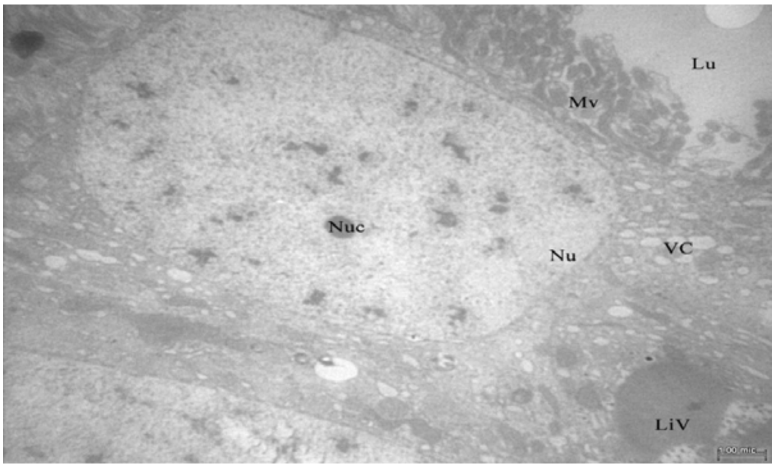

Fig. 9: Transmission electron micrograph of the midgut of the late 6th instar larva treated with *B. thuringiensis kurstaki* and *Spli*NPV. (x3000)
Nucleus (Nu) - Nucleolus (Nuc) - Lipid vesicle (LiV) - Microvilli (Mv) - Lumen (Lu)

ARABIC SUMMARY

التأثيرات الهيستوباثولوجية على المعي الأوسط لدودة ورق القطن الكبرى نتيجة المعاملة بخليط من مبيدين حيويين

عاطف على البنا[1] - سارة محمد إبراهيم عبد الكريم[2] - عادل صبحى العقاد[1] - محمد عادل حسين[1]
عادل رمزي فهمى[1] - حسن قاسم بخيت[2]
1- كلية العلوم – جامعة عين شمس – القاهرة
2- معهد بحوث وقاية النبات – الدقى - جيزة

تعتبر دودة ورق القطن من أكثر الحشرات ضرراً على إنتاج المحاصيل والخضروات. وقد أدى الاستخدام المتزايد للمبيدات الكيمائية لمقاومة هذة الافة إلى ظهور مستويات عالية من المناعة لدى هّذة الحشرة مع وجود متبقيات لهذه المبيدات فى البيئة. و لهذا اتجهت الأنظار إلى استخدام المركبات التى لها أساس حيوى.
المركب الحيوى بروفكت (Profect®) هو خليط من ***Bacillus thuringiensis* var. *kurstaki*** و ***Spodoptera littoralis* NPV** .

أظهرت النتائج وجود تغيرات في تركيب المعي الأوسط ليرقات العمر السادس نتيجة المعاملة بالتركيزات القاتلة للنصف للمركب، فقد لوحظ أن الخلايا العماديه للمعي الأوسط قد فقدت شكلها المميز وترتيبها. كما انفصلت الmicrovilli عن حافة الخلية و سقطت فى التجويف المعوى بالاضافة الى فقدان النواة للشكل المركزى والبيضاوى كما أن بعض عضيات الخلية قد تأثرت بسبب المعاملة.