Histopathological Effects of Two Different Neem Products on the Midgut Tissues of the Black Cutworm, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae)

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ABSTRACT

Effect of the two Neem products, Neem-Azal-T/S and Neemix on the midgut tissues of the black cutworm, *Agrotis ipsilon* (Hufn.) 4th instar larvae was evaluated at LC_{50} concentrations. Longitudinal and circular layers of muscles were strongly affected and separated largely from the epithelial cell walls. The epithelial cells, digestion and absorption cells in addition to the regenerative cells were enlarged and destroyed. The peritrophic membrane surrounded a bulk of indigested food in both treatments, while the food was nearly completely digested in untreated larvae. In general, Neemix was more effective than Neem-Azal-T/S.

Key Words: Neem-Azal-T/S, Neemix, Histopathology, *Agrotis ipsilon*.

INTRODUCTION

Numerous phytophageous insects react highly sensitive to the extracts of the tropical Neem tree (*Azadirachta indica* Juss.) and its most active principle Azadirachtin (AZA). Intensive research work has been done on Neem and diverse biological activities have been reported (Schmutterer, 1995). Unlikely, many of these studies on insects were conducted with unformulated Neem extracts and with unknown AZA concentration. However, a standardized quality and an efficacious formulation were presuppositions for a possible registration as a commercial insecticide which clearly obtained in the choose products, Neen-Azal-T/S (1% AZA) and Neemix (4.5% AZA).

The present work aims to study the histopathological effects of two common Neem products on the midgut of the black cutworm, *Agrotis ipsilon* (Hufn.) 4th instar larvae as one of the key pests on vegetables and several field crops in Egypt.

MATERIALS AND METHODS

Stock culture of target insect

A culture of *A. ipsilon* was reared under the laboratory conditions (25±2°C and 50-60% R.H. with normal day photoperiod).

Tested Materials

A) Neem-Azal-T/S (Trifolio-M GmbH, D-Lahnau) is a standardized insecticide based on neem with an Azadirachtin content of 1% and plant oils (51%) in addition to emulsifiers (Schulz, *et. al.*, 1997).

B) Neemix 4.5% EC, Azadirachtin 4.5% (W/V) + Inert 95.5%, produced by: Thermo Trilogy Corp. /Nichimen.

Experimental Technique

Adequate numbers of newly molted 4th instar larvae of *A. ipsilon* were fed on castor oil leaves for 48 h; one group on leaves dipped in the LC_{50} of Neem-Azal-T/S (0.17 ml/100 ml water) and another group on leaves dipped in the LC_{50} of the Neem-product, Neemix (0.13 ml/100 ml water). Other larvae from the stock culture were fed on untreated castor oil leaves for also 48 h as control.

Preparation of the treated and untreated larvae for histological studies was carried out according the following steps as described by Romeis (1968):

1- Larvae were fixed in ethyl alcoholic Bouin's solution for 48 h and washed in 70% ethyl alcohol, then dehydrated by dipping in 90% followed by absolute ethyl alcohol 4 h for each.
2- After dehydration, the larvae were transferred to a mixture of ethyl/benzol (1:1) for 3 hours (3 replacements) and then to benzol for 15 minutes followed by a mixture of equal parts of benzol and liquid paraffin wax (Bp 56-58°C) for 30 minutes.
3- The larvae were transferred from the mixture of benzol and liquid paraffin to pure paraffin wax for 24 h (renewed 2-3 times) and then embedded in paraffin blocks.
4- The wax block containing the specimen was fixed in the block holder of a rotary microtome and sectioned at 5µm thickness (cross sections).
5- Sections were stained by Erlich's haematoxylin and counterstained with methylene blue and then covered with glass covers fixed with Canada balsam. Slides were kept for 4 hours at 40°C for dryness.

RESULTS AND DISCUSSION

A) Description of a cross section in the midgut of untreated *A. ipsilon* 4th instar larvae

A cross section in the midgut of *A. ipsilon* 4th instar larvae, fed on the untreated castor oil leaves showed that the epithelial cell walls consist of two types of cells, large columnar cells with spongy cytoplasm and large nuclei, in addition to smaller cells (regenerative cells) occur either singly or in groups between the bases of larger cells, (Fig.1 & Fig.2).

The large cells, having their inner ends exposed or projecting into the stomach lumen, are the digestive cells, which play an active role in the processes of secretion or absorption. The smaller basal cells are the regenerative cells (rg), which propagate cells to replace the digestive cells when the latter are exhausted by secretory activities or shed at the time of ecdysis. The epithelial cell walls surrounded by an outer layer sheath of muscles which contains the longitudinal muscles (lmcl) and the circular muscles (cmcl) (Fig. 1 & Fig. 2). The two types of muscles play the main role in food movement in the alimentary canal for digestion and absorption, (Snodgrass, 1935). The epithelial cells rest upon a membrane which appears to be a tunica propria, or product of the cell bases, differing in no respect from the basement membrane (BMb) of the body wall from the ectodermal parts of the alimentary canal. The food content of the stomach of noctuid larvae is separated from the ventricular epithelium by a thin membrane, which, though often in more or less intimate contact with the inner ends of the epithelial cells, typically surrounds the food mass as a cylindrical sheath for the most part free from the stomach walls. This food envelope is known as the peritrophic membrane (PMb). The peritrophic membrane is separated from the epithelium by a space (a) as shown in Fig. (1).

The fat body tissues - the principle tissue - that serves for the deposit of nutritive and energy. Normally, this adipose tissue consists of loosely aggregated or compact masses of cells. The fat cells, however, are usually closely adherent, except at the time of metamorphoses, and are so densely packed that they assume polygonal shapes. The external surfaces of the cell masses are often smooth and regular, and in such cases they appear to be covered by a delicate membranous sheath (Fig. 2).

B) Effect of Neem-Azal-T/S on the midgut tissues of 4th instar larvae of *A. ipsilon*

The layers of the muscles (longitudinal and circular) were clearly affected as a result of feeding of the larvae on the treated castor oil leaves dipped in the LC_{50} of this Neem product for 2 days. The muscles were separated largely from the epithelial cell walls. This means that the movement of the midgut was no longer assumed, though the food content could not be stirred. Therefore, the larvae lost their appetite or the desire to eat, which was clearly shown in figures (3&4).

A bulk of indigested food surrounded by the PMb was found (Fig. 3), while in control a little rest of food was seen (Fig. 1). This agree with that found by Babu *et al.*, (1997), who stated that, methanolic extract of *Azadirachta indica* resulted anti-feeding effect against 4th instar larvae of *Achaea janata* and also agree with the results of Schmidt *et al.* (1997), who stated that, methaolic extract of *Melia azedarach* fruits contained some anti-feedant activity when tested against *A. ipsilon* larvae. Such results were reported by Mordue and Blackwell (1993) in their research on the insecticidal effects of azadirachtin and limonoids from the Indian Neem tree.

The separation of the muscle layers of the midgut in addition to the breakdown of the epithelium are in agreement with that found by Salem *et al.* (2003). They reported that feeding of 6th instar larvae of *S. littoralis* on treated food with water the extract of *Peganium harmala* at 5% concentration gave the same results of this study.

Fat bodies in the treatment with Neem-Azal-T/S seem to be spongy, digested with unclear cells shape or form in addition to completely disappearance of the fat cells in some locations of the cross section as shown in figures (3&4) in comparison with that in the control (Figs. 1&2). These observations in fat bodies might be due to the consumption of the cells as a reserve of food source in case of indigestion of food as described by Snodgrass, 1935. This agrees also with that stated by Koul *et al.*, (1996), Amr (2001) and Abd El-Zaher (2005).

C) Effect of Neemix on the midgut tissues of 4th instar larvae of *A. ipsilon*

As shown in figures (5&6), Neemix was more effective on the muscular sheath, the fiber layers were largely separated from the epithelial cells which obtained more destruction than that observed in Neem-Azal-T/S treatment (Fig. 3&4). This highly efficacy of Neemix may be due to the increase of the active ingredient (Azadiractin, 4.5%) in the product, it's only 1% in Neem-Azal-T/S (Schulz *et al.*, 1997) or due to unknown additives which may enhance the activity of Azadirachtin than that used (3 different plant oils and emulsifier) in Neem-Azal-T/S

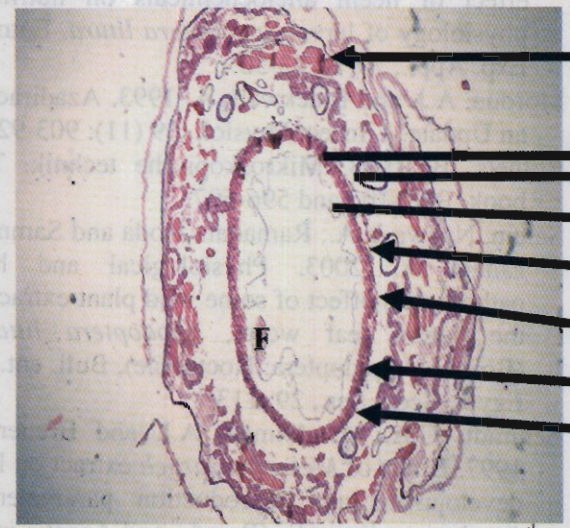

Fig. (1): Cross section in the midgut of untreated 4th instar *Agrotis ipsilon* larva (10 X 4x).

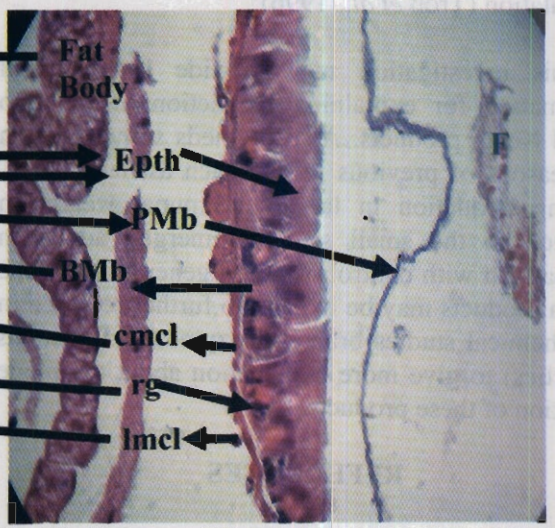

Fig. (2): Focus on different tissues in a cross section of the midgut of untreated *A. ipsilon* larva (10 X 40x).

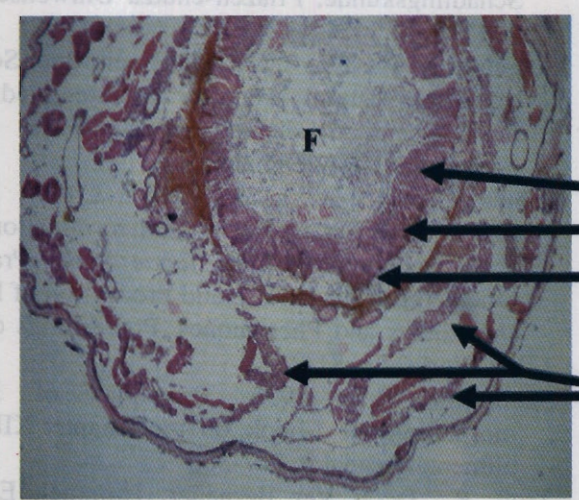

Fig. (3): Cross section in midgut of 4th instar *A. ipsilon* larva fed on treated food with Neem-Azal-T/S (10 X 4x).

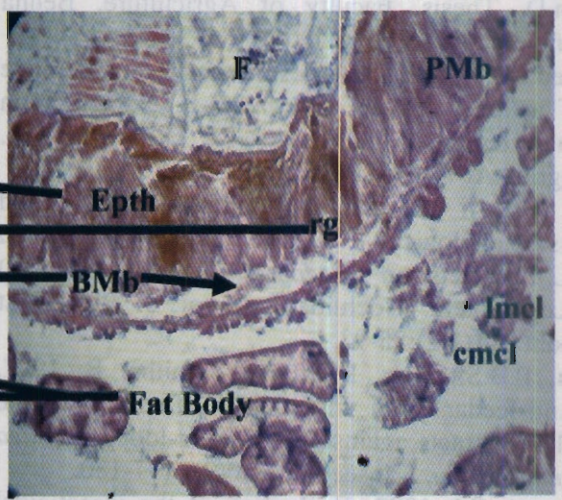

Fig. (4): Focus on affected tissues in the midgut of 4th instar larva of *A. ipsilon* fed on treated food with Neem-Azal-T/S (10 X 40x).

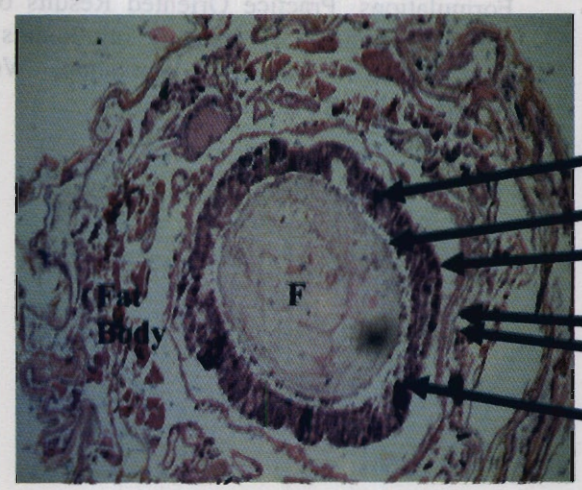

Fig. (5): Cross section in midgut of 4th instar *A.ipsilon* larva treated by feeding with Neemix (10 X 4x).

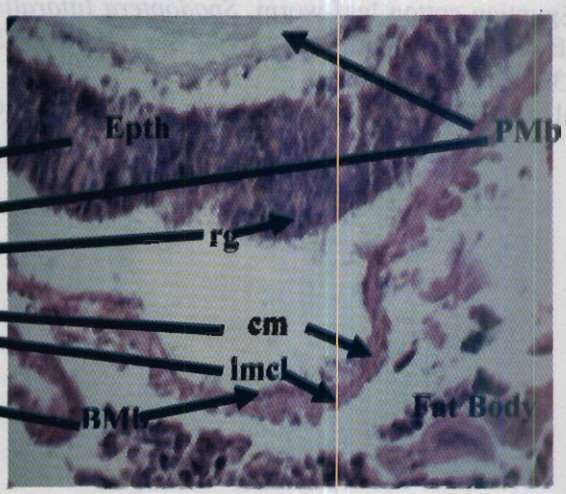

Fig. (6): Cross section in midgut of 4th instar *A. ipsilon* larva fed on treated food with LC_{50} of the neem product Neemix (10 X 40x).

formulation (Troß *et al.*, 1996).

This investigation may provide some useful information for explaining the action of the two tested Neem products. Also, it sheds some light on the reasons of previous results such as, larval anti-feeding, reduction in larval and pupal weight in addition to the small size of emerged adults in comparison with control. Modern generations of the Neem products may be in need to further research in bio-chemical studies branch (Enzymes and Proteins activities) to give more explanation about the mode of action of these products.

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