Histopathological Changes in Larvae of the Greater Wax Moth, *Galleria mellonella* L. Caused by *Bacillus thuringiensis kurstaki*

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ABSTRACT

Larvae of *Galleria mellonella* showed midgut epithelial cells with disorder of the peritrophic membrane 24 hrs post ingestion of diet treated with *B.t. kurstaki* that became intensive in the next following days, where vacuoles in the cytoplasm were observed and separation of the cells from each other with elongation towards the gut lumen led to detachment from the basement membrane, where they fall in lumen showing disintegration of the nuclei. By death, most of the midgut epithelial cells were found separated in large numbers in the lumen. Silk glands showed a slight decrease in the fibroin content, but a highly decreased content of the sericine. This effect was also associated with enlargement of the epithelial cells, formation of vacuoles, and disintegration of both the cytoplasm (cytolysis) and the nuclei (caryolysis). Malpighian tubules showed disintegration and lysis of the brush border, cytoplasm and nuclei leading to dysfunction of this organs and most probably to death of the infected larvae.

Key Words: *Bacillus thuringiensis kurstaki, Galleria mellonella,* histopathology, midgut, silk gland, Malpighian tubules.

INTRODUCTION

The remarkable success of using the environmentally safe entomopathogenic spore forming bacterium *Bacillus thuringiensiss* Beliner in controlling certain lepidopteran pests drew the attention to use it successfully against larvae of *Galleria mellonella* as specific lepidopteran bio-insecticide proved safe to bees, natural enemies, and mammals (Lautenschlager and Podwaite, 1980; Burges, 1980; El-Husseini, 1981; and Abou Bakr and EL-Shemy, 1991).

Although the larvae of *G. mellonella* are widely reared in insectaries as common test insect for bioassay of chemical and biological pesticides, it received no attention for histopathological studies with *B.t.* On the other hand, many other insect species were histopathologically examined post ingestion of spores and delta endotoxin of *B. thuringiensis,i.e., Earias insulana* (Saad *et al.*,1985), *Heliothis virescens* (Ryerse *et al.*, 1990), *Bombyx mori* (Hoque *et al.*,1994),and *Dacus oleae* (Dimitriadis and Domouhtsidou, 1996).

The present work aims to investigate the changes caused by *B. thuringiensis kurstaki* on the structure of mid-gut, silk glands and Malpighian tubules in larvae of G. *mellonella*.

MATERIALS AND METHODS

Rearing the Greater Wax Moth, *G. mellonella*

Larvae were reared on a semi-synthetic diet composed of 90 g wheat flour, 20 g corn flour, 10g milk powder, 10g dry yeast, 20 ml bee honey and 20 ml glycerin as described by Ibrahim *et al.* (1984). Dry components were mixed, and then honey and glycerin were mixed and added gradually to the dry material and well mixed in the form of a delicate paste. The diet could be freezed till needed. A diet layer of 5-7 cm thick was placed in a 2 L glass container, on which the eggs of *G. mellonella* were placed. The containers were covered with plain paper fitted in place with 2 rubber bands. Rearing containers were incubated at 28-30°C associated with 60-70% relative humidity. Surplus diet was added to the developing larvae as needed till pupation took place. Emerged adults were collected and kept in similar empty glass containers (egg laying cages) provided with a paper cone having lids to the outside of the glass container, and covered also with plain paper. Eggs were laid at base of the paper cone around the lid of the egg-laying container. Paper cones carrying the eggs were removed for egg collection, and replaced by new ones.

The Tested *Bacillus thuringiensis*:

The commercial formulation Dipel 2X-wettable powder based on *B. thuringiensis kurstaki* (Abbott Laboratories. Illinois, Chicago, USA) was used to treat the larvae of *G. mellonella* (L_3) by mixing into the experimental diet, from which the bee honey was excluded because of its known anti-bacterial effect.

The LC_{50} from Dipel-2X was estimated by a bioassay test as 4.784 g / 100 g diet (Omar, 2004) and used for infesting larvae (L_3) of *G. mellonella* to produce a slow killing time that facilitates the histological follow up of induced changes in the different tested tissues of the larvae.

Histopathology:

Ten larvae from both the treated with LC_{50} and the control were fixed daily in Bouin's alcohol fixative during the next successive 8 days after treatment. Fixation time was 12 hrs, and then larvae were processed in the common way for paraffin serial sections of 6-8μ with double staining by eosin and hematoxylin; and mounted in Balsam of Canada. To reach light microscopic three-dimension view somewhat like that of scanning electron microscopy, thick sections of 13-15μ were

prepared and stained over night in hematoxylin (El-Husseini, 2002). Mid-gut, Malpighian tubules, and silk glands were examined with light microscope in both *B.t.* treated larvae and those of the control.

RESULTS AND DISCUSSION

Midgut:

Cross sections in midgut of untreated larvae of *G. mellonella* (Fig.1) showed the classical normal architecture of the epithelial cells surrounded by the basement membrane, circular and longitudinal muscles. Also, a well defined secreted peritrophic membrane is present; and the brush border membrane of the epithelial cells could be seen at different locations of the midgut according to the position of tissues by sectioning. One day after feeding on diet treated with *B.t. kurstaki* (LC_{50}), the peritrophic membrane showed alterations and became thicker on several locations of the midgut (Fig.2); and one day later, the epithelial cells showed an obvious swelling and elongation that was associated with vacuolization of the cytoplasm as seen in Fig. (3). Elongation of the midgut epithelial cells is clearly observed in thick cross sections of 13-15μ as a three-dimension view presented in Fig. (4). Progressing action of the *B.t.* toxin on the midgut epithelial cells led to separation of the elongated swollen cells from each other (Fig. 5) and later on from the basement membrane. The extremely swollen and elongated epithelial cells detached from the epithelium could fill the lumen of the midgut as shown in Fig. (6) at the 5^{th} day after ingestion of *B.t.* treated diet. On the 6^{th} day post treatment, large parts of the epithelial cells fall in the lumen (Fig.7) as a result of cell lyses; and at the end as the treated larvae died, all midgut cells showed a complete lyses, separated from each other and from the basement membrane as seen in Fig. (8).

The above obtained results in larvae of *G. mellonella* are similar to those reported by Shen and Qian (1994) and recorded in other *B.t.* susceptible lepidopteran larvae described by El-Husseini (1976), Saad *et al.* (1985), and Knowles and Ellar (1987), as well as in dipteran larvae as described by Dimitriadis and Domouhtsidou (1996) in larvae of the olive fly, *Dacus oleae* infected by *B.t. fuhuokaensis*.

These histopathological malformations in the architecture of midgut when infected with *B.t.* endotoxin-spore-complex are launched by the action of the delta endotoxin crystals (parasporal bodies). The ingested crystals are dissolved in the alkaline midgut lumen of the susceptible larvae and are cleaved by the trypsin like enzymes into trypsin-resistant toxin molecules. Then, the toxin diffuses across the peritrophic membrane and binds to specific proteins of the brush-border membrane (Knowles and Ellar, 1987; Wolfersberger, 1992). This binding forms cation-conducting pores, which disrupt the defense permeability of the membrane. Thus, the driving force for amino acid uptake by the midgut cells was removed, allowing the redistribution of cations between the midgut lumen and cell cytoplasm, and removing the force energizing maintenance of the 1000-fold proton activity gradient across the apical membrane of midgut cells. This mechanism caused a change in cytoplasmic properties great enough to disrupt normal cell metabolism, and led to the above described symptoms (Figs. 1-8) that ended by death of the infected larvae (Saad *et al.*, 1985; Rie-Van *et al.*, 1990 and Wolfersberger, 1992). Similar histopathological changes were also described in dipteran larvae (Dimitriadis and Domouhtsidou, 1996).

Silk and silk glands:

Cross sections in silk glands of *G. mellonella* larvae showed the typical histological structure (Fig. 9-A), where the epithelial cells secreted the 'fibroin' (silk thread) surrounded by the adhesive protein 'sericin' necessary to glue the thread while forming the silky cocoon. Larvae infected with *B.t. kurstaki* showed first a swollen shape of epithelial cells associated with vacuole formation in the cytoplasm (Figs. 9-B and 9-C). Also, nuclei became enlarged as a sign of lyses. At the same time, the secretion of the fibroin was slightly affected, while that of sericin was extremely decreased as shown in Fig. (9-D). As infected larvae died, a complete lysis of the epithelial cells was observed leaving only a thin cytoplasmic layer attached to the basement membrane around the fibroin that was surrounded by a thin layer (decreased content) of the sericin as shown in Fig. (9-E).

The above described histopathological malformations in the silk glands of *G. mellonella* larvae can be explained by the data presented by Omar (2004) concerning the highly decreased weight of the silky cocoon produced by *B. t.* infected larvae compared to those of the untreated control. Although the greater wax moth is widely reared in insectaries as common test insect for insecticides and entomopathogenic tests, it received no attention for histopathological studies with *B. t.* concerning effect on the silk glands. Thus, the present results are, so far, the first on this subject. Decreased content of both fibroin and sericin were observed by Hoque *et al.* (1994) in larvae of the silkworm, *B. mori* ingested diet treated with the commercial product Dipel (*B.t. kurstaki*), and Harris *et al.* (1997) reported that frass production (faeces) and spinning of silken shelters in larvae of *Epiphyas postivittana* were delayed when ingested diet treated with Dipel.

Malpighian tubules:

As shown in Fig. (10), cross sections in Malpighian tubules in healthy larvae of *G. mellonella* showed the normal histological structure of one cell layer, with enlarged region of the nucleus, and a clear brush border around the lumen. Larvae infected with *B. t.* showed first a

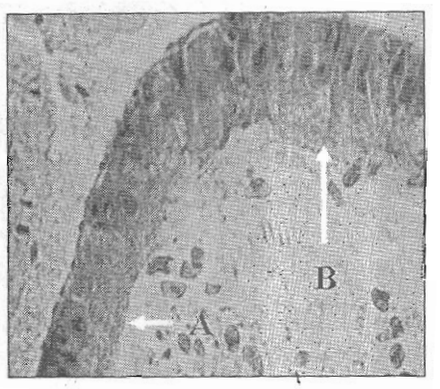

Fig. (1): Normal mid-gut epithelial cells showing a thin peritrophic membrane (A) and the brush membrane (B).

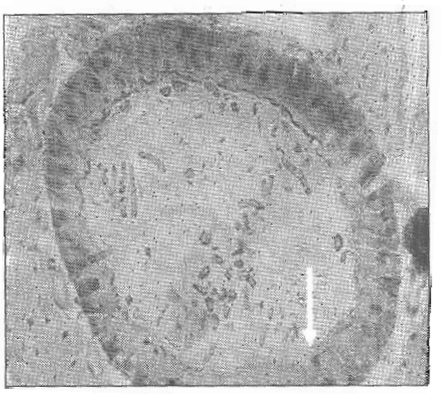

Fig. (2): One day postingestion of *B.t.*; peritrophic membrane turned thicker.

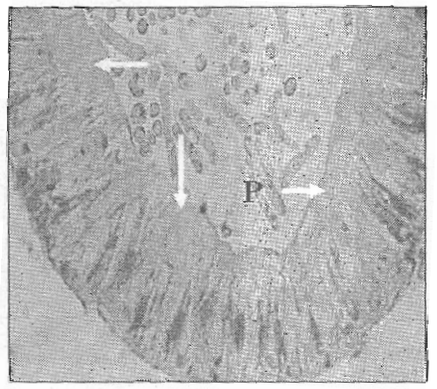

Fig. (3): Two days post infection; epithelial cells started elongation, secreting more material into the peritrophic membrane (P).

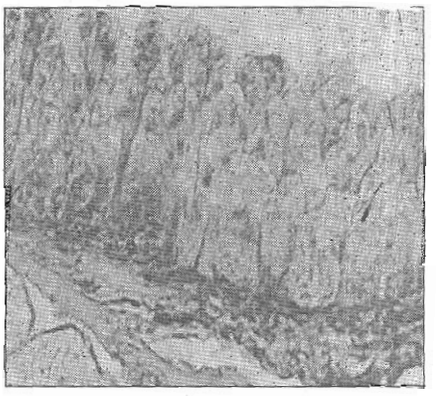

Fig. (4): Thick section after 3 days showing the elongation of mid-gut epithelial cells.

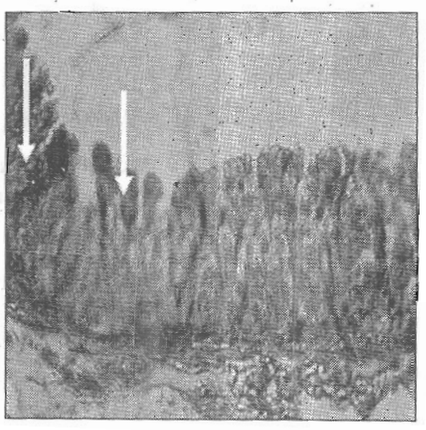

Fig. (5): More elongation of epithelial cells, 4 days after infection; with separations between them.

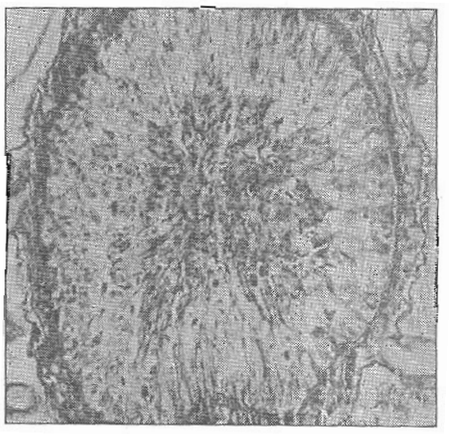

Fig. (6): Extreme elongation of mid-gut cells nearly blocking the lumen; 5 days after infection.

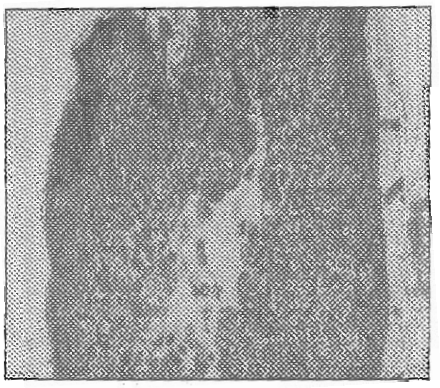

Fig. (7): Thick section showing rupture of mid-gut cells 6 days post infection; falling in the lumen shortly before death of the infected greater wax moth larvae. *G mellonella.*

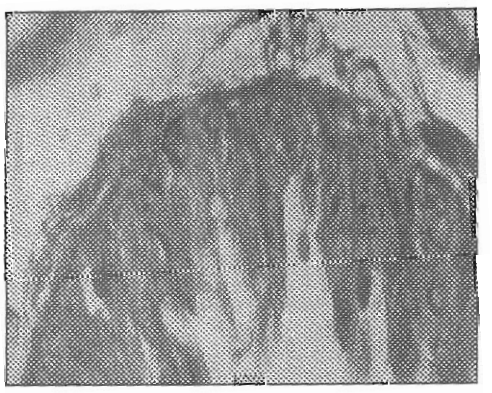

Fig. (8): Remains of the mid-gut cells in dead *Galleria* larva on the 7th day post infection.

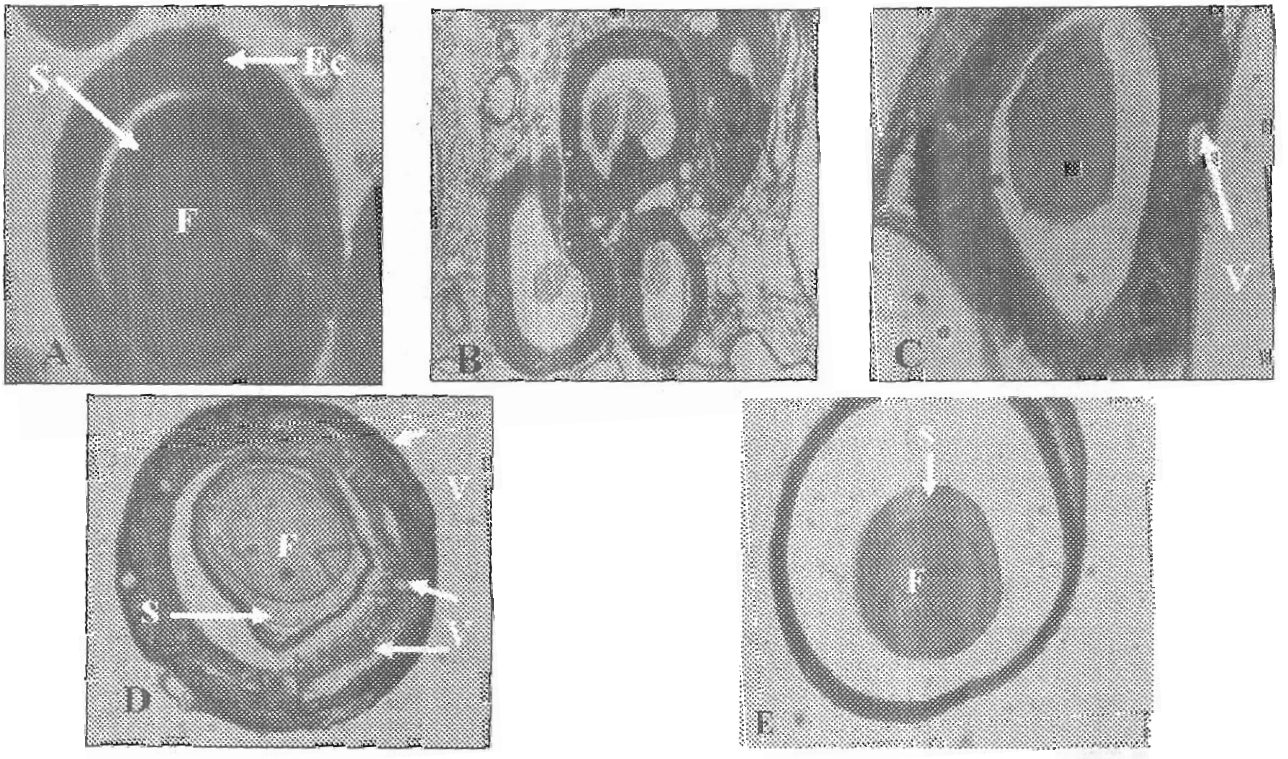

Fig. (9): Gradual damage caused to silk glands and silk thread in *G. mellonella larvae* due to infection with *B. huringiensis*. (A: Control; B, C,D and E: successive 4 days post treatment. Bm = basement membrane, Ec = epithelial cells, F= fibroin, S = Sericin, V = vacuoles).

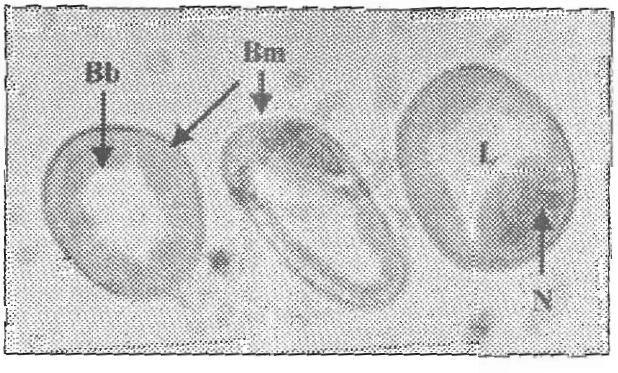

Fig. (10): Cross section in Malpighian tubules of untreated larvae of *G. mellonella* showing normal structure. Bb=brush border. Bm=basement membrane, L='umen and N=nucleus.

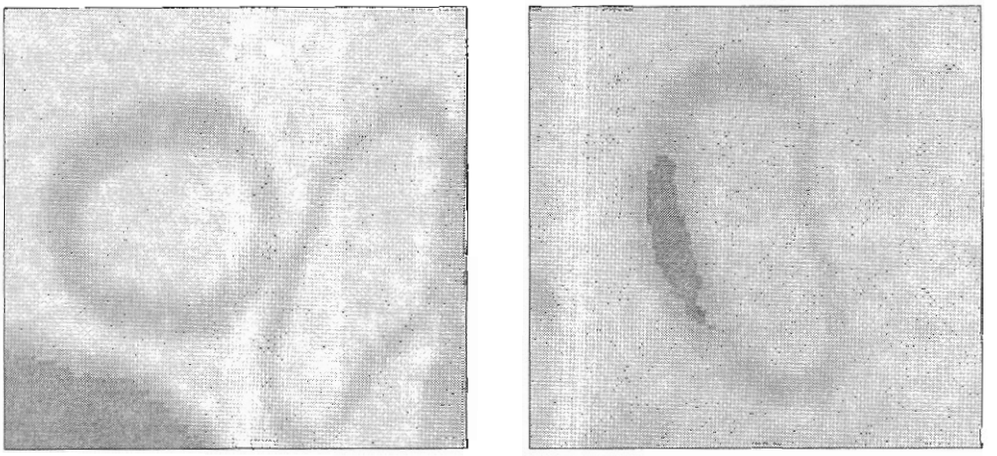

Fig. (11): Malpighian tubules in larvae of *G. mellonella* treated with *B. thuringiensis* var. *kurstaki*.

disintegration of the brush border structure and fusion of the cytoplasmic content associated with elongation of the nucleus (Fig. 11) as a sign for caryolysis and vacuolization followed by cytoplasmic disintegration (cytolysis). Reisner *et al.* (1989) treated Malpighian tubules of the hesperiid *Calpadis ethilus* by injecting endotoxins (134 and 64 kda) into the tubule lumen and found that the toxin 63 kda (10 µg/ml) completely inhibited urine secretion and induced massive cytolysis. They recorded variable cytopathological changes with progressed severity from low to high toxin concentrations. Their observation coincidence with the present findings where tubules in infected larvae showed enlargement of spaces (vacuoles) in the cytoplasm and basal enfolds. The authors stated that it was followed by alterations in microvilli, mitochondria, lyses and enlargement of the formed vacuoles. They recorded more changes such as apical and basal membrane lyses, swelling of the rough endoplasmic reticulum and mitochondria and leakage of cytoplasmic material into the lumen. They also stated that 63 kda endotoxin affected a fluid-transporting epithelium other than midgut. Similar results were recorded by Ryerse *et al.* (1990) in larvae of *H. virescens*, and by Wang and Cheung (1994) in *Pieris canidia*.

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