HISTOPATHOLOGICAL STUDIES ON DEAD LARVAE OF *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) EXPERIMENTALLY KILLED WITH δ-ENDOTOXIN OF *BACILLUS THURINGIENSIS* (SOTTO)

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SUMMARY: Newly hatched larvae of pink bollworm (Pectinophora gossypiella) were fed in 10–20 μg/ml δ-Endotoxin, B. thuringiensis in an artificial diet. Microtomical studies on dead larvae were carried out. 5 μ thick sections revealed varied histopathological changes leading to disorientation and rupture of epithelial cells of the gut which ultimately may have lead to paralysis and death of the test insect.

Key Words: δ-endotoxin, gut epithelium, pectinophora gossypiella, bacillus thuringiensis.

INTRODUCTION

Bacillus thuringiensis has been studied most intensively during the last three decades because of its promising application in the biological control of Lepidoptera (1–10).

The parasporal body of *Bacillus thuringiensis* being an endotoxin, (δ-endotoxin) is proteinaceous and is formed inside the cell during sporulation. It is toxic for susceptible host and capable of paralyzing the gut of most lepidoptera larvae. According to Heimpel and Angus (15). Lepidopterous species susceptible to δ-endotoxin of *B. thuringiensis* are divided into three types. X-ray studies showed that both type I and type II insects suffer from mid gut paralysis few minutes after ingestion of toxin along with general paralysis resulting in death in 1–7 hours. Type III insects die after 2–4 days without any symptoms of general paralysis. The present microtomical studies have been carried out to determine the histopathological changes produced in pink bollworm pectinophora gossypiella (15) when fed on 10 and 20 μg/ml of purified δ-endotoxin *B. thuringiensis* Sotto a local isolate.

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MATERIALS AND METHODS

Microtomy

Dead larvae were procured from 10 and 20 μg/ml toxin containing diet vials after 48 days Sheikh *et al.* (11). The dead larvae were fixed on Bion's fluid Ethyl alcohol (80%) 150 ml, formalin (40% sol.) 60 ml, glacial acetic acid 15 ml, picric acid crystals 1 gm for an hour. After fixation, the dead larvae were transferred into 70% ethanol for preservation.

Embedding

The fixed and preserved specimens were treated with 50% ethanol and kept in it for about 10 hours. They were then passed through 7 different grades of tertiary butyl alcohol (TBA) having decreasing quantities of distilled water in TBA and ethanol in a series, ranging from 10 to 100 v/v. The specimens were kept 10 to 14 hours in each grade in the series.

Paraffin max (E. Merck) of known melting point 59–62°C was added bit by to TBA (100%) and kept at room temperature for 24 hours. TBA containing dead larvae was placed in an incubator and some more paraffin wax flakes were added to it on dissolution in TBA the material settled at the bottom and nearly half of the total quantity of dissolved wax was transferred into another vessel and more wax flakes were added, the process was continued till the entire quantity of TBA was removed. Thus the

material was completely embedded in 100% wax. The temperature of incubator was maintained just 5°C higher than the melting point of paraffin wax.

Block making

Paraffin wax (M. Merck) used in block making was first placed in a beaker and kept in an oven for 36–48 hours having a temperature 2°C higher than its melting point in order to remove air bubbles. Cavity type staining glass blocks were used for block making. After coating a thin film of glycerine on the surface of the cavity of the staining glass block the melted wax was poured into the cavity and then a processed larvae was embedded in the center of the block. The wax blocks so prepared containing the larval material were cooled in water. The wax blocks were then cut to appropriate size so that it could be fixed on to microtome for section cutting.

Section cutting and staining

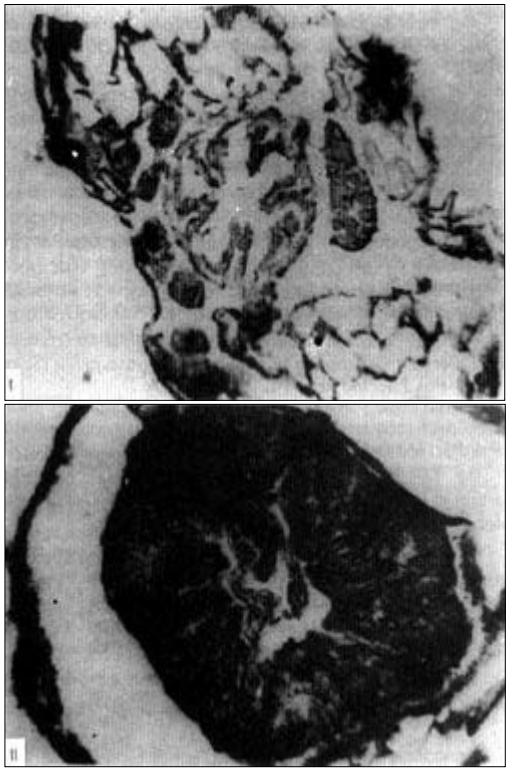
Microtomical sectioning of the larvae of the control and those fed on 10 and 20 μg/ml of toxin mixed in diet was done to 5 μu thickness using (Minot Microtome Model 1212 Ernst Leitz optical works. Wetzler, Germany) The transverse section were stuck on the slides with egg albumen. Slides were allowed to pass through different grades of ethanol for rehydration and dehydration keeping the slides for about 5 minutes in each grade except in hematoxylin. The sections were kept in this stain for 45–50 seconds and 5 minutes in eosin. Canada balsam was used for permanent mounting.

RESULTS AND DISCUSSION

The δ-endotoxin is known to act on the gut of the larvae where it causes paralysis and tissue disintegration. Microtomical studies of the dead larvae from 10 to 20 μg/ml concentration of test and control vials were carried out (Figures 1–5).

Results of the histopathological investigation showed that the section from the control vial (Figures 1 and 2) are clear and the epithelium projecting into lumen of gut uniformly stained from proximal to distal end, is in tonus condition and the gut muscles seem to be intact and no relaxation of muscles is seen. This can be easily differentiated from Figure 3 where the evidence of gut paralysis has set in since the epithelium is to some extent disorganized and the muscles around the epithelium seen to be relaxed. Figure 4 shows further changes in the gut indicat-

Figures 1 and 2: 5 μ thick t.s. of 1st stage larvae of P. gossypiella unexposed to the toxin 45–50 sec. staining in hematoxylin and 5 mins in eosine. X800.



Figures 3 and 4: 5 μ thick t.s. of 1st stage larvae of P. gossypiella procured from bioassays of 10 μg concentration of the toxin/ml 45–50 sec. staining in hematoxylin and 5 mins in eosine. X800.

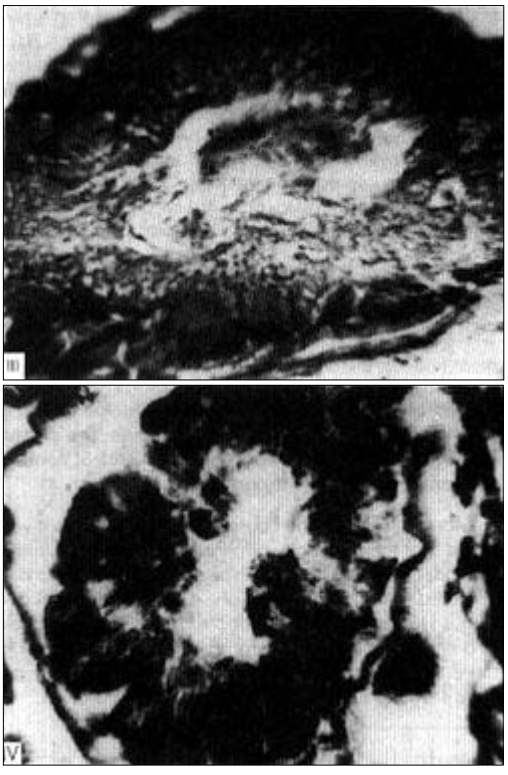
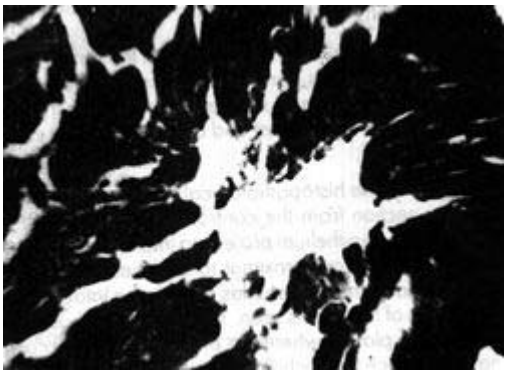


Figure 5: 5 μ thick t.s. of 1st stage larvae of P. gossypiella procured from bioassays of 20 u.g. concentration of the toxin/ml of the diet. 45–50 sec. staining in hematoxylin and 5 mins in eosine. X800.



ing that the distal end of the epithelium has undergone disintegration the muscular layer seems to become thick Figure 5 shows that the extensive damage is done to the gut, it gets ruptured and epithelial cells get disorganized, distorted and appear to have taken a higher stain as compared to the cells of proximal part of epithelium.

These results are in confirmation with the work of Heimpel and Angus (15) they observed that the epithelium disorganized and gut muscles are often seen coupled with a lack of staining ability. Tanada (12) described similar findings in *Pieris rapae*, and reported that soon after ingestion of *B. thuringiensis*, the portion of mid gut epithelium becomes disorganized. The histopathological studies of Toumaroff and Vago (13) on silkworm larvae fed on *B. thuringiensis* also agrees with our findings. The present studies are also in accordance with the work of Vankora (14) and Heimpel and Angus (15).

Moreover during the course of present studies it was found that the pink bollworm *Pectinophora gossypiell*a (Saunders) belongs to type I species Lepidoptera as it has died due to mind gut paralysis indeed Heimpel and Angus (15) reported that lipidoptera species susceptible of δ-endotoxin of crystalliferous bacteria are divided into three types on the basis of response to the toxin type. Type I and II insects suffer from mid gut paralysis.

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