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# HISTOLOGICAL CHANGES OF *LEUCINODES ORBONALIS* TREATED WITH ACTINOBACTERIAL SECONDARY METABOLITES

**Helen Diana I, Syed Jahangir H***

Post Graduate and Research Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

**ABSTRACT:** Prepupal *L. orbonalis* (n=16) treated (four days) with different concentrations (30, 35 and 45ppm) of secondary metabolites obtained from JMCHA8 strain showed various alterations in the gut histology as degenerated epithelial cells and gastric ceacum with enlarged gut lumen with less bolus content with disoriented peritrophic and basement membrane. Also the cuboidal epithelial cell arrangement with numerous foldings in Malpighian tubules were altered in the JMCHA8 strain secondary metabolites treated group than compared to the control group larvae. These results evidenced that digestive tract and excretory organs of the larvae were affected due to reduced energy and unbalanced ionic content which resulted in complete lack or disturbed function of the organs.

**KEYWORDS:** *L. orbonalis;* Histology; Gut; Malpighian tubules; actinobacterial secondary metabolites.

**Corresponding Author: Dr. Syed Jahangir H*** Ph.D.

Post Graduate and Research Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamil Nadu, India. Email Address: Syedbot2000@gmail.com

## 1.INTRODUCTION

In Tamil Nadu, the most commonly used insecticides in brinjal field to control pest are Alanto, Exodus, Acetamiprid and Regent. *Leucinodes orbonalis* (Guen.) cause serious damage and reduces upto 60% of the annual productivity of brinjal plants [1-2]. While excessive application of pesticides affected the whole ecosystem by bioaccumulation of its residues in food chains and also in soil, water and air [3-4]. Instead of synthetic chemical pesticides, nowadays the new idea of "bioinsectides" emerges out to protect our ecosystem from gregarious insects. Actinobacteria showed a wide range of biocontrol action against a range of phytopathogens [5] and also it holds a prominent position due to their diversity and proven ability to produce new compounds [6]. Insect

histology acts as a good model to study the mode of action of foreign particles or substance to their body and their digestive system, main physiochemical barriers invading compounds [7]. Insect gut is a hollow tube starts from the buccal cavity and act as the principal site for digestive enzymes secretions to digest food and absorb nutrients [8]. Lepidopteran insect digestive canal composed of three parts such as foregut, midgut and hindgut which are lined by a single layer of epithelium and connective tissue which are surrounded by muscle tissue for contraction of the digestive tract [9]. The exterior of the basement matrix is bathed in hemolymph that carries nutrients to the rest of the body of insect [8]. Cuticle layer is absent around Midgut region and it actively interfacing with various pathogens or foreign substances while performing the essential digestion and nutrient absorption functions [10].The main osmoregulatory and excretory organs of insects are malpighian tubules (MT) also responsible for the isosmotic filtrate from their hemolymph into the hind gut [11]. In larvae, the osmotic and ionic regulations carried out by thin finger like extensions called MT, present between midgut and hindgut which connects to the intestinal tract. The larval instars showed increased rate of excretion during their maturation phase by enlargement by cell growth of a few tubules [12]. Due to these reasons, in this study, the gut and malpighian tubules of *Leucinodes orbonalis* larva treated JMCHA8 strain secondary metabolites were focussed.

## 2. MATERIALS AND METHODS

### 2.1. Sample collection and processing

From brinjal fields, aseptically one kilogram (each site) of insecticide exposed rhizospheric soil collected in Paithur village, Athur Taluk, Salem district, Tamil Nadu (Lat: 11°30’54.71”E Long: 78°33’19”E) are transported to the Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli district for further preparation. The soil samples were air dried for four days and sieved in a mesh and stored in the refrigerator until further use. Based on the primary and secondary screening, the isolated actinobacteria designated as JMCHA8 and their secondary metabolites were obtained for further analysis.

### 2.2. Lethal dose concentration and Experimental Dose

The median lethal concentration of JMCHA8 strain secondary metabolites on *Leucinodes orbonalis* larva was identified as 75ppm [13] with the help of probit analysis. Three different concentrations such as 30ppm, 35ppm and 40ppm of JMCHA8 actinobacterial secondary metabolites were finalized as experimental dose and the larval groups consist of 3$^{rd}$ and 4$^{th}$ instars larva (n=16). Three different concentrations of the solvent extracted secondary metabolites was exposed to the larva through feed for four days and finally the treated and control larva were collected and fixed.

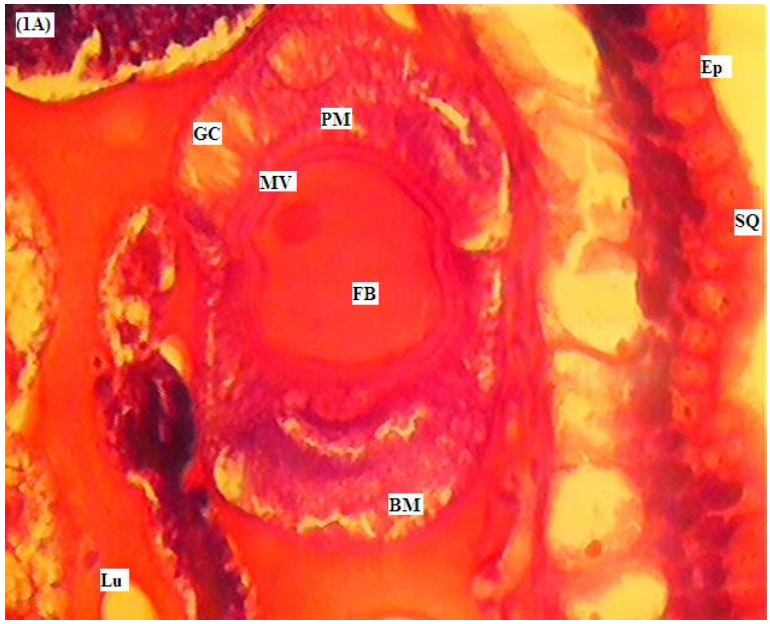
### 2.3. Histological analysis

For light microscopic studies, the larva fixed in 10% formalin, washed and processed through series of alcohol (30%, 50%, 70%, 80%, 90%, 100%) for dehydration, cleared in xylol and finally embedded in paraffin wax. Sections of 5µm thickness were cut using Leica ultramicrotome and

stained with Haemotoxylin counterstained with Eosin [14]. DPX mounted sections were observed and photographed using Olympus photomicrography unit.

## 3. RESULTS AND DISCUSSION

In larva, the midgut constitutes the largest part of the digestive system where the digestion takes place. Histological sections of midgut, malpighian tububes and their neighbouring organs or parts of larva (400x magnification) were mainly focused here to study about the effect of entered actinobacterial secondary metabolites through feed. Mid gut cross section of control larvae of 4$^{th}$ instar larva (Figure 1A) showed epidermis (Ep) with squamous cells (SQ) present in the outer layer. Brush border membrane composed of numerous and regularly placed microvilli (MV) observed in gut lumen (Lu) filled with food bolus (FB). The gastric caecum (GC) showed a well-preserved layer of epithelial cells and innerside of the caecum, the peritrophic membrane (PM) and basement membrane (BM) were observed. In control larva, the malpighian tubules walls composed of a single layer of cuboidal epithelial cells with prominent basal lamina and their apical membrane showed numerous short and irregular projections. Figure 1B showed external folding with uniformly arranged epidermal (Ep) layers with elongated squamous (SQ) cells with lumen extends into the malpighian tubule layer (MGL). Similar histological findings observed in various Lepidoptera [15-17], Coleoptera [18-19], Diptera [20] and Hymenoptera [21-22] instar larva.



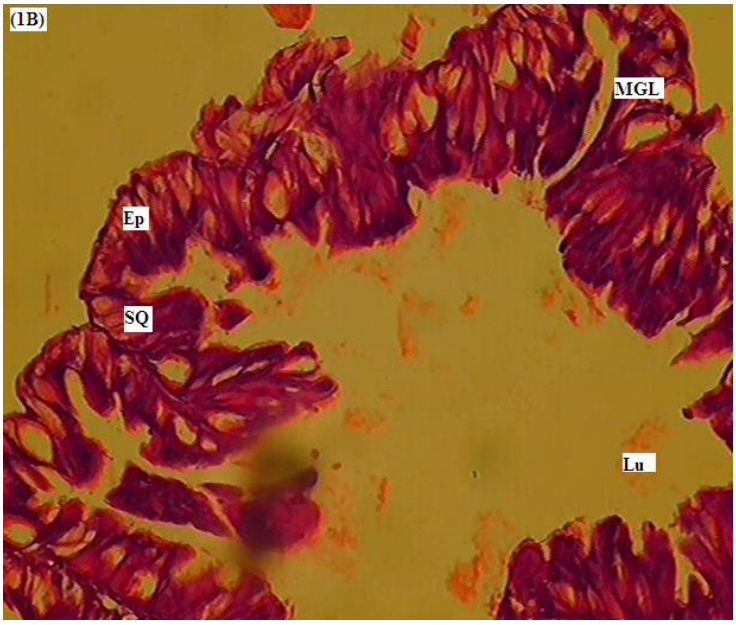
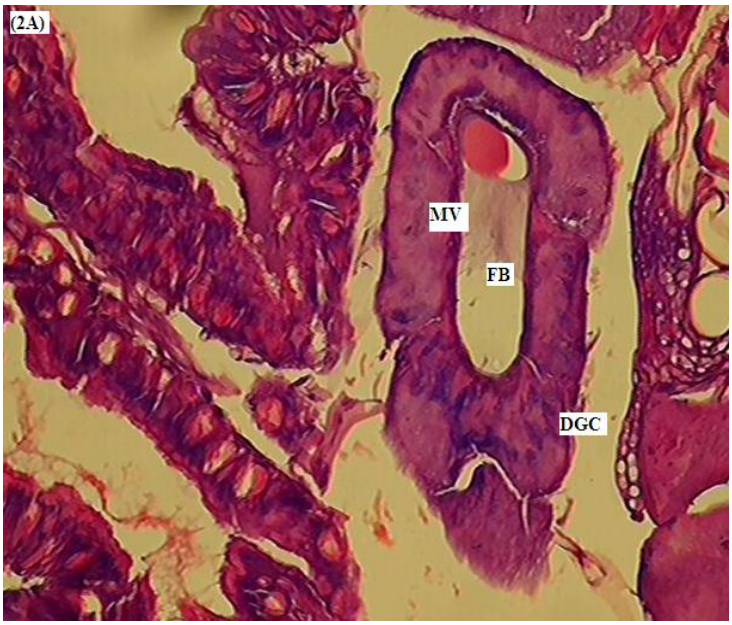



**Figure 1. Mid gut cross section and epidermal layers of control larvae of *L. orbonalis* 4th instar larva (400x)**

Treated Larval midgut (Figure 2A) showed degenerated microvilli (MV) and destroyed gastric caecum (DGC) with reduced food bolus (FB) content and increased space observed between the inner linings of gut. Degenerated malpighian tubule (DMT) and the nearby midgut linings contains perforated (Pf) epidermal layers (Figure 2B) were observed.



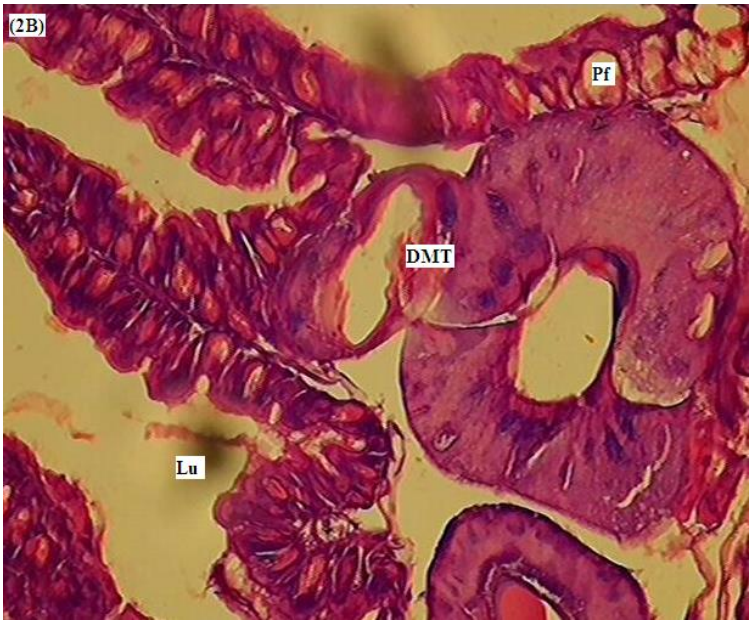
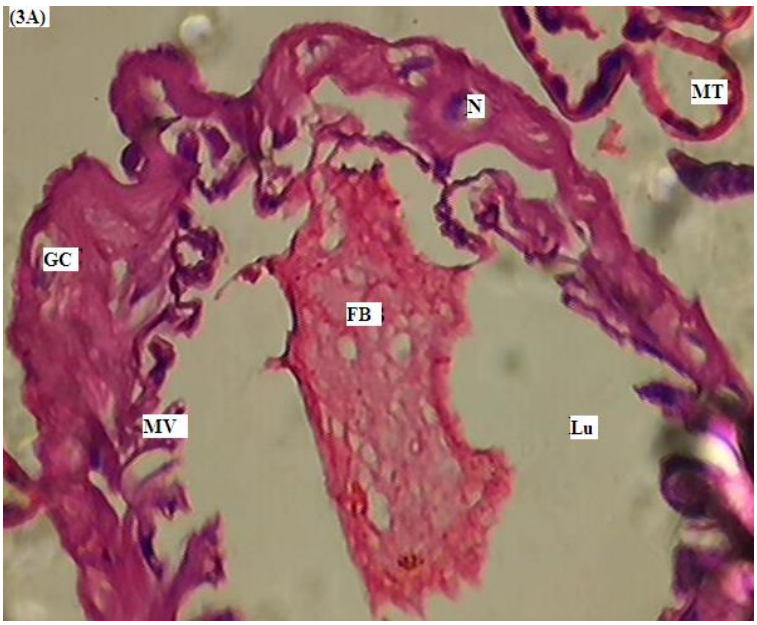



**Figure 2. Mid gut cross section and epidermal layers of *L. orbonalis* 4th instar larva treated with 30ppm of secondary metabolites of JMCHA8 strain (400x)**

In Figure 3A, the food bolus (FB) content severely fragmented with increased lumen (Lu) in the gut and their nucleus (N), microvilli (MV) and gastric caecum (GC) were severely degenerated. The malpighian tubules (MT) lost its regular arrangement of cuboidal epithelial cells. Vacuolated (V) and severely degenerated muscle layers (DML) were observed. Muscle tissues showed perforations (Pf) in their gut regions (Figure 3B). Similar to our results, Al-Mehmadi and Al-Khalaf [23] reported apical degeneration in midgut region than compared to controls dipteran larva and also brush border, basal membrane, nucleus, and cytoplasmic organelles were lysed in the gut lumen. Destruction of the peritrophic membrane also observed.



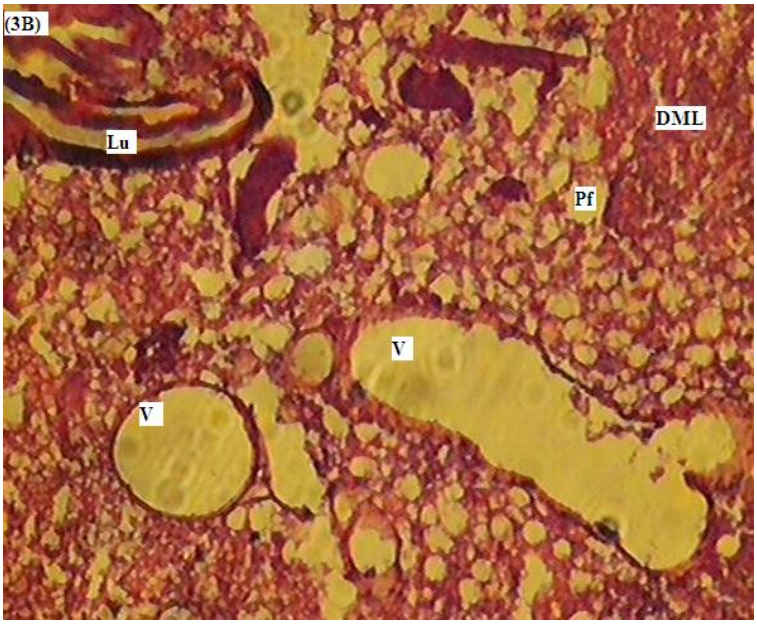
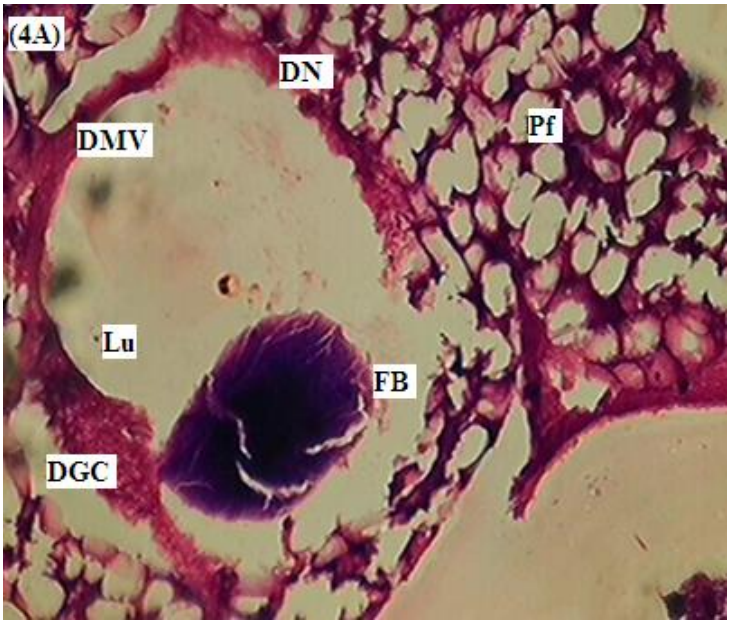



**Figure 3. Mid gut cross section and epidermal layers of *L. orabonalis* 4th instar larva treated with 35ppm of secondary metabolites of JMCHA8 strain (400x)**

In higher concentration exposure i.e. in 40ppm JMCHA8 strain (Figure 4A) secondary metabolites exposure the midgut was completely ruptured with perforated (Pf) layers around the gastric ceacum. Enlarged lumen (Lu) with degenerated microvilli (DMV) and destroyed gastric caecum (DGC) with disoriented nucleus (DN). Highly degenerated muscle layers (DML) with completely shrinked muscle tissues in the gut regions and degenerated epidermis (DEp) (Figure 4B) were also observed. Degeneration signs like cell shrinkage, vacuole formation and apoptotic bodies observed in larval midgut of *Anticarsia gemmatalis* [24] and *Bombyx mori* [25].



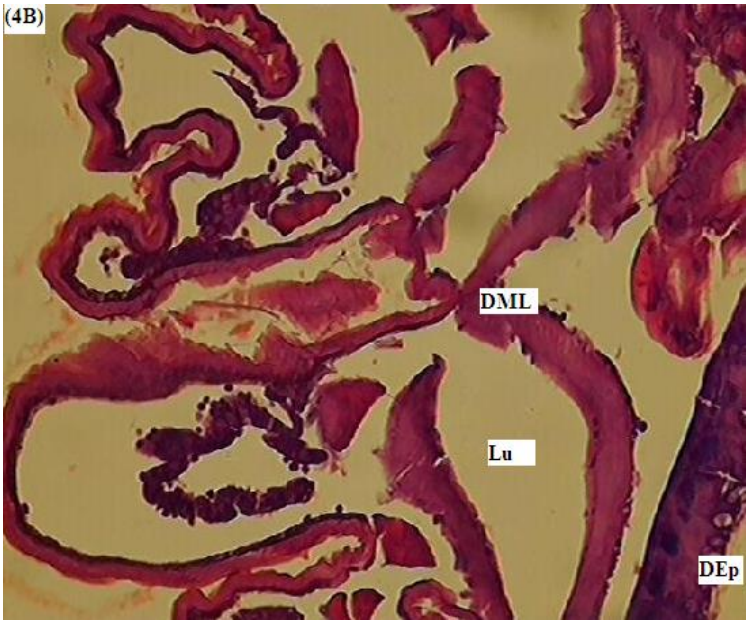



**Figure 4. Mid gut cross section and epidermal layers of *L. orabonalis* 4th instar larva treated with 40ppm of secondary metabolites of JMCHA8 strain (400x)**

Malpighian tubules filter the hemolymph to remove the excretory products produced from metabolism into the hindgut [26-27]. MTs disturbances highly related with midgut and hindgut activities of insects due to the accumulation of toxic products [28-30]. The degeneration of malpighian tubule and other tissues acts as an evidence for necrosis of tissues [12, 31] in the insect system.

## 4. CONCLUSION

Histological analysis of prepupal (instar) stage of *L. orbonalis* showed various alterations in the gut region as degenerated epithelial cells and gastric ceacum with enlarged gut lumen. Cuboidal epithelial cell arrangement in malpighian tubules were altered in the JMCHA8 strain secondary metabolites treated group than compared to the control group larvae. These results concluded that midgut in digestive tract and malpighian tubules morphology were severely altered which resulted in complete lack or disturbed function of the organs.

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## CONFLICT OF INTEREST

No conflict of interest

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