

Original Research Article

Histological investigations on Potato Tuber Moths treated with a botanical extract of basil (*Ocimum basilicum* L), with particular attention to the alterations in the Ultrastructure of the larval digestive tract

Abstract

Background: *Phthorimaea operculella* (the potato moth (PTM)) is well known as one of the most important pests that attacks potato, *Solanum tuberosum* L. crops worldwide. Adult moths attack tubers through harvest and/or through entering the store facilities. Infested tubers became unsuitable for trade or consumption, and then tuber damage increased gradually after several generations of the pest during storage time. Botanicals have been found to be an efficient control method especially when they act as antifeedant or repellent, or even stomach poisons. The estimation of the mortal impact of some extracts against PTM was examined by emphasizing the Ultrastructure changes in the larval digestive tract by using scanning and transmission electron microscope.

Results: Structure changes throughout the entire digestive tract (Fore-, Mid- and Hind-gut), induced by Basil extract were detected through both microscope, these changes leading lastly to the death of the treated larvae. Many different ruptures and/or malformations were detected throughout all the digestive tract assessment.

Conclusions: Studies of the changes occurred revealed some histopathological changes occurred through all the digestive tract (Fore-, Mid- and Hind-gut), the attached muscular layer, tracheal system, and visceral fat bodies of the larvae fed tubers treated with Basil extract. Starting here from this point of view, it could be

recommended the use of some plant extracts, especially for their highly protection for the tubers against the moths.

Keywords: Potato tuber moth, botanical extracts, alimentary canal, Ultrastructure changes, scanning and transmission electron microscope.

1. INTRODUCTION

Phthorimaea operculella Zeller, (Gelechiidae: Lepidoptera) is an extremely serious pest for potato crop. Rondon (2010)[1] reported that yield losses reached 100% because of infestation during storage and/or marketing. Where tubers become unfortunate for sale or consumption after infestation, and then their damage increases gradually after several insect generations during storage. Application of Chemical insecticides induces wide contamination and pollution to the environment causing intoxication of non-target arthropods and other beneficial insects, leading to the development of pesticide resistance among target insects [2,3].

The plant extracts of some wild plants are considered as a powerful tools as a biopesticide against many insect pests. Studies cleared that, there are several aromatic and medicinal plants have harmful effects, *i.e.*, antifeedant, repellent, growth regulation impact and/or potentially harmful action on a wide-ranging of insect pests [4,5,6,7,8].

Herein, the current research is performed to estimate and emphasize the potential impact of Basil plant extract on the Ultrastructure changes of the whole larval digestive alimentary canal which were undertaken using scanning and transmission electron microscope [7].

2. MATERIALS AND METHODS

2.1- Insect culture: Using fresh un-infested potato tubers, *Solanum tuberosum* L (var. Spunta), as a substratum for stock laboratory colony of PTM was established. According to confident descriptions by some authors [9,10], the culture was kept in the National Research Centre rearing Lab, equipped with wooden cages within room-storage under controlled conditions ($27\pm 2^{\circ}\text{C}$ and $70\pm 5\%$ RH) with a light-dark cycle of 10–14 hours. Periodically, every ten days, fresh potato tubers were added to maintain a fresh, clean culture and allow for egg deposition.

2.2- Plant extract: Basil plant (*Ocimum basilicum* L.) locally available in the field was air-dried under a shad at ambient conditions. The air-dried material was ground into fine powders and kept in a tight glass container until use. Exclusively prepared plant powders were extracted with ethanol 80% by using the soaking technique (Freedman et al. [11]). The crude extract was mixed at 2.5% with talcum powder (Magnesium silicate) as an inert carrier substrate, Potato tubers were mixed thoroughly with 25g of the extract-treated talcum powder/1 Kg tubers until tubers have been covered uniformly, and the treated powders stuck properly to the tuber surfaces.

2.3- Electron microscopic studies: The remaining surviving treated larvae which were obtained from inside the treated tubers after 10 days post-mining inside the tubers were used. Larvae were taken for Ultrastructure investigations using the scanning electron microscope after Boulton and Brabazon [12] and transmission electron microscope (TEM) as described by Sharaby et al. [7], then fixed immediately in 4% glutaraldehyde fixation. Untreated larvae of the same age were used as a control group. The larval alimentary canal (the whole body behind the head) from the treated and normal (untreated) larvae were examined. Specimens

were kept in the fixative at 4°C till processed. To investigate the Ultrastructure changes, the entire alimentary canal components (fore-, mid- and hind-gut) were examined using the method described by Sharaby et al. [7] after being investigated by JEOL JSM-T20 Scanning microscopy ScanZies and Transmission EM/10 electron microscopy at 60 KV.

3- Results

3.1- The Ultrastructure observations: Moribund larvae, which were fed tubers treated with talcum powder containing Basil-ethanolic extract were chosen for the Ultrastructure investigations. Cleared histological changes inside the alimentary tube were observed.

The alimentary canal of the larvae: The alimentary canal in the larvae is a tube extending from the mouth to the anus and is formed from three embryonic elements, the stomodaeum and proctodeum that form the fore- and hind-gut respectively (they are lined by intima layer or body cuticle), and the middle endodermal derivative, mesenteron or mid-gut [13].

3.2- The fore-gut: Serves as a storage chamber and may be the site of preliminary digestion, in untreated larvae, forms a small sac (Crop), consisting of a layer of epithelial cells resting on the basement membrane, their apical portion lined with thin chitinous intima) (Figs. 6 A,B) provided with many micro spines projected directed towards the lumen (Fig. 1A) and surrounded by a muscular layer. Ultrastructure changes were detected by obvious damage and separation of the chitinous intima with their microspines from the basal epithelial cells was observed, many vacuoles and holes were also noticed (Figs. 1 B,C,D and 6-C).

3.3- The mid-gut: Is the most important site of digestion (essentially with the production of digestive enzymes) and absorption of nutrients, forming the longest part of the digestive tract it is folded, with a unicellular epithelial layer (Fig. 7B) resting upon a basement membrane (Fig. 7C). This membrane is surrounded externally by circular and then by longitudinal muscles fibers (Fig. 7C). The epithelium consists of columnar (Fig. 7B) and goblet cells (Fig. 7D), at the base, clusters of small regenerative cells each of which contains a relatively large nucleus and strongly basophilic cytoplasm (Fig. 7C). The epithelium is also protected from the food particles by a detached sheath Pritrophic membrane, surrounding the lumen. Also in Figure (7 A,B,C,D) (normal mid-gut) appears with the luminal surface of the epithelium cells which are provided with a striated border (Fig. 2A and 7A, D) constituting long microvilli. Such microvilli are projected inwards into the lumen to increase the absorption surface of cells, in addition, the space between them acts as a kind of sieve. Most changes were detected in the fore-, mid-, hind-gut, fat bodies, outer and inner cuticle layers and on some of the trachea that connected with the hindgut and in muscular layers. The most affected tissue was in the mid-gut epithelium compared with the untreated mid-gut. Treated larval Epithelial cells were destroyed in some parts, twisted cell membrane (Fig. 7 I), and disappear of the cell boundaries, irregular in adhering cell membrane, large vacuoles were found in cytoplasm and appearance of fat droplets the epithelium cells (Fig. 7 G,I), appearance of elongated and damaged mitochondria with many vacuoles (Fig. 7 H,J), many lysosomes are observed in the cell cytoplasm (Fig. 7 F) the muscular fiber layers became shrinkage in some areas (Fig. 8 B), compared to the control (Fig. 8 A). Severe destruction of the epithelium, and the goblet cells increased their

secretion (Fig. 2C) and their apical swelling into the gut lumen as a bulbous eversion (Fig. 2B).

3.4- Changes on the hind-gut: For water absorption and faecal formation, shows four distinct regions, pylorus, ileum, colon and rectum. The colon consists of a layer of epithelial cells with a big nucleus based on a basement membrane and lined inwards toward the canal lumen at their internal side with a thick layer of chitinous intima (Fig. 3A and 9A) and surrounded with a layer of visceral muscular (Fig. 8A), sever damage in the circular muscles layer in surrounded muscles (Fig. 8 C), separation on the intima layer (Fig. 3B, 9 B,C), great damage in the epithelial cell nucleus, vacuolization, shrinkage and rupture in the basal basement membrane are observed (Fi-g. 8B). Clear lyses of fiber muscles induced to gut paralysis and disruption in food digestion and movement inside the alimentary canal, reflect on the nutrition and assimilation of food and larval development.

3.5-Changes on the fat body: The untreated hatchlings show an instinctive fat body comprised of cellular bunches which frequently take the structure of cell line (Fig. 4A). The cells seem rectangular or sporadic cells encompassed by membranous sheath with center and cytoplasm filled with numerous lipid beads (Fig. 10A). The treated fat cells became isolated from each other, causing perceptible vacuoles, annihilation of the membranous sheath (Fig. 4B, 10A). The destruction of the cell-structure is most likely because of the layer interruption followed by the membrane burst and the release of the cell contents that incite cell demise. Chaieb et al.(2007) uncovered a cytotoxic impact of *Certum perqui* saponins on the fat group of *S. littoralis* larvae.

3.6-Changes on muscles: Instinctive muscles encompass the alimentary channel and conduits, the muscles are striated, and the muscles cells are massed into muscle filaments into practical joins (Fig. 8A). Instinctive muscles produce peristalsis as checked in the stomach related framework. These muscles are otherwise called neurogenic or coordinated muscles. The progressions in the solid system (Fig. 8 B,C) that separating of the muscles into little parts are ascribed to the devastation of the sarcolemma, halfway mix and break of the muscles fiber and cells leading to deformity and turmoil of the epithelial layer where the muscles lost their function in processing activity.

3.7-Changes on the windpipe: The Tracheal framework is extraordinarily connected to the entire stomach related tract, a tracheal cylinder is contain edge like circumferential circles (taenidia) that breeze spirally through the wall membranous to forestall its breakdown and keeping it open against the inside pressure of the body liquids. This shape empowers tracheal cylinders to flex and stretch without creating crimps that could limit wind current (Figs. 5A, 9A). In the treated hatchlings the observed changes in the tracheal cylinder demonstrated halfway break in the taenidia (Fig. 5B,), wrapped in the cylinder and diminishing in their lumen, shrinkage of the tracheal lumen. The ventilation cycle conveying the oxygen development from the environment up the windpipe tissues and tracheoles through the body bug and the entry of carbon dioxide in the opposite path (Chapman, 1988), harmed windpipe prompting disturbance in bug breath, likewise damages of the joined muscles disturbs the ventilation cycle. Disturbance in breath affects al-I natural cycles in the bug body including processing. Past perceptions appeared that ethanol 80% of basil or colocynth separates prompted no direct poisonous impact as in conventional insect sprays. The concentrates showed a particular method of activity associated

with disruption in the assimilation cycle and breath framework and digestion of fats. The extracts showed closeness in certain perspectives to development controllers and adolescent chemical analogues since they caused formative impediment with disturbance in assimilation. This is due to a decrease pace of endocuticular statement and chitin that has been affirmed histopathologically on the front and rear stomach, and might be caused unbalance in the compound system as their impacts on all parts.

4- Discussion

The obtained observations have shown that digestion is a process in which ingested macromolecules by insects break down into smaller ones- to be absorbable via epithelial cells of the mid-gut. Numerous enzymes based on food materials have a significant roles in this process. Any disruption in their activity hinders insects to provide their nutrients for natural requirements. The present results agreed with the results of Abdullah [14] and Zibae & Bandani [5]. This outcome is in accord with Sharaby et al. [16] who observed severe effects in the alimentary duct and fat bodies of *H. littoralis* first nymphal instar after treated with garlic, mint and eucalyptus essential oils at the LC₅₀ concentration. The current studies possessed that, obvious separation of the epithelial cells from the basement membrane with damage of the peritrophic membrane leading to mixing of the gut contents with the haemolymph causing the larval mortality, while microvilli enhanced the rate of absorption as mentioned by DeRobertis et al. [17], the puffy and elongation protruding of villi into the lumen as a bulbous eversion are a results of enzymes activities of the epithelial cells. These results are in agreement with the findings of Sutter & Raun [18] and Shoukry et al. [19].

The Changes in the hind-gut observations were agreed with that mentioned by Sharaby et al. [16] on the grasshopper, *Heteracris littoralis*.

On the other view, all Changes on the fat body were matched with Chaieb et al. [20] who revealed a cytotoxic effect of *Certum perqui* saponins on the fat body of *S. littoralis* larvae.

Our results for Changes in muscles were in accord with those obtained by Sharaby et al. [16], Ranjini & Nambair [21] and Mohamed et al. [22].

Results of the Changes on the trachea were matched with those reported by Corbet et al. [23] for their work with Eucalyptus essential oils as mosquito larvicides. He added that, the mosquito larvae were susceptible to surface materials inflowing through their tracheal system, and observed that essential oils of *Eucalyptus* sp. enlarged the attraction to tracheal flooding and chemical poisonous. Our findings were in accordance with those mentioned by Sharaby and El-Nujiban [24] in their histological study on the effects of some plant extracts against *Agrotis ipsilon*. Also, the outcome results were matched with Mohamed et al. [22] for their work in applying some essential oils against *Culex pipiens* larvae.

5- Conclusions:

In Nature, there are many plant extracts prompted whole protection for the treatment of the potato tubers from PTM invasion throughout storage. Extraordinary consequences have been located in the improvement and metamorphosis of the larvae. Electron microscopic Ultrastructure study revealed some histopathological changes occurred throughout the entire digestive tract (Fore-, Mid- and Hind-gut), the attached muscular layer, tracheal system, and visceral fat bodies of the larvae treated with Basil extract. The acquired outcomes suggested an interesting opportunity for developing new biological organic insecticides based on plant

extracts, which are safe, limit the build-up of insect resistance, available and environmentally unpolluted in order to control this serious lepidopteran and many other pests either in open fields or during storage, which affect production, marketing, and national income. Starting here from this point of view, it could be recommended the use of the aforementioned plant extract for their highly protection to the tubers and as oviposition inhibitors against insect pests.

List of abbreviations

Figures abbreviations:

Cell Cytoplasm (CC), Goblet Cell (GC), Pores (PO), Villi (v), Spines (sp), Intima (IN), Nucleus (N), Mitochondria (MT), Cell membrane (cm), Rough Endoplasmic Reticulum (RE-R), Mucous secretion (MU), Basement Membrane (BM), Regenerative Cells (RC), Base of epithelial cells (BEC), Muscular Layer (ML), Cavity (CA), Exocuticle of intima (EXC), Endocuticle of intima (ENC), Autofajic Vacuole (OV), Golgi Sacule (GS), Vacuole (VC), Lysosome (LY), Muscle (MC), Damaged Area (DE), Pore Canal (PC), Taenidia (TN), Fate Body (FB), Trachea (T), Connective tissue (CT), Muscles fibers (MF).

Declaration: All authors declare that this script has not been published before somewhere else and it is unique research.

Ethics approval and consent to take part: Not applicable.

Consent for guide: Not applicable.

Availability of facts and materials: All materials are available.

6- References

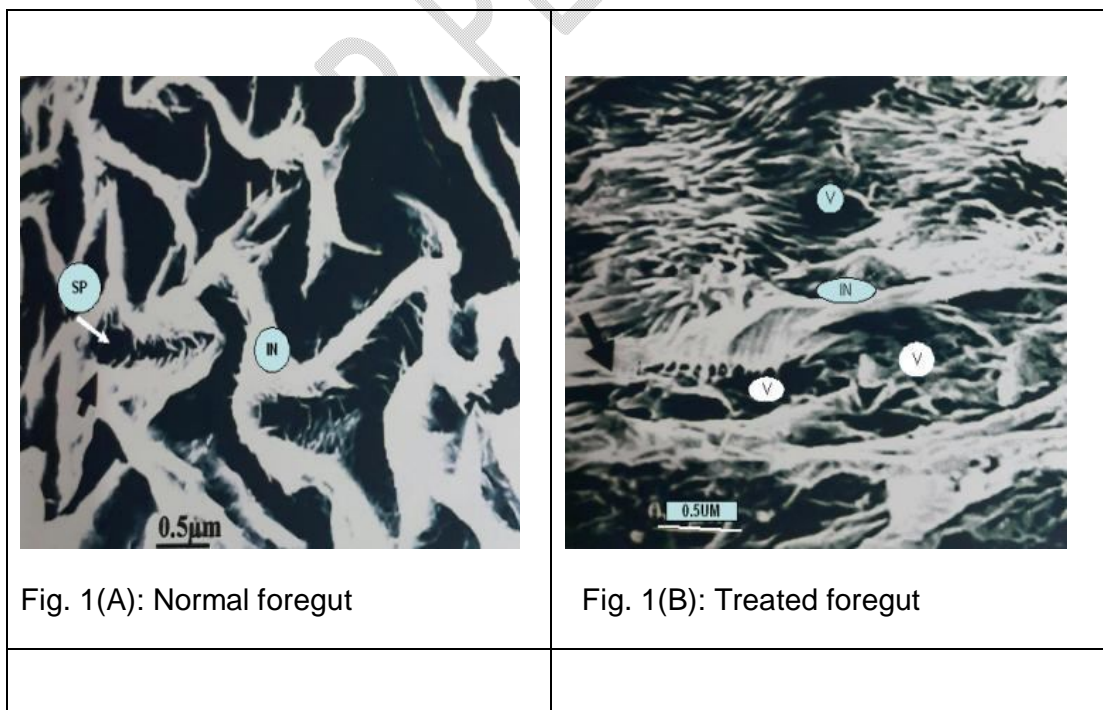
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Fig. (1) to Fig. (5) Observations using Scanning electron microscope



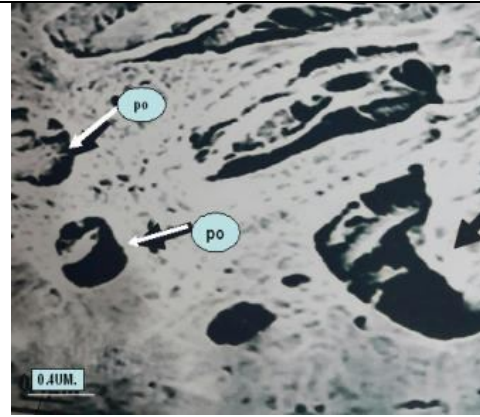


Fig. 1(C): Treated foregut

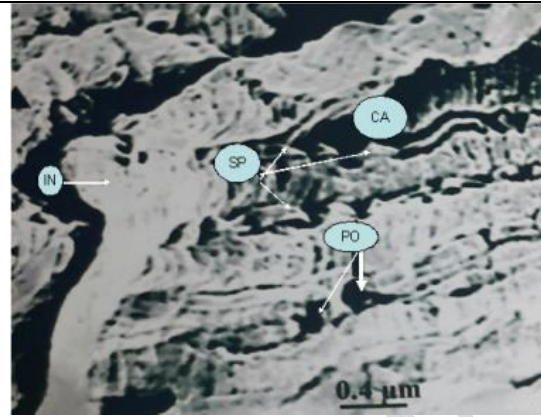


Fig. 1(D): Treated foregut

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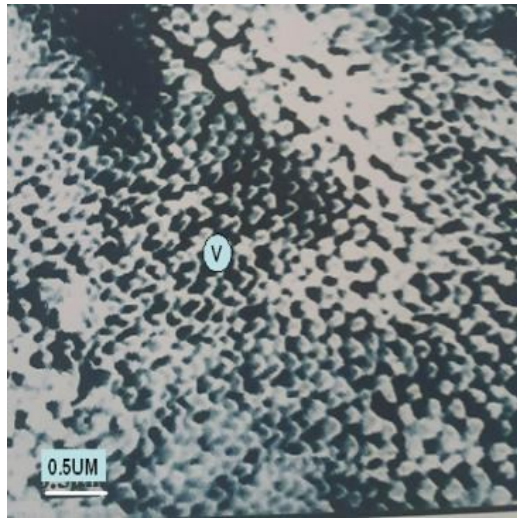


Fig. 2(A): Normal midgut showing intact microvilli

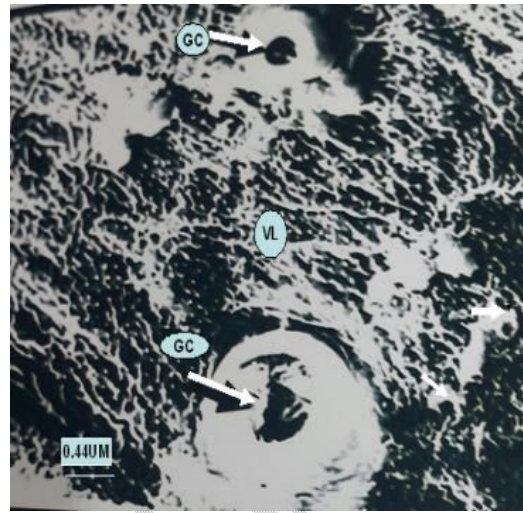


Fig. 2(B): Treated mid gut showing swollen goblet cavity and damaged villi

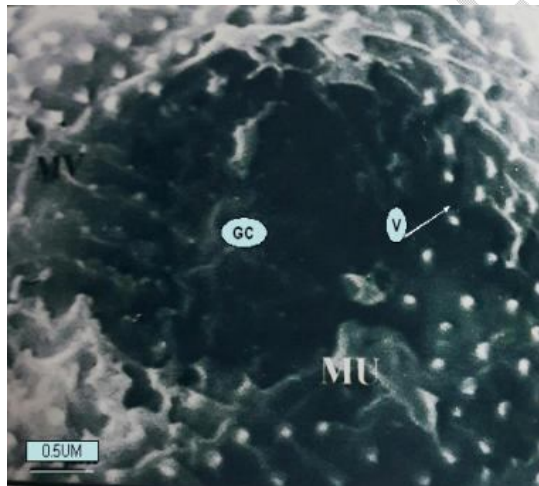
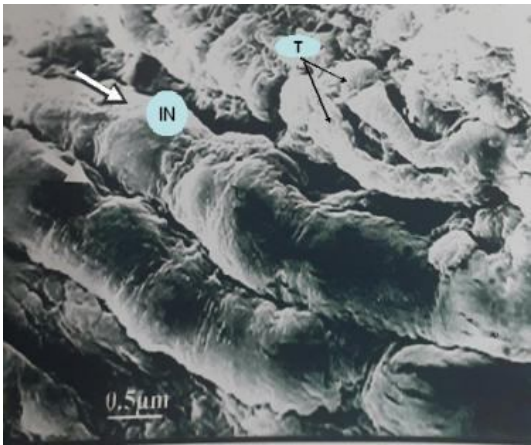
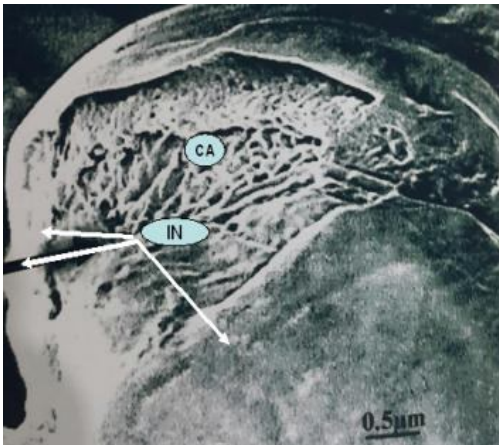
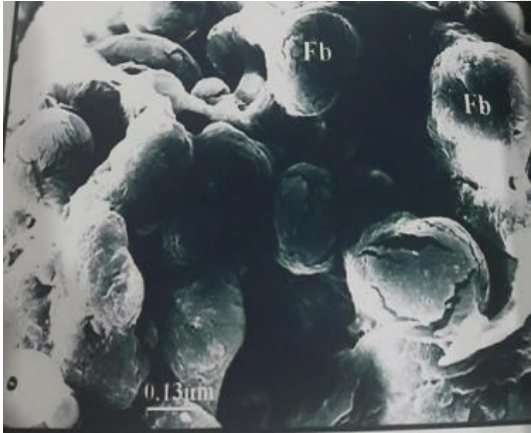



Fig. 2: (C): Treated mid gut showing mucous secretion covered villi surface

	
<p>Fig. 3(A): Normal hind gut showing intima folds</p>	<p>Fig. 3(B): Treated hind gut cleared shrinkage in intima layer</p>
	
<p>Fig. 4(A): Normal fat bodies</p>	<p>Fig. 4(B): Treated fat bodies</p>

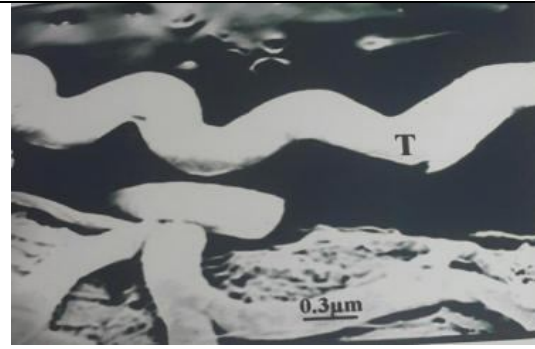


Fig. 5(A): Normal Trachea

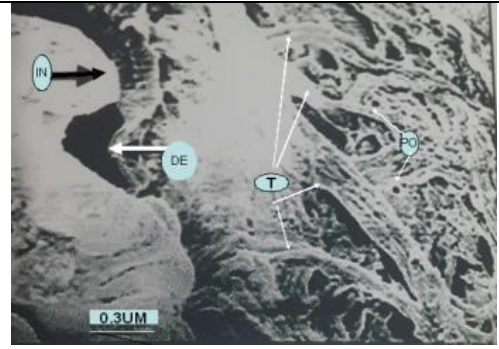
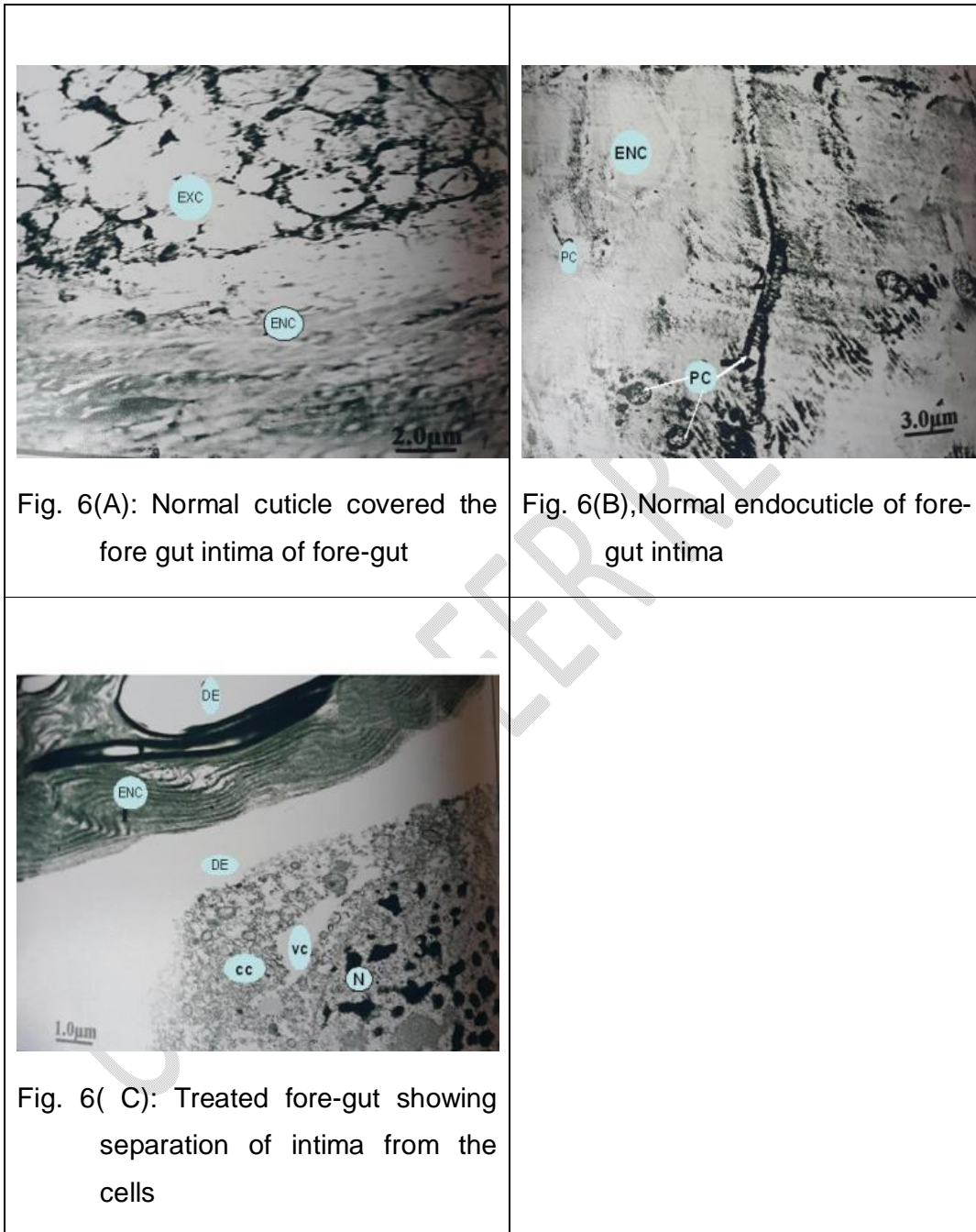


Fig. 5(B): Treated Trachea

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Fig. (6) to Fig. (10) Sections studied by Transmission electron microscope.



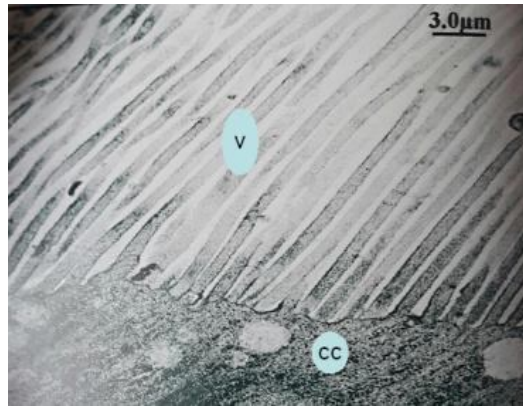


Fig. 7(A): Normal microvilli of mid gut epithelial cells of normal larvae

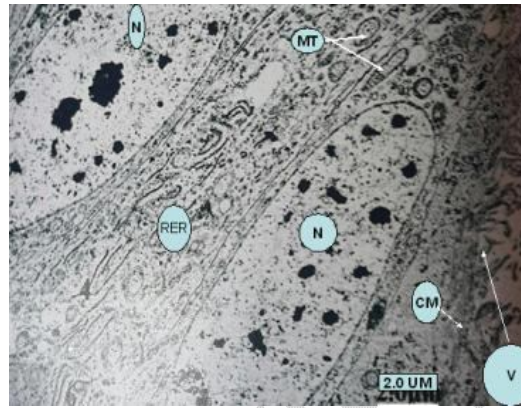


Fig. 7(B): Epithelial cells of mid gut of untreated larvae

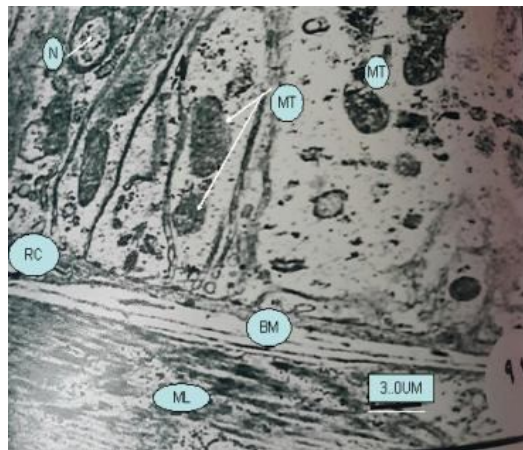


Fig. 7(C): Base of Epithelial cells of normal untreated larvae, showing intact basement membrane, muscles and mitochondria

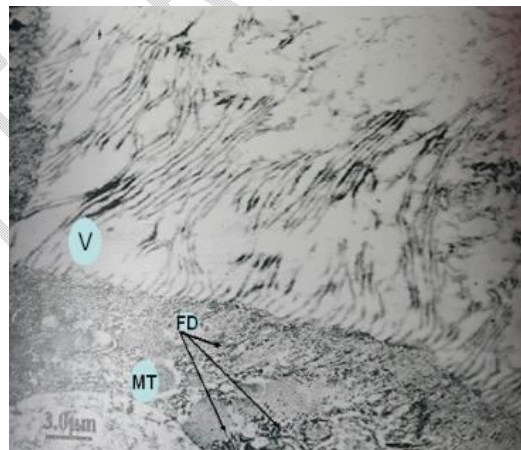


Fig. 7(D): Damaged villi on the apical portion of epithelial cells of mid gut of the treated larvae

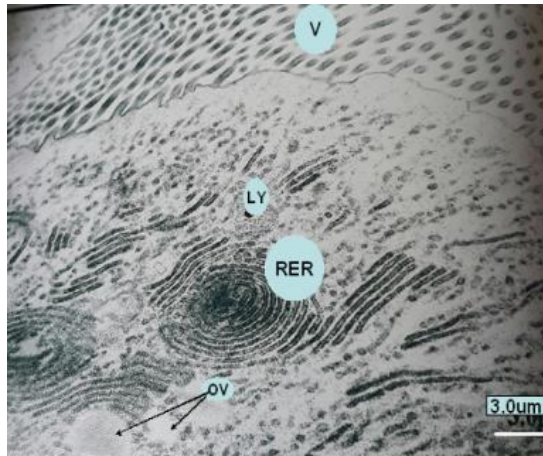


Fig. 7(E): Treated mid gut showing many vacuoles and lysosome

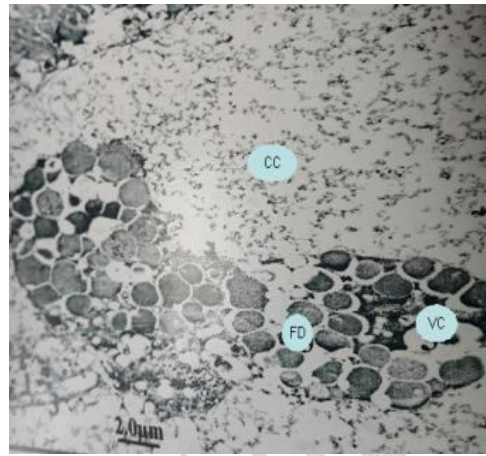


Fig. 7(F): Treated mid gut cells showing accumulation of fat droplets in the cytoplasm

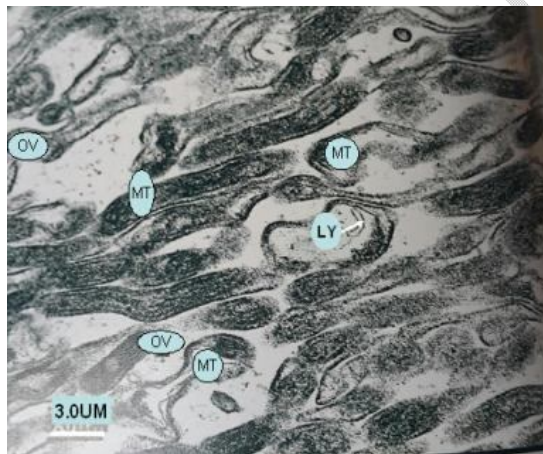


Fig. 7(G): Treated epithelial cells of the mid gut showing degenerated and elongated mitochondria

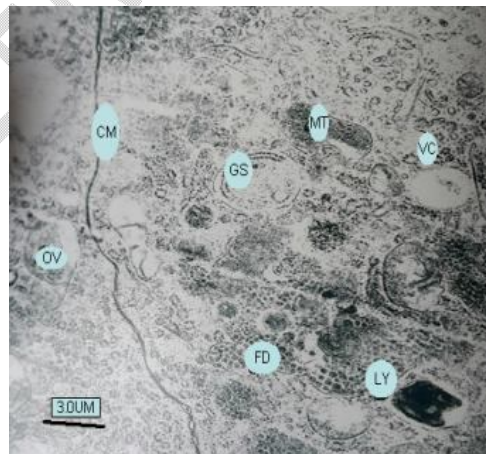


Fig. 7(H): cytoplasm of epithelial cells of treated larvae

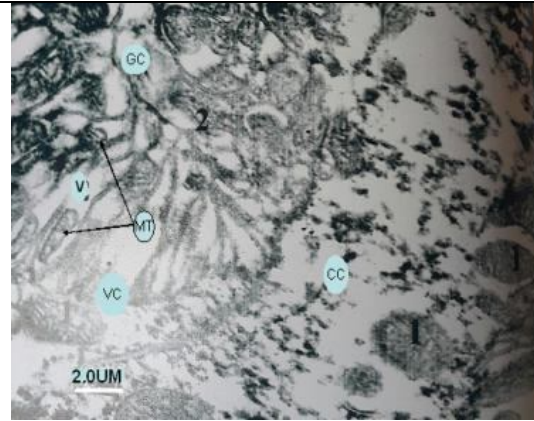


Fig. 7(I): Treated goblet cell, showing degenerated microvilli and mitochondria

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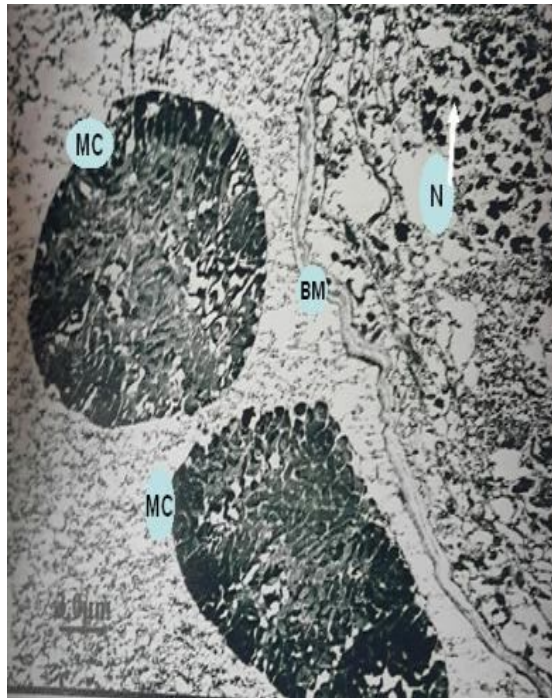


Fig. 8(A): Normal muscles of normal larva

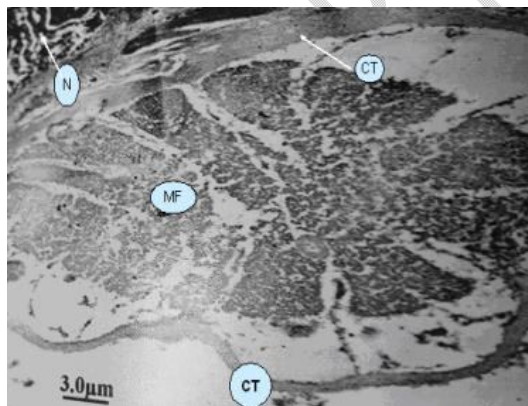


Fig 8: (B), Damaged muscles surrounded the mid-gut of the treated larvae

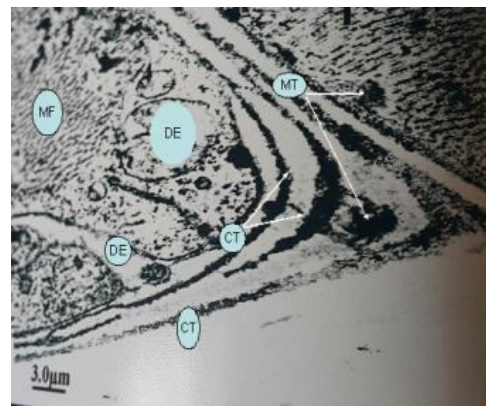


Fig. 8: (C) Damaged muscles surrounded the hind- gut of treated larvae

UNDER PEER REVIEW

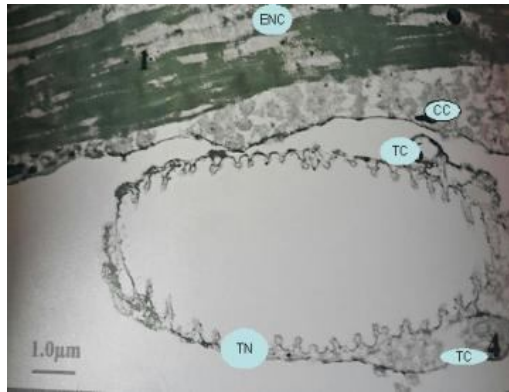


Fig 9: (A), Normal endocuticle and trachea attached the hind-gut

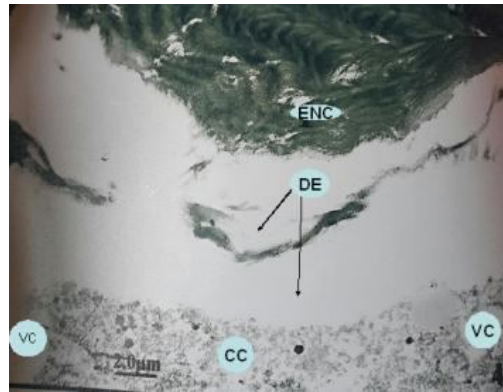


Fig. 9: (B) Treated hind-gut showing detached of intima layer and separation on their cuticle.

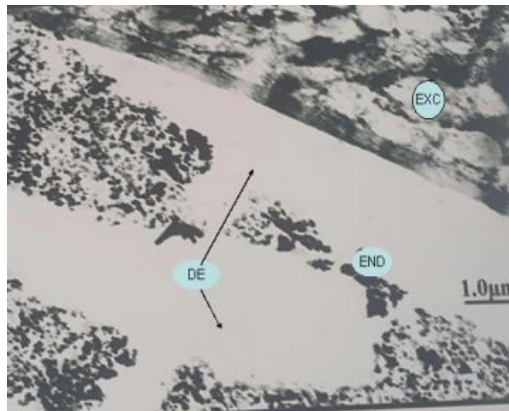


Fig. 9: (C) Damaged cuticle of hind-gut

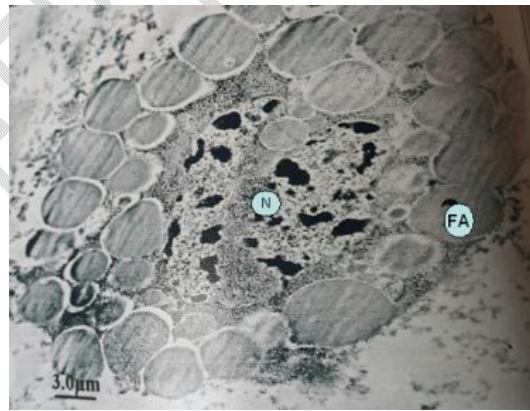


Fig. 10: (A) Normal fat body