Asian Journal of Biology

Volume 20, Issue 11, Page 25-36, 2024; Article no.AJOB.124648
ISSN: 2456-7124

Histological Investigations on Potato Tuber Moths, *Phthorimaea operculella* Treated with a Botanical Extract of Basil (*Ocimum basilicum* L), with Particular Attention to the Alterations in the Ultrastructure of the Larval Digestive Tract

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SAF and FSB recommended the studies concept, designed the experiments. Authors SAF, GMA and FSB wrote the manuscript, reviewed the records, and edited and permitted the manuscript, reviewed the information, managed figures. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajob/2024/v20i11446

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/124648

Original Research Article

Received: 05/08/2024
Accepted: 07/10/2024
Published: 14/10/2024

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Cite as: *MA, Gesraha, Sharaby AF, and Fallatah SB. 2024. "Histological Investigations on Potato Tuber Moths, Phthorimaea Operculella Treated With a Botanical Extract of Basil (Ocimum Basilicum L), With Particular Attention to the Alterations in the Ultrastructure of the Larval Digestive Tract". Asian Journal of Biology 20 (11):25-36. https://doi.org/10.9734/ajob/2024/v20i11446.*

ABSTRACT

Background: *Phthorimaea operculella* (Zeller), the potato tuber moth (PTM) is one of the most important pests that attacks potato, *Solanum tuberosum* L. crops worldwide. Adult moths attack tubers in fields as well as in storage. Infested tubers become unsuitable for trade or consumption, the tuber damage increases 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: Structural 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 malformations were detected throughout all the digestive tract assessment.

Conclusions: Studies of the occurred changes revealed some histopathological changes through out all the digestive tract (Fore-, Mid- and Hind-gut), the attached muscular layer, tracheal system, and visceral fat bodies of the larvae when fed with tubers treated with Basil extract. From this findings, the use of plant extracts can be recommended for protection of potato tuber against the infestation by the tubers moths.

Keywords: Phthorimaea operculella; botanical extracts; Ocimum basilicum; Solanum tuberosum; Spunta; digestive tract; Ultrastructure changes; scanning microscope; transmission electron microscope.

1. INTRODUCTION

Phthorimaea operculella Zeller, (Gelechiidae: Lepidoptera) is an extremely serious pest for potato crop. Rondon [1] reported that yield losses reached 100% because of infestation during storage and marketing. The tubers become unfit for sale or consumption after infestation, and 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 tool as a biopesticide against many insect pests. Studies have clearly shown that, there are several aromatic and medicinal plants which have harmful effects, *i.e.*, antifeedant, repellent, growth regulation impact and potential harmful action on a wide-ranging of insect pests [4-8].

Herein, the current research was 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 which 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±2ºC and 70±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 shade at ambient conditions. The air-dried material was ground into fine powders and kept in a tight glass container until further use. Exclusively prepared plant powders were extracted with 80% ethanol 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-mixed talcum powder/Kg of tubers until tubers were 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 (ca 20 larvae for both treated and control tubers). Larvae were taken for Ultrastructure investigations using the scanning electron microscope after Boult 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] using 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. Clear 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

The Fore-gut serves as a storage chamber and may be the site of preliminary digestion. In untreated larvae, it 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 directly towards the lumen (Fig. 1A) and surrounded by a muscular layer In treated larvae. Ultrastructure changes were detected by obvious damage and separation of the chitenous intima with their microspines from the basal epithelial cells, many vacuoles and holes were also observed (Figs. 1 B,C,D and 6-C).

3.3 The Mid-Gut

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 Peritrophic membrane, surrounding the lumen. Also in Fig. (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 disappearance of the cell boundaries, irregular in adhering to cell membrane, large vacuoles in cytoplasm and appearance of fat droplets in 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), shrinkage of the muscular fiber layers in some areas (Fig. 8 B) were observed as compared to the untreated larvae in control (Fig. 8 A). Severe destruction of the epithelium, and increased secretion by the goblet cells (Fig. 2C) resulted in their apical swelling into the gut lumen as a bulbous eversion (Fig. 2B).

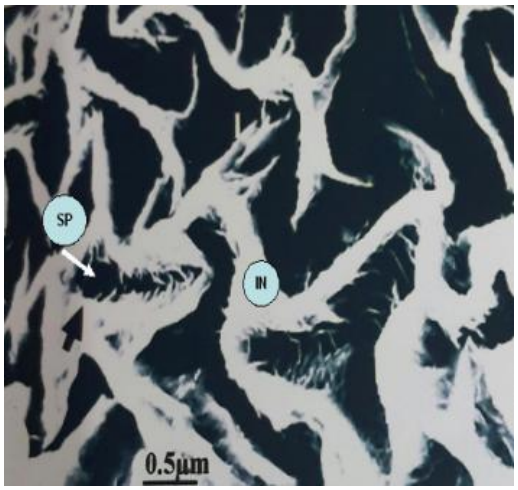

Fig. 1(A): Normal foregut

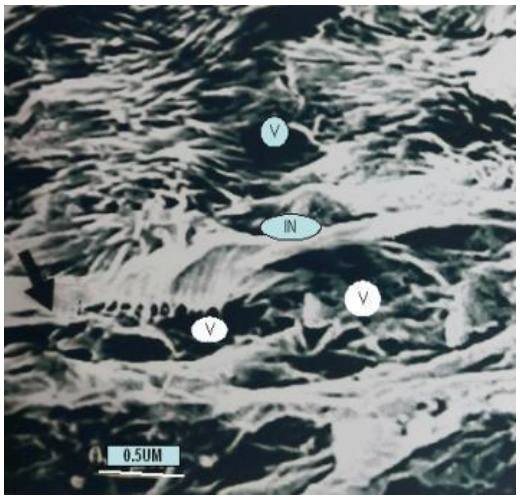

Fig. 1(B): Treated foregut

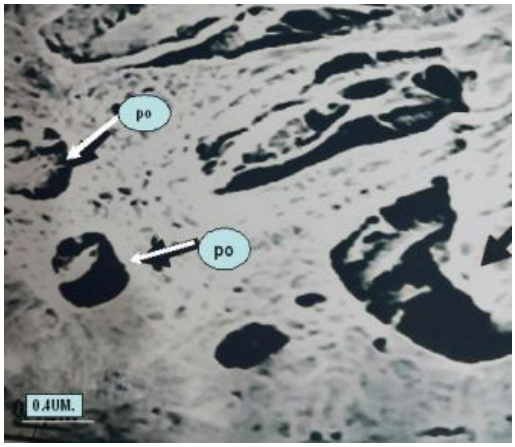

Fig. 1(C): Treated foregut

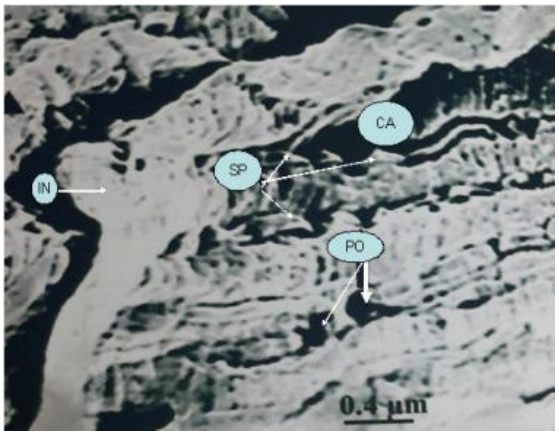

Fig. 1(D): Treated foregut

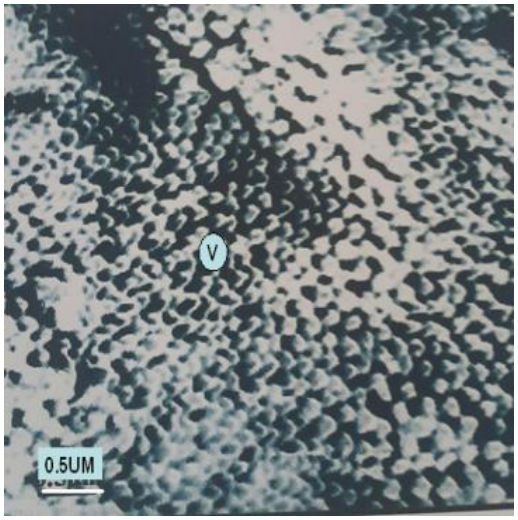

Fig. 2(A): Normal midgut showing intact microvilli

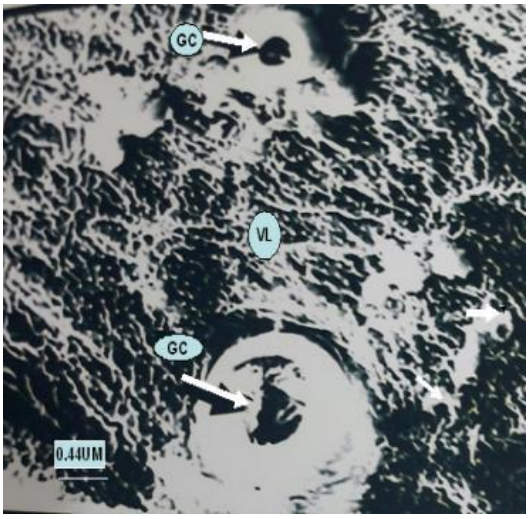

Fig. 2(B): Treated mid gut showing swollen goblet cavity and damaged villi

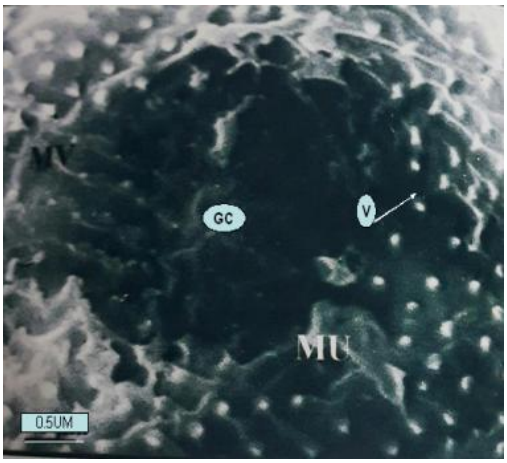

Fig. 2. (C): Treated mid gut showing mucous secretion covered villi surface

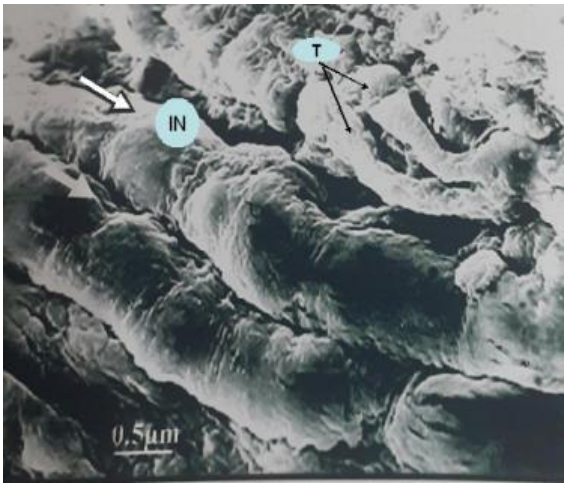

Fig. 3(A): Normal hind gut showing intima folds

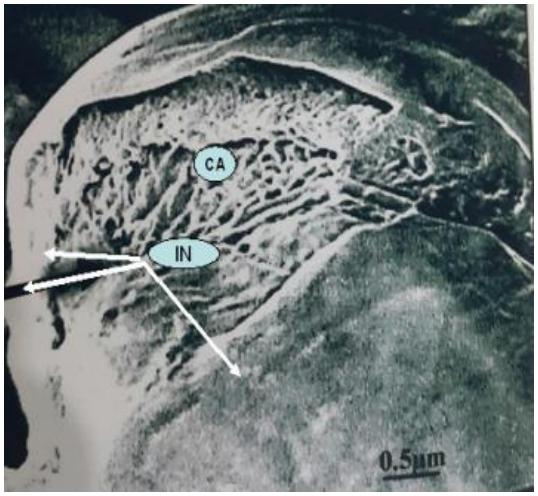

Fig. 3(B): Treated hind gut cleared shrinkage in intima layer

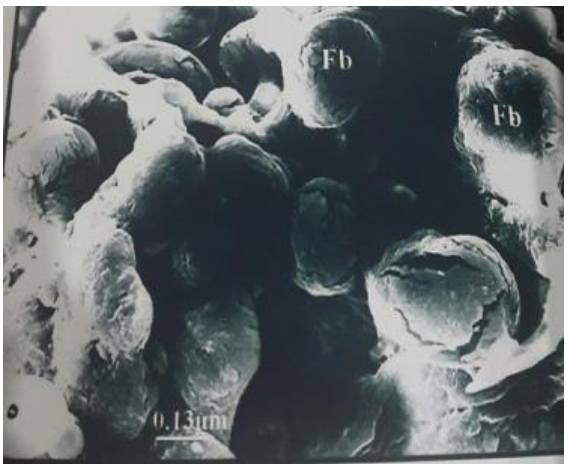

Fig. 4(A): Normal fat bodies

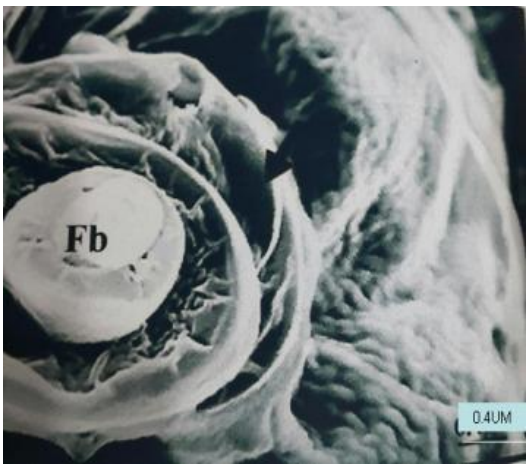

Fig. 4(B): Treated fat bodies

Fig. 5(A): Normal Trachea

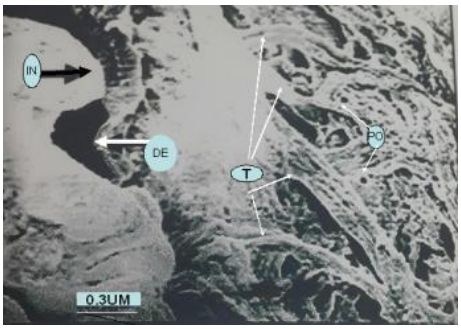

Fig. 5(B): Treated Trachea

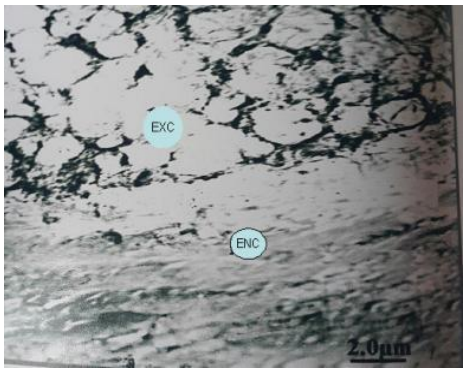

Fig. 6(A): Normal cuticle covered the fore gut intima of fore-gut

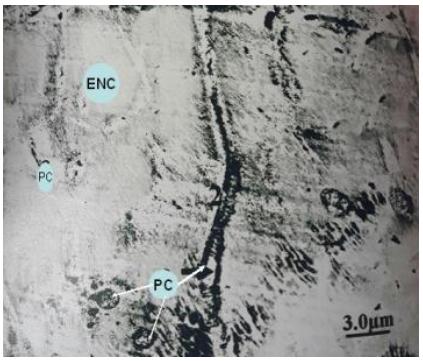

Fig. 6(B): Normal endocuticle of fore-gut intima

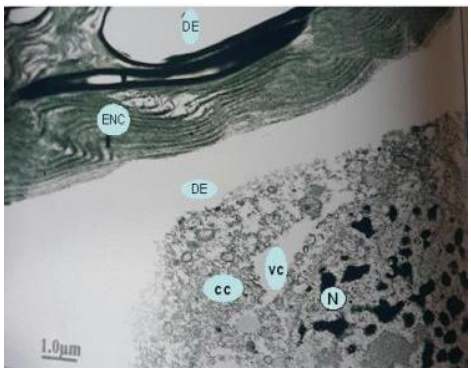

Fig. 6(C). Treated fore-gut showing separation of intima from the cells

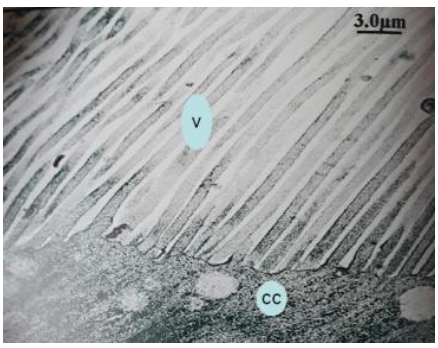

Fig. 7(A): Normal microvilli of mid-gut epithelial cells of normal larvae

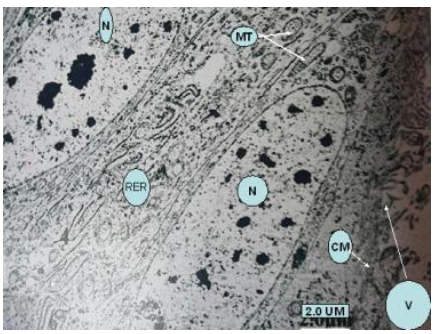

Fig. 7(B): Epithelial cells of mid-gut of untreated larvae

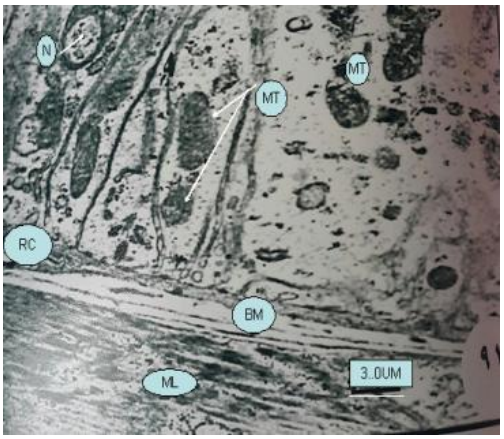

Fig. 7(C): Base of Epithelial cells of normal untreated larvae, showing intact basement membrane, muscles and mitochondria

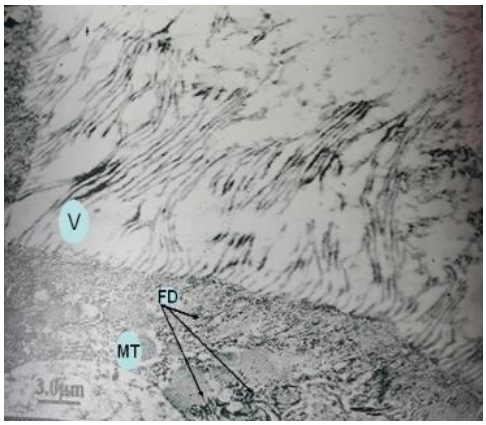

Fig. 7(D): Damaged villi on the apical portion of epithelial cells of mid gut of the treated larvae

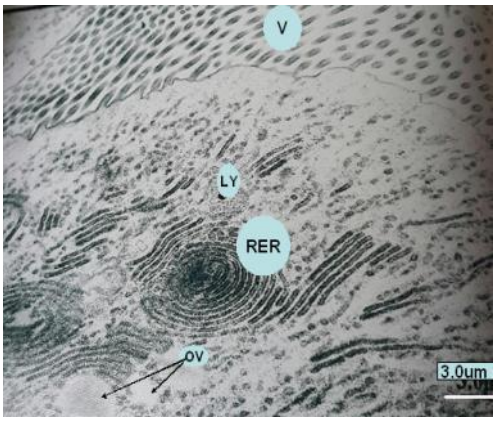

Fig. 7(E): Treated mid gut showing many vacuoles and lysosome

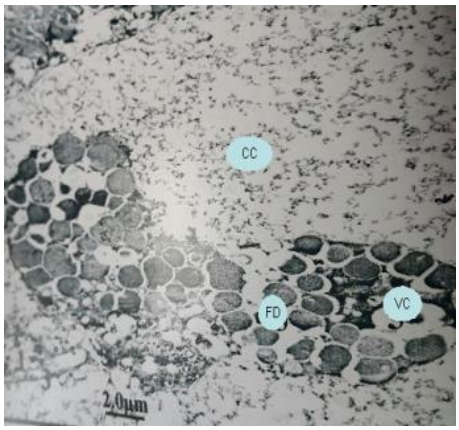

Fig. 7(F): Treated mid gut cells showing accumulation of fat droplets in the cytoplasm

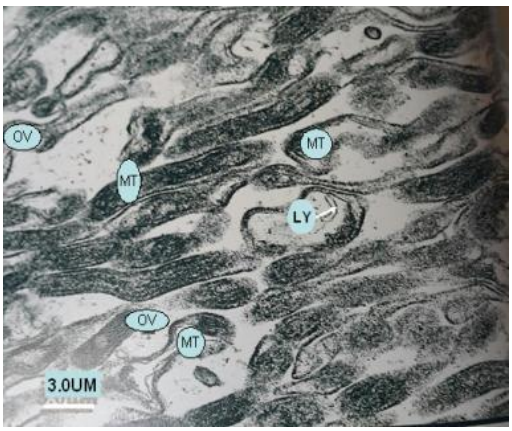

Fig. 7(G): Treated epithelial cells of the mid gut showing degenerated and elongated mitochondria

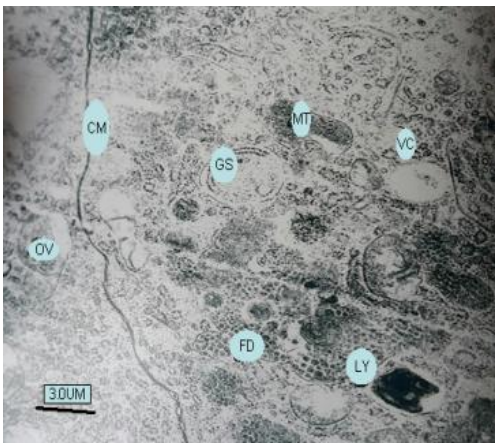

Fig. 7(H): Cytoplasm of epithelial cells of treated larvae

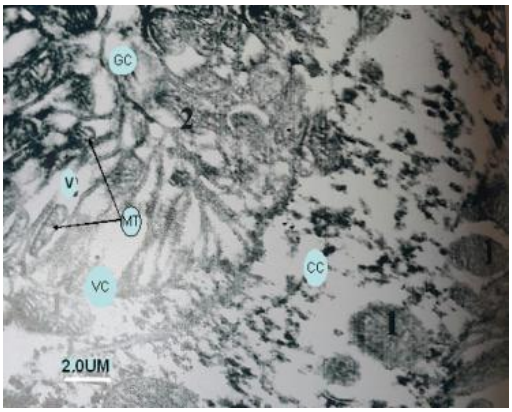

Fig. 7(I): Treated goblet cell, showing degenerated microvilli and mitochondria

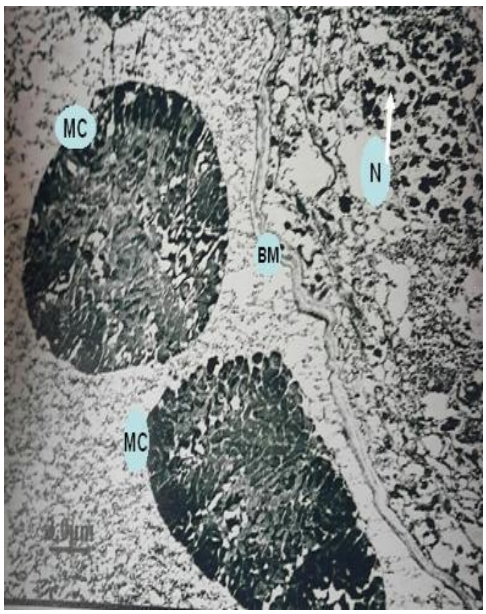

Fig. 8(A): Normal muscles of normal larva

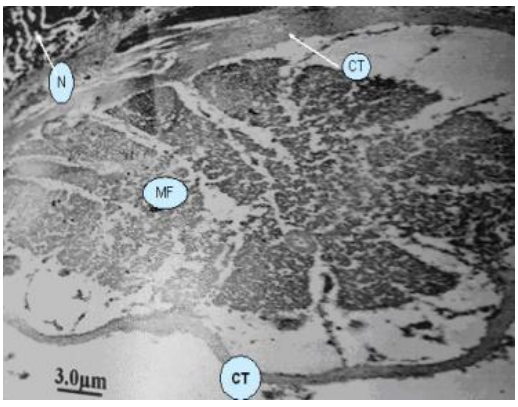

Fig. 8 (B): Damaged muscles surrounded the mid-gut of the treated larvae

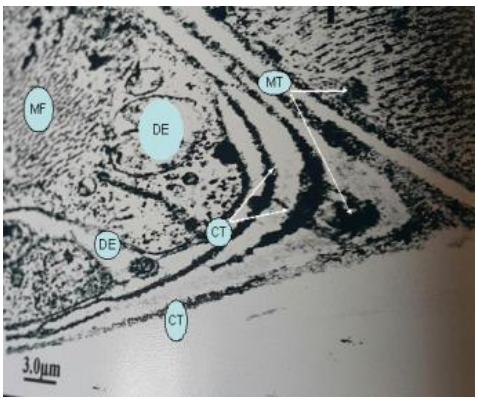

Fig. 8 (C): Damaged muscles surrounded the hind-gut of treated larvae

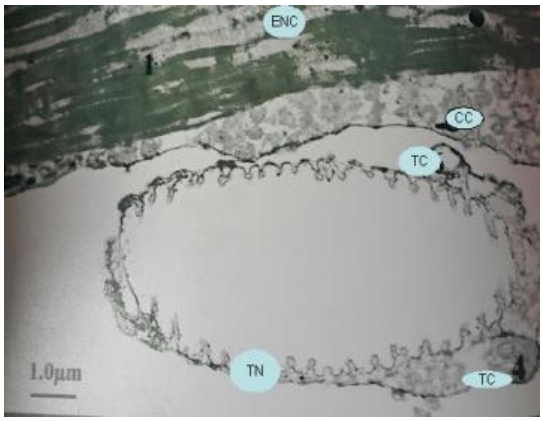

Fig. 9 (A): Normal endocuticle and trachea attached the hind-gut

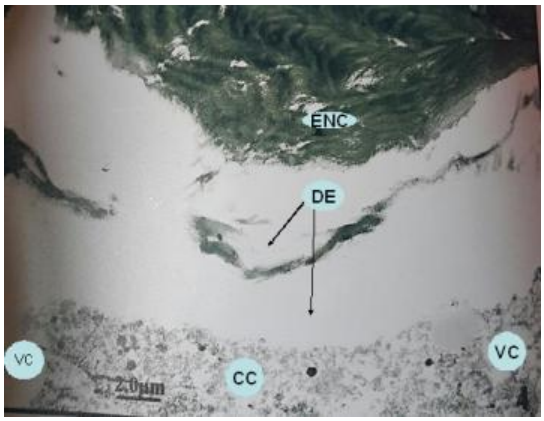

Fig. 9 (B): Treated hind-gut showing detached of intima layer and separation on their cuticle.

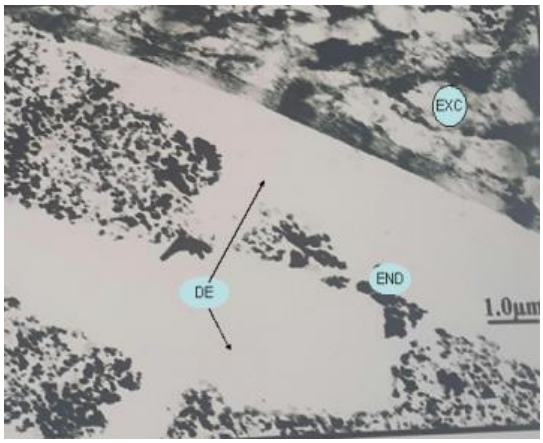

Fig. 9 (C): Damaged cuticle of hind-gut

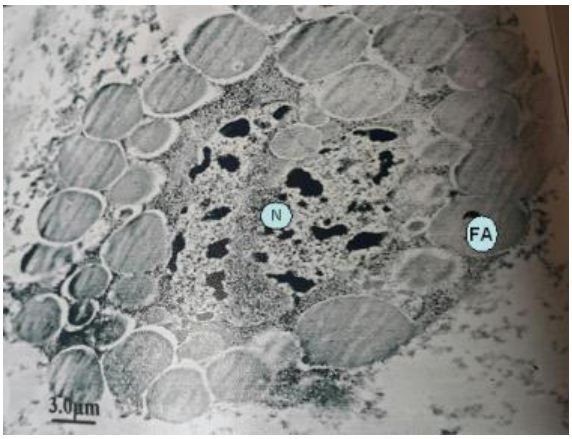

Fig. 10 (A): Normal fat body

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

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

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)*

3.4 Changes on the Hind-Gut

For water absorption and faecal formation, four distinct regions, pylorus, ileum, colon and rectum are shown. 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 muscles (Fig. 8A). Severe damage in the circular muscle layer in surrounded muscles (Fig. 8 C), separation of the intima layer (Fig. 3B, 9 B,C), extensive damage in the epithelial cell nucleus, vacuolization, shrinkage and rupture in the basal basement membrane were observed in treated larvae (Fig. 8B). Clear lyses of fiber muscles induced 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 distinctive 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 and annihilation of the membranous sheath (Fig. 4B, 10A). The destruction of the cell-structure was 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. [14] uncovered a cytotoxic impact of *Certum perqui* saponins on the fat group of *S. littoralis* larvae.

3.6 Changes on Muscles

Distinctive 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). Distinctive 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 separate the muscles into little parts are ascribed to the devastation of the sarcolemma, halfway mix and break of the muscle fibers 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 contains edge-like circumferential circles (taenidia) that breeze spirally through the wall membranous to forestall its breakdown and keep 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 and shrinkage of the tracheal lumen. The ventilation cycle conveying the oxygen development from the environment up the windpipe tissues and tracheoles through the insect's body and the entry of carbon dioxide in the opposite path [13], harmed windpipe prompting disturbance in insect respiration, likewise damages of the joined muscles disturbs the ventilation cycle. Disturbance in the respiration affects all natural cycles in the insect's body. Past perceptions appeared that 80% ethanol extract 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 respiration processing 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 imbalance 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 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 [15] and Zibaee & Bandani [16]. This outcome is in accord with Sharaby et al. [17] who observed severe effects in the alimentary duct and fat bodies of *Heteracris littoralis* first nymphal instar after treated with garlic, mint and eucalyptus essential oils at the LC_{50} concentration. The current studies showed 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. [18]. The puffy and elongated protrudes of villi into the lumen as a bulbous eversion were the results of enzymatic activities of the epithelial cells. These results are in agreement with the findings of Sutter & Raun [19] and Shoukry et al. [20].

The changes in the hind-gut observations were agreed with that mentioned by Sharaby et al. [17] on the grasshopper, *Heteracris littoralis*.

All changes on the fat body were matched with Chaieb et al. [14] 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. [17], Ranjini & Nambair [21] and Mohamed et al. [22].

Results of the changes on the trachea were in accord with those reported by Corbet et al. [23] for their work with Eucalyptus essential oils as mosquito larvicides. They reported that, the mosquito larvae were susceptible to surface materials entering through their tracheal system, and observed that essential oils of *Eucalyptus* sp. increased 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 provided a complete protection for the treated potato tubers from the invasion of the potato tuber moth throughout the storage period. Unexpected consequences have been found in the development and metamorphosis of the treated 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, easily available and environmental friendly for the control of this serious lepidopteran and many other pests either in open fields or in storage, which affect production, marketing, and income. Starting here from this point of view, it could be recommended the use of the aforementioned plant extract for its highly protection to the tubers and as oviposition inhibitors against insect pests because of its deterrent impact.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/124648