



## Histological Studies on *Heteracris littoralis* (Rambur) Treated with Silica Nano-particles / Challenger Formulation

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### Authors' contributions

This work was carried out in collaboration between all authors. Author MAG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AFS and ARE managed the analyses of the study. Author MAG managed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

**Aim:** Local diatomaceous earth (Silica nano-particles) and commercial pesticide (Challenger 36% SC) formulation was tested against alfalfa grasshopper, *Heteracris littoralis* (Rambur, 1838) (Orthoptera: Acrididae) under laboratory conditions.

**Study Design:** Histological study for alimentary tract using light microscope.

**Place and Duration of Study:** Department of Pests and Plant Protection, National Research Centre, Egypt- 2016-2017.

**Methodology:** Fourth instar nymphs of *H. littoralis* were taken from laboratory culture reared on semi-artificial diet [1] for the experiments. The nymphs were fed on diet mixed with 1% concentration of Silica nano-particles/Challenger formulation. The tissue specimens of the alimentary canal were dissected in 0.9% NaCl solution and fixed in Bouin's solution for 24 hours [2] then dehydrated in ascending alcoholic series and cleared in Xylen for few seconds, and then specimens were infiltrated in three changes of paraffin wax each lasted 20 minutes. With Ehrlich's acid haematoxylin and alcoholic eosin. The stained sections were dehydrated, cleared and mounted using D.P.X. For microscope examination.

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**Results:** The Silica nano-particles induced great irregularity or deformity in the alimentary tract, that leading finally to death. All parts of the alimentary tract were affected by silica treatment (fore-gut, mid-gut and associated gastric caeca and hind-gut).

**Conclusion:** Histological changes were observed as a result of Silica effect on the enzymes and the physiology of the treated insect. So, it could be concluded that Silica nano-particles may be used through the integrated pest management programme against *H. littoralis* by spray on the plants or as a food bait traps.

*Keywords: Silica nano-particles; grasshopper; Heteracris littoralis; histological study.*

## 1. INTRODUCTION

Grasshoppers are cosmopolitan destructive insect pest, it destroys crops else where yearly, during outbreaks they cause losses totalling millions of pounds. It belongs to family Acrididae that includes more than 100 species; most of them are existing in almost all Egyptian Governorates. This pest causes great injuries to most crops, especially Egyptian clover plants (*Trifolium alexanderinum* L.) which was considered the main fodder for most livestock animals in Egypt. The control of grasshoppers was studied by many authors [3,4,5,6,7].

Chemical insecticides are still the main weapon against grasshoppers, especially during their outbreaks. However, pest damage could also be reduced through an expert management of the crop nutrient requirements or alteration with mineral nutrients that reduces crop susceptibility to pest's infestation [4]. These insect pests such as the stem-borer, different plant-hoppers and other pests such as spider mites [5].

Diatomaceous Earth causes small abrasions on the cuticle of any insect pest that contact its powder [8]. The pest gradually loses its body fluids, dehydrates then dies. Silica as a food baits, the silica crystals abrade their digestive tract and finally lead to the death of the insect. It may take a few days to eliminate pests after pesticide application. They also concluded that the Silica leads to increase abrasiveness of foliage, so deterring insect feeding by emanating the volatile odours of the plant.

The aim of the present search was to observe the structure changes in the tissues of the alimentary tract of grasshopper, *H. littoralis* following treatment with Silica nano-particles/Challenger formulation.

## 2. MATERIALS AND METHODS

Fourth instar nymphs (3 replicates each comprised 15 individuals) of *H. littoralis* were

taken from laboratory culture reared on semi-artificial diet [1] for the experiments. The nymphs were fed for three days on diet mixed with 1% concentration of Silica nano-particles/challenger formulation, which was obtained from Refractories, Ceramics and Building Materials Department, National Research Centre, Giza, Egypt, and then fed on normal diet. The formulation of Silica Nano-326 particles/Challenger (Silica Nano-particles was served as a carrier for Challenger) were applied. Challenger 36% SC is a commercial formulation of chlorfenapyr pesticide, manufactured by BASF - The Chemical Company, New Jersey, USA. Untreated diet was used for the nymphs as a control (20 individuals). Treated and untreated nymphs were examined daily. After 12 days, moribund nymphs which were near death chosen, dissected and extracted the alimentary canal for histological examination. All treated nymphs were died within 15-19 days post treatment.

The tissue specimens of the alimentary canal were dissected in 0.9% NaCl solution and fixed in Bouin's solution for 24 hours [2] then dehydrated in ascending alcoholic series and cleared in Xylen for few seconds, and then specimens were infiltrated in three changes of paraffin wax each lasted 20 minutes. With Ehrlich's acid haematoxylin and alcoholic eosin. The stained sections were dehydrated, cleared and mounted using D.P.X. for microscope examination.

## 3. RESULTS AND DISCUSSION

It is known that the alimentary tract is constructed of three main parts, Fore-gut, Mid-gut and Hind-gut.

### 3.1 Normal Fore-gut

The cavity which called (pharynx) it continues as oesophagus then enlarges into a crop which are thin-walled of cells rested on a basement membrane and externally surrounded by

muscular layers (Fig. 1A and C). The entire fore-gut is lined with chitin. The crop opens into short muscular organs which called the gizzard. At the joint of the gizzard-stomach there are 6 pairs of gastric caeca. These are small bag like structure arranged in a ring-like shape around the anterior end of the stomach (Fig. 1C).

### 3.2 The Treated Fore-gut

Fig. 1B and D show the layer of epithelial cells of the fore-gut, which was completely destroyed and ruptured, with vacuolated cytoplasm and large area of necrosis were noticed, then separated from the basement membrane.

### 3.3 Normal Mid-gut

Fig. 2A and B consists entirely of stomach or ventriculus. The mid-gut is not lined by chitin layer, but lined with the peritrophic membrane. This membrane protect the stomach from

abrasions. The mid-gut cells consist of three kinds of epithelial cells they are columnar, goblet and regenerative cells. The inner surface of the columnar and goblet cells lined by microvilli. All cells were rested on basement membrane and surrounded externally by muscular layers.

The gastric caeca cleared (Fig. 1D) histological abnormalities, there were shrinkage, showing decreasing in the lumen surface accompanied with epithelial bursting and epithelial folds. Destruction happened in the muscular layer surrounding the caeca. Fat cells appeared small in size, dark color and loss their structure. Bursting of some fat tissue membrane and release of the cellular contents.

Our results was in accordance with that reported by [9], on their work on *Spodoptera littoralis* and *Locusta migratoria migratoria* to evaluate the oral toxicity of the gibberellic acid - a plant growth regulator - as a control agent.

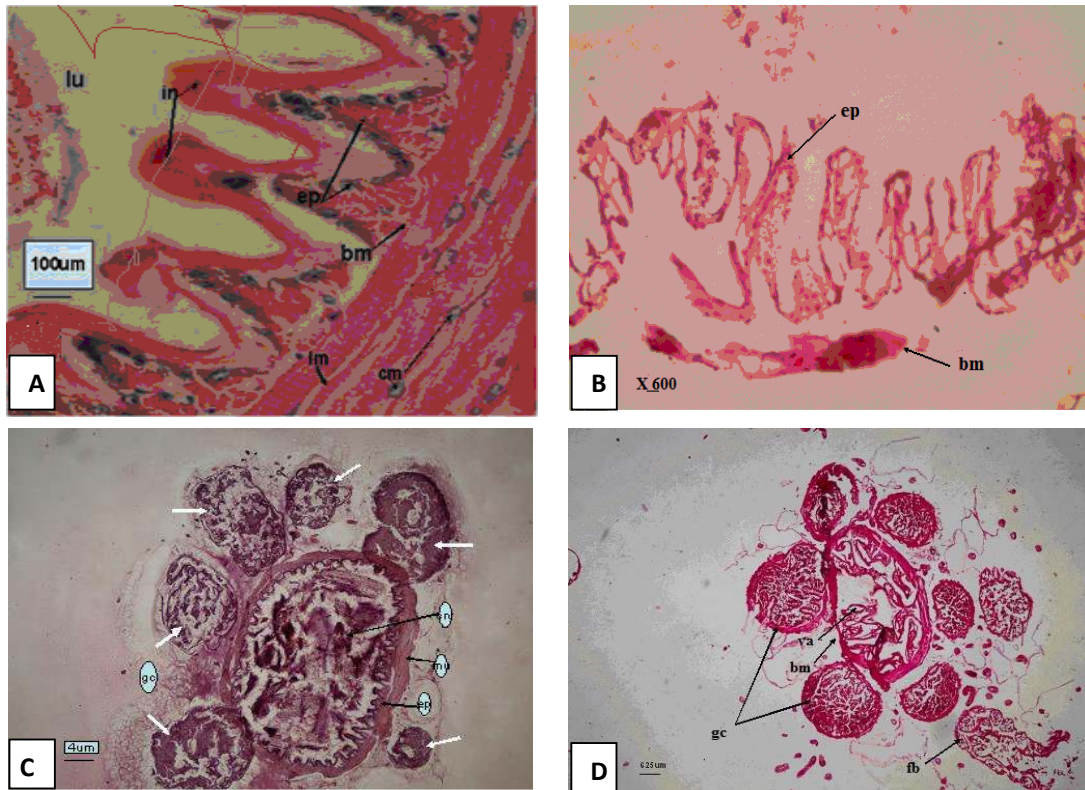


Fig. 1. (A) Normal foregut cells, (B) Treated foregut cells, (C) T.S. through normal crop and the 6 gastric caecae white arrows, (D) T.S. treated foregut and gastric caeca

### 3.4 Treated Midgut (Fig. 3A and B)

Degeneration of most of epithelial cells, breakdown of some and separation from each other, large vacuole were noticed in the cells, hypertrophy and hyperplasia were observed too and fading of cell boundaries.

This result was agreed with that mentioned by [6,7] on their work using natural oils and some plant extracts on *H. littoralis* as control agents, and also matched with that reported by [10] on their study on compounds containing silicon to control *Tuta absoluta* larvae in Brazil. Also, these results were in accordance with that reported by [11] on their work on *Helicoverpa armigera* Hübner using commercial Neen formulation (PONNEEM).

### 3.5 Hind-gut

It is a coiled section comprised three main parts: the anterior part (the ileum), the middle part (the colon), and the posterior part (the rectum). This last part (the rectum) opens exteriorly through the anus. The hindgut consists of a big layer of epithelial cells lined with cuticle, rested on basement membrane and was surrounded by a thick muscular layer (Fig. 4A).

In the treated hindgut, partial changes was observed including the intima layer that lined the cells which were affected to great extents, a partial abortion occurred, also degeneration of small number of their cell (Fig. 4B). These remarks are to be brought closer the observation on *Schistocerca* carried out by [12].

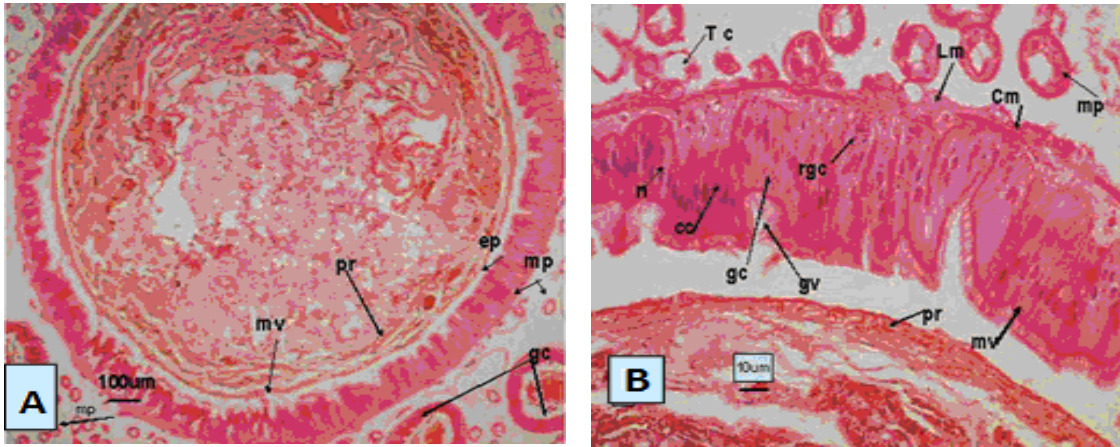


Fig. 2. Normal Midgut; (A): T.S. In control midgut of *H.littoralis* nymph. (B) T.S. In the control midgut of *H.littoralis* grasshopper nymph (magnification from A)

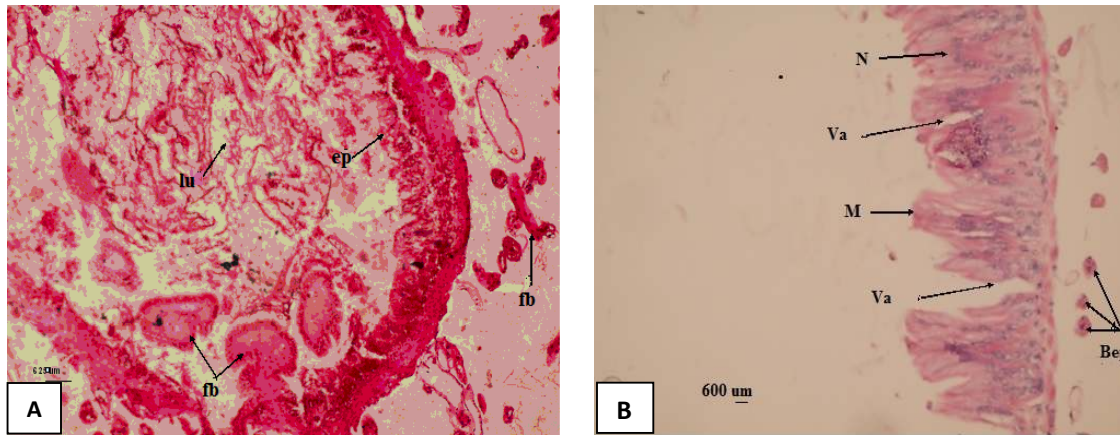
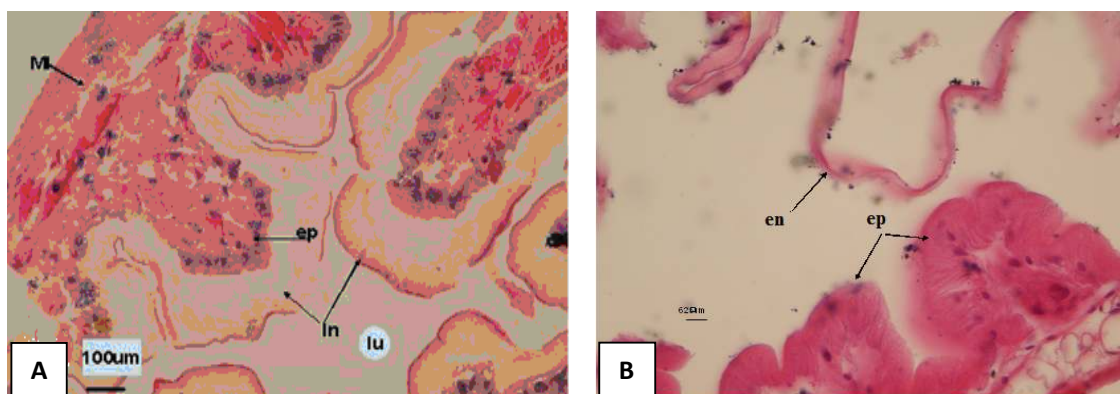


Fig. 3. (A) Treated midgut, (B) Magnified portion from (A)



**Fig. 4. (A) Normal hindgut, (B) Treated hindgut of *H. littoralis* nymph**

Abbreviations: Lumen (Lu) - Intima (In) - Epithelial cell (ep) - Basement membrane (bm) - Longitudinal muscle (Lm) - Circular muscle (cm) - Gastric caeca (gc) - Peritrophic membrane (pr) - Microvilli (mv) - Malpighian tubule (mp) - Trachea (tc)- Nucleus (n) - Regenerative cell (rgc) - Goblet cavity (go) - Fat body (fb) - Vacule (va) - Muscular layer (mc)

All the present harm changes that observed in almost all parts of the alimentary tract of *H. littoralis* led to interruption in the digestive system and their enzymes and metabolism process, this causes unbalance in the insect physiology, which influence the growth and the development of the treated insects and leading finally to the death.

From the previous mentioned results it could be concluded that Silica nano-particles may be used through the integrated pest management programme against *H. littoralis* by spray on the plants or as a food bait traps.

#### 4. CONCLUSION

The treated foregut was completely destroyed and ruptured, with vacuolated cytoplasm and large area of necrosis were noticed, and then separated from the basement membrane. The treated midgut showed degeneration of most epithelial cells, breakdown of some and separation from each other, large vacuole were noticed in the cells, hypertrophy and hyperplasia were observed too and fading of cell boundaries. The treated hindgut showed partial changes including the intima layer that lined the cells which were affected to great extents, a partial abortion occurred, also degeneration of small number of their cell.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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