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Histoarchitectural alteration in midgut of *Dysdercus koenigii* (Fabricius) (Hemiptera: Pyrrhocoridae) treated with chlorpyrifos

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Abstract
Dysdercus koenigii is a species of true bug in the family Pyrrhocoridae. It is a serious pest of cotton crops. This study aimed to investigate the midgut of adult *D. koenigii* at the ultrastructural level using light microscopy. Insecticide chlorpyrifos of the concentration 0.0002% and 0.0008% were provided in food to adult bugs to examine the alteration from normal histology of midgut. This study indicated that midgut was highly affected after 24h post-treatment and the intensity of the histopathological effects was dependent on the concentration used. On applying insecticide, deformation in the shape of midgut was seen. Midgut gets more deformed in 0.0008% as compared to 0.0002% concentration. In midgut, epithelial layer gets detached from muscular layer and this detachment was more pronounced at higher concentration. Degeneration was observed in basement membrane. The results suggest that chlorpyrifos could be used as an effective insecticide for the control of *D. koenigii.*

Keywords: *Dysdercus koenigii*, midgut, chlorpyrifos

Introduction
D. koenigii is distributed in Pakistan, South-eastern Asia and various states of India *viz*., Gujarat, Uttar Pradesh, Bihar, Madhya Pradesh and Tamil Nadu. *D. koenigii* feeds on okra, maize, and pearl millet etc. The adult *D. koenigii* are brick-red in colour with their antennae and thoracic scutellum dark-coloured, abdominal sterna white-banded and terga uniformly red. The nymphs moult five times as they grow. The adult has a laboratory life-span of 30-35 days. The most serious disease caused by this bug is internal boll disease. The adults and older nymphs feed on the emerging bolls and the cotton seeds as they mature and they transmit cotton staining fungi *Nematospora gossypii* that develops on immature lint and seed [1]. Chlorpyrifos inhibits the activity of enzyme acetylcholinesterase and targets on the nervous system of the insects [2, 3]. It is considered moderately hazardous to humans by the World Health Organization. As a consequence, Chlorpyrifos has been accounted for as one of the commonly utilized organophosphate pesticide [4, 5]. It is an organophosphate insecticide utilized both in farming and household pest control agents because of its non-carcinogenic and non-teratogenic nature [6]. Chlorpyrifos has been accounted for as one of the commonly utilized organophosphate pesticide [5]. Ultrastructure studies on the gut of bugs have been performed by many scientists. These include milkweed bug *Spilostethus pandurus* [7], *Graphosoma lineatum* [8], *Cimex lectularius* and *C. pipistrelle* [9], adult female of *Rhodnius prolixus* [10]. Present study investigates histopathological effect of insecticide chloropyrifos on the midgut of red cotton stainer, *D. koenigii.*

Material and Methods
The adults and nymphs of *D. koenigii* were collected from the okra field located near the Aligarh Muslim University campus and brought to the laboratory for the research work. These insects were kept in glass rearing jars containing a thick layer of damp sterilized sand at the bottom and the jars were maintained at 28±2°C temperature and 70±5% relative humidity. The insects were fed on overnight soaked cotton seeds, which were changed on alternate days. Over-crowding was avoided for proper culture of the insect. 10-12 replicates per batch for a concentration were studied at a time.

Sampling of Experimental Insects

For the project work adults were maintained in a separate jar. Then they were treated with different concentrations of chlorpyrifos. 2 ml of each concentration (0·0002% and 0·0008%) was given in feed (cotton seeds). Then the treated insects were kept under observation.

Application of Insecticide

Cotton bugs were kept in a jar containing 2 cm thick layer of sterilized sandy soil at the bottom of the jar. This soil layer was kept moist to provide the suitable environment for cotton bugs. Cotton bugs were fed with cotton seeds infused with 2 ml of insecticide placed in a Petri dish within the glass jar. Finally, glass jar was covered with muslin cloth.

10 cotton bugs were exposed to insecticide (chlorpyrifos) in two setups each *i.e.*, one set containing feed with 0.0008% conc. and second setup with 0.0002% concentration. In addition to this one controlled setup was also run.

Preservation and histological preparation

The cotton bugs were dissected after 24 hours of examination. The midgut was dissected out from cotton bugs and was immediately fixed in Bouin's solution for 18-20 hours.

After this dehydration proceeded in ascending grades of alcohol i.e., 30%, 50%, 70%, 80%, 90% for 5 minutes each while in 96% and 100% for half an hour each followed by mixture of 100% and xylene solution (1:1) for 10 minutes than incubating the tissue in xylene and paraffin (1:1) at 60° C for 15 minutes then incubate tissue in pure wax for 2 hours. The midgut was embedded in paraffin wax in the shape of cubes. After a day 5 µm sections were cut using microtome from each prepared block. The ribbons were then placed on the glass slide lubricated with a solution of albumin and glycerin (1:1). The slides containing section were warmed slightly to straighten the creases. The slides were processed in 2 changes of xylene and then descending grades of alcohol series 100%, 96%, 90%, 80%, 70%, 50%, 30% for 5 minutes each then in distilled water for 5 minutes. Slides were stained in Delafield's haematoxylin for 10 seconds and then kept in tap water and distilled water for 5 minutes each and counterstained with Eosin for 20 minutes followed by upgrade dehydration of alcohol and then 2 changes of xylene for 5 minutes each. After air drying, slides were mounted in DPX and observed under microscope. Photomicrograph were taken using Nikon Eclipse Ci.

Result and Discussion

Normal histology of midgut of *D. koenigii* (Fig. 1&2)

The gut is subdivided into three functional regions: stomodeum, mesenteron and proctodeum. Midgut or mesenteron has no cuticular lining. The oesophagus leads into a swollen first region of midgut. The posterior region of midgut, which connects with hindgut, is relatively short and bears a number of small caecae. The arrangement of the four malpighian tubules in *Dysdercus* is unusual in that the two tubules on each side of the animal are connected to each other at their distal ends to form a continuous loop.

In midgut, only a single layer of epithelial cells is noticed. Epithelial cells rest on a basement membrane. The external surface of midgut is lined by an inner circular and an outer longitudinal muscle layer. The epithelial layer is fitted firmly together along the lumen cavity. The lumen is wide and columnar epithelial cells are elongated and uniform. Epithelial cells possess well defined nucleus. The Circular muscle layer consists of one strand and the longitudinal muscles are also few and scattered. Both the muscle layers are well developed with a very thin margin compared to size of cells.

Histopathological effects of chlorpyriphos on midgut of *D. koenigii* (Fig. 3-6)

At 0.0002% concentration (Fig. 3&4), treated midgut gets deformed in shape and this deformation become greater on increasing the concentration i.e., 0.0008% (Fig. 5&6). Similar changes were reported in *P. americana* treated with N-nitroso-N-methylurea [11], *Schistocerca gregaria* treated with fenitrothion [12]. Degeneration is observed in basement membrane at lower concentration but basement membrane is feebly visible at higher concentration. Similar findings were previously mentioned in *S. exi*gua larvae treated with diflubenzuron, malathion and cypermethrin [13], in *Blatella germanica* treated with acetylcholinesterase [14], in *Synthesiomyia nudiseta* treated with the volatile oils of *Cupressus macrocarpa* and *Alpinia officinarum* [15].

At 0.0002% concentration, circular and longitudinal muscles became narrower and fragile but can be distinguished, whereas at 0.0008% concentration, circular and longitudinal muscles cannot be distinguished. Similar histopathological changes were reported in *Spodoptera littura* treated with organophosphate insecticides [16], in *Oxya nitidula* treated with monochrotophos [17], in *Chrysomya megacephala* treated with deltamethrin and chlorpyrifos [18, 19].

Detachment of the epithelial layer from muscular layer is more pronounced at higher concentration in comparison to the lower concentration. Similar observations were reported in *Culex pipiens* treated with various fractions of *Artemisia judaica* and *Anagallis arvensis* mixed with water [20], in the midgut of *Spodoptera exigua* with action of diflubenzuron, malathion and cypermethrin [13], in *Helicoverpa armigera* fed with leaf extract of plant *Lantana camara* [21], in *Rhynchophorus ferrugineus* treated with zinc sulfate [22].

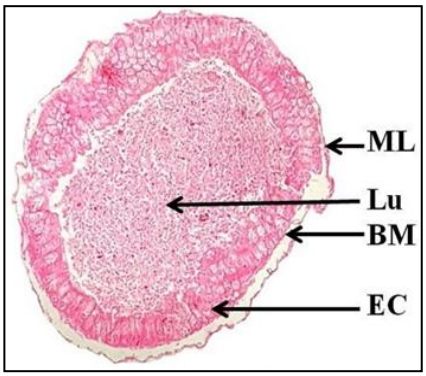

Fig 1: T.S. of midgut of *D. koenigii:* Control (10X)

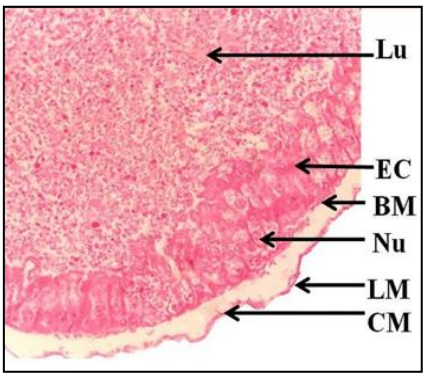

Fig 2: T.S. of midgut *koenigii*: Control (40X)

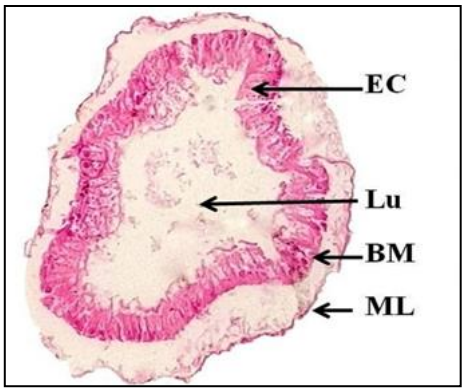

Fig 3: T.S. of midgut of *D. koenigii* treated with 0.0002% chlorpyrifos (10X)

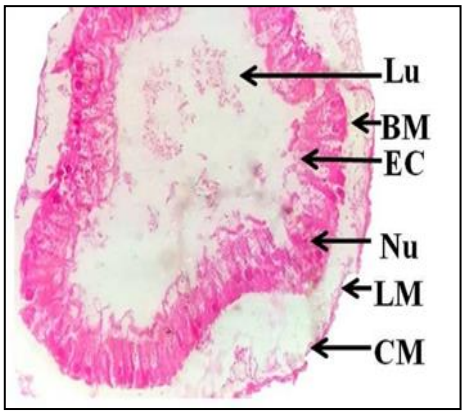

Fig 4: T.S. of midgut of *D. koenigii* treated with 0.0002% chlorpyrifos (40X)

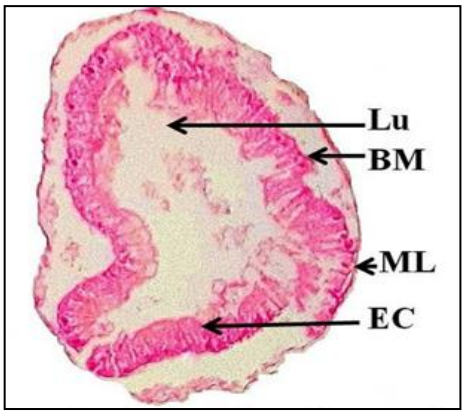

Fig. 5: T.S. of midgut of *D. koenigii* treated with 0.0008% chlorpyrifos (10X)

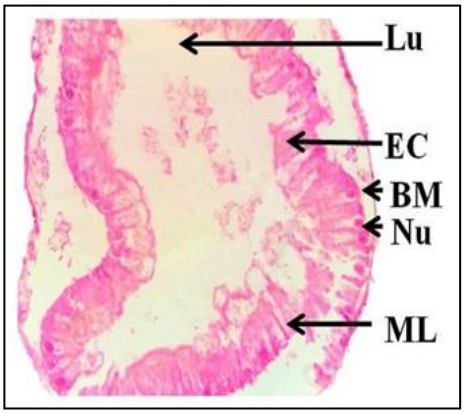

Fig 6: T.S. of midgut of *D. koenigii treated with* 0.0008% chlorpyrifos (40X)

Abbreviations: BM. Basement membrane: CM. Circular muscles: EC- Epithelial cells: LM- Longitudinal muslces, Lu- Gut lumen; Nu- Nucleus

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