

ORIGINAL ARTICLE

Pyrethroid Pesticides Induced Impairments in Midgut Histo-Architecture of Naiad of *Trithemis aurora* (Burm.) Dragonfly (Odonata : Libellulidae)

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ABSTRACT

The midgut of last instar naiad of *Trithemis aurora* (Burm.) was exposed to LC_{50} concentrations 2.69×10^{-5} and 2.50×10^{-3} ppm. of cypermethrin and deltamethrin pesticides respectively for 40 hrs, exhibited positive histo-pathological derangements in various midgut tissues. High accumulation of cell contents was seen at apical ends of epithelial cells. The displacement of nuclei was observed and nuclear membrane was found damaged. The circular muscles become contracted and longitudinal muscle bundles remained damaged under cypermethrin constrain. The deltamethrin reacted with epithelial folds, separating their latero-apical ends for more pesticidal activity. The intercellular boundaries of epithelial cells were perfectly damaged at the basal portion of epithelial folds. The peritrophic membrane was damaged. The circular and longitudinal muscles were affected by the pesticide.

Key words : *Trithemis aurora*, pyrethroid, naiads, nidi, mesenteron, odonata, cypermethrin, deltamethrin.

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INTRODUCTION

The odonates are the amphibious, most conspicuous, predaceous insect group, could be sighted in large numbers hovering over the water bodies particularly in sunny days during the rainy season. These insects have remarkable sub-equal two pairs of translucent net veined wings and large compound eyes. Their developmental period spend in fresh water moats. The larval phases are also predaceous. They are considered promising organisms in the water pollution monitoring due to their sensitivity to the toxicants. Being predaceous in habit these are equipped with powerful grasping labium, together with other gnathal appendages like labrum, mandibles and maxillae for effective hunting and manipulation of their prey.

The premature stages of dragonfly, *Trithemis aurora* are aquatic and carnivorous in nature have been placed on the top of the food chain in fresh water ecosystem. These premature stages, called naiads, check the population of mosquito larvae and other harmful insects in aquatic bodies. For more production in agriculture sector today farmers use pyrethroid pesticides to control the population of insect pests to protect food grains, pulses, vegetables and fruit plants, from insect pests. These toxic pesticides enter the aquatic ecosystem either through the runoff or through rain water and pose serious threat to the aquatic organisms including dragonfly naiads. The present pesticides cypermethrin and deltamethrin were observed to reveal the histopathological derangement in midgut tissues of last instar naiad of *T. aurora* after continuous exposure upto 40hrs. under LC_{50} constrain 2.69×10^{-5} and 2.50×10^{-3} ppm. respectively. The midgut region, where various digestive and absorptive activities take place was prone to these pesticides.

In different tissues of nymph *Tetragonfugia Sp.* accumulation of DDT [1]; toxicity level of organo-chlorine insecticides to pre adult stage of *Brachythemis contaminata* (Fabr). [2]; effect of Metasystox on the digestive system of nymph of *Brachythemis contaminata* (Fabr.); toxicology and safety evaluation of carbaryl to the last nymph of *Brachythemis contaminata* (Fabr.); effectiveness of sumithion on the rectal

gill basket of nymph *Brachythemis contaminata* (Fabr.) Shukla *et al.*, [3]; metabolism of pesticides and its accumulation in different tissues of larvae of *Tholymis tillagra* [4] have been established. The effect of organophosphate like, Folithion, Ekalux, Lebaycid, Demecron, Malathion and chlorinated hydrocarbons such as BHC, DDT has been studied on anisopteran nymph and aquatic hemipteran. The folithion reported to be more toxic to both the insects, while malathion and DDT were the least toxic to *Sphactodema rusticum* (Fabr.) and anisopteran naiads, respectively [5].

Bhardwaj and Tyagi [6] have observed loss of ability to fly, acute dorsoventral convulsions, forward forewing deflection and clockwise cyclic rotation in adult *Pantala flavescens* (Fabr.) when exposed to various concentrations of Malathion.

Andries [7] has established the occurrence of microvilli in the mesenteron epithelium of larva of *Aeschna cyanea* (Muller). Differentiation of cellular characteristics in metamorphosing mesenteron of *Aeschna cyanea* (Muller) has been elucidated by Andries [8]. Bhardwaj and Tyagi [9] have mentioned ultrastructural details of mesenteron of *Pantala flavescens* (Fabr.)

Cypermethrin and deltamethrin are substantially more resistant to degradation by Light and air, thus making them suitable for use in agriculture but they have significantly higher mammalian toxicities.

MATERIAL AND METHOD

The last instar naiads were collected through a small net having nylon cloth cone (mesh size 40-80 cm²) from benthic littoral zone of fresh water pond. These visually healthy naiads were released into aquaria. The *Daphnia sp.*, *cyclops sp.*, *Paramecium sp.*, and mosquito larvae were provided them as food *ad libitum*. For histological studies, naiads in control and treated with LC₅₀ constrain for continuously 40 Hrs. exposure were dissected under stereoscopic binocular microscope in water so to take out the alimentary canal on plane slide. The midgut region was assessed and incised from foregut and hindgut. The midgut region cleaned from extraneous tissues was fixed in Bouin's fluid (alcoholic) for about 12 hrs. The excess fixative from the tissues was removed by washing several times in 30% alcohol. The tissue was dehydrated in ascending grades of alcohol. It was cleared in xylene and embedded into molten paraffin 58⁰C – 60⁰C and finally paraffin blocks were casted [23]. Nicely trimmed paraffin block was sectioned at 8-10 μ m through rotary microtome (Weswox). The serials bearing sections were stretched over a prealbuminised microslide and dried in air. The slides with serial sections were dewaxed in xylene and passed through descending grades of alcohol. The tissues were stained in haematoxylin and eosin and treated through ascending alcoholic grades. The sections were cleared in xylene and mounted in DPX. The histoarchitectural and histopathological characteristics of midgut epithelium were studied using trinocular microscope.

RESULTS

The middle translucent part of alimentary canal was called the mesenteron. It was demarcated into promesenteron, meso-mesenteron and meta-mesenteron. It has the usual histological configuration. The promesenteron was lined with tightly packed epithelial folds of uniform height. In a fold top cells, lateral cells and base cells were recognized. (Plate- I).



Plate- I- Microphotograph of T.S. promesenteron with different types of cells. (10 × 10X).

The Mesomesenteron has compactly arranged epithelial folds. The folds attain variable height. The trimorphic nature of epithelium comprising of the top cells, the lateral cells and the base cells was recognized in the mesomesenteron also. The basement membrane was perceptible and never observed

inside the intra fold spaces. The circular muscles are much elaborated and striated as compared to longitudinal muscles which were grouped into bundles. The peritoneum was continuously perceptible. (Plate- IIa & IIb).



Plate- IIa :- Microphotograph of T.S. mesomesenteron showing different types of cells. (10 × 10X).

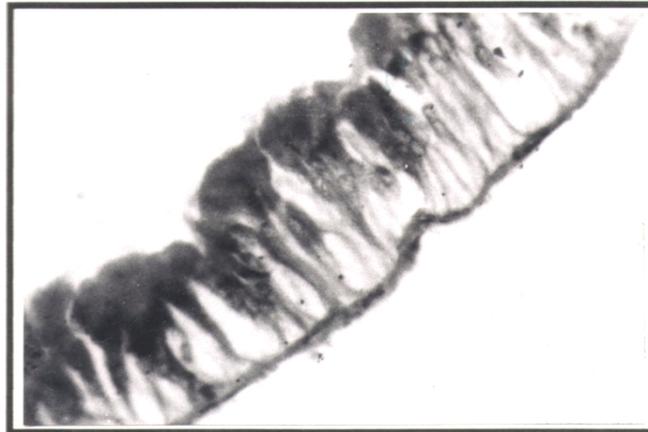


Plate IIb:- Microphotograph of T.S. mesomesenteron with nidi, cellular boundaries & epithelial folds. (10 × 10X).

The epithelial folds were not observed in metamesenteron so it lost the folded nature. The single layered straight epithelium made up of narrow columnar cells which can not be further differentiated. The intercellular boundaries were usually broken. The basement membrane was poorly sighted. The musculature was as usual, the peritoneum was present. The peritrophic membrane was sighted as concentric layers. The regenerative cells were congregated into a roughly triangular area known as nidus. The nidi were always confined at the base of epithelial folds. In metamesenteron they are embedded into the thickness of the epithelium. (Plate- III).



Plate III:- Microphotograph of T.S. metamesenteron with cellular configuration. (10 × 10X).

The present pyrethroid pesticides have affected severely to midgut tissues and generated histopathological derangements after continuous exposure of naiads to LC_{50} constrains for 40 Hrs. The folded contour of the midgut epithelium on exposure to the cypermethrin was not altered. There was high accumulation of cell contents at the apical ends of the epithelial cells. This movement has caused intense vacuolation at the basal portion of the epithelial cells. The displacement of nuclei was observed and nuclear membrane was also damaged. The intercellular boundaries remained intact. (Plate IVa, IVb).



Plate IVa:- Microphotograph of T.S. Mesenteron after 40 hours of exposure under cypermethrin. (10×10X). Intense apical accumulation of cell contents and displacement of nuclei.



Plate IVb:- Microphotograph of T.S. Mesenteron after 40 hours of exposure under cypermethrin. (10×10X). Intense vacuolation at the base of epithelial folds and intact cell boundaries.

The turgid apical portion of epithelial cells was torn off, thus cells expelled their contents violently into the midgut lumen. The peritrophic membrane was completely damaged therefore not observed. The nidi or regenerative cells showed hyperactivity. The basement membrane persisted but was very thin. The circular muscles become contracted, their striations and nuclei were disappeared. The muscle fibres lost their integrity. The longitudinal muscle bundles were observed to reduced to their diameter. The peritoneum was not observed. (Plate V).



Plate- V:- Microphotograph of T.S. Mesenteron after 40 hours of exposure under cypermethrin. (10×40X). Violent squeeze of cell contents into the lumen. Hypernidial activity. Damage of muscular integrity, disappearance of peritoneum and basal vacuolation of epithelial cells.

The deltamethrin reacted with the epithelial folds and their latero-apical ends were separated, therefore the folds were exposed for more pesticidal activity. The cellular contents of epithelial cells were moved towards the apical periphery of epithelial cells. This movement of cellular contents has created vacuolation at the basal, middle and apical portion of epithelial cells. The nidi or regenerative cells were observed shrunken and clamped together. They were also exposed due to the damage of basal cell boundaries. (Plate VIa, VIb). The nuclei were also displaced. The internal pressure of epithelial cells expelled their cellular contents violently into the midgut lumen. The intercellular boundaries of epithelial cells were perfectly damaged at the portion of the epithelial folds. The peritrophic membrane was completely damaged. The basement membrane was moderately damaged. The circular muscle turned weak, lost their striations but maintained their integrity. The longitudinal muscles were affected by the present pesticide and turned feeble. (Plate VIIa, VIIb).

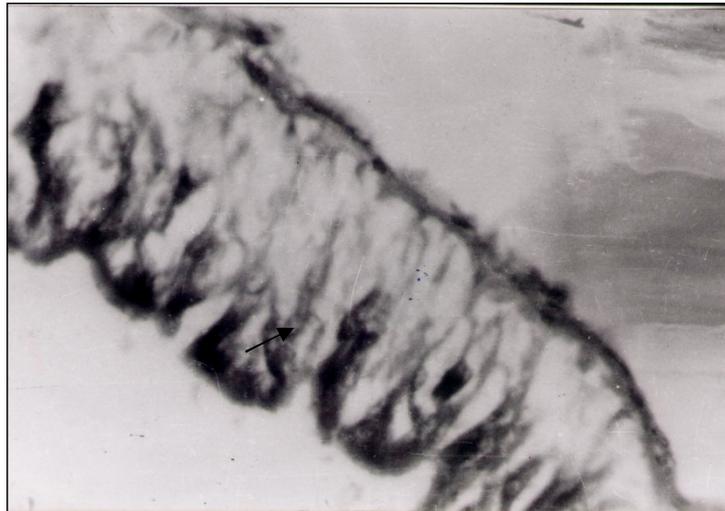


Plate VIa:- Microphotograph of T.S. Mesenteron after 40 hours of exposure under deltamethrin. (10×10X). Separation of apical interfold spaces, apical accumulation of cellular contents and vacuolation at the base of epithelial folds.



Plate VIb:- Microphotograph of T.S. Mesenteron after 40 hours of exposure under deltamethrin. (10×10X). Damaged intercellular boundaries at the basal portion of epithelial folds. Shrunken nidi.

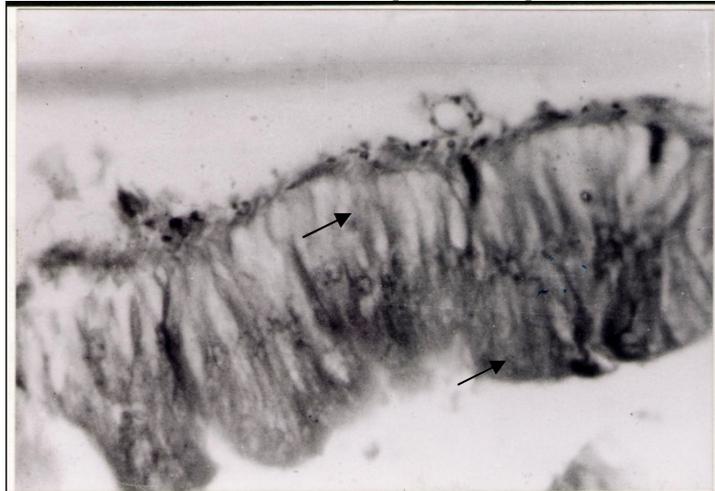


Plate VIIa:- Microphotograph of T.S. Mesenteron after 40 hours of exposure under deltamethrin. (10×40X). The accumulation reached upto the apex of epithelial folds, musculature became contracted and nuclei displaced.



Plate VIIb:- Microphotograph of T.S. Mesenteron after 40 hours of exposure under deltamethrin. (10×40X). Brisk expulsion of cellular contents into the midgut lumen, damaged epithelial folds and musculature.

DISCUSSION

The mesenteron of last instar naiad of *T. aurora* was simple having uninucleate, columnar epithelial cells. The epithelium constituted well elaborated compact epithelial folds. The present three segmented nature viz pro-, meso and metamesenteron of mesenteron supports the three subdivisions of midgut of aquatic predatory bugs i.e. *Notonecta undulata* say, *Belostoma flumineum* say, *Pelocoris femoratus*, P.B., *Rantara fusca* P.B., by Parson [101959] and *R. elongata* (Fabr.) by Rastogi [111962]. The contour of epithelium in different parts of mesenteron resembled to the type I and type II epithelium illustrated and reported in aquatic Hemipteran bugs by Parson [101959] respectively. The functional characterization of various epithelial cells into top, lateral, basal and nidial cells has been made during the present study. The highly distended without striae top cells are termed as secretory cells. These cells usually discharge their cytoplasmic contents more violently into the mesenteron lumen and have been called as monophasic as described in *Coccinella septempunctata* Linn. [12Pradhan, 1939]. The lateral cells with less distension and distinct striae are also secretory in behaviour and release their contents several times into the mesenteron lumen. Such groups of secretory cells have been presumed polyphasic as established in *Epilachna indica* (Fabr.) [12]. The base cells occasionally engage in secretory activity rather than they engaged themselves in the absorption of nutrients. The nidial cells situated in the nidi are new proliferated young cells. These cells replace old exhausted, damaged top, lateral or base cells in cyclophasic manner. Jones [13] has reported cyclic morphological changes in the epithelial cells of *Diploptaxi liberta* (Burm.). The peritrophic membrane provides protection to self, delicate mesenteron epithelial cells from erosion by hard food particles [14,15]. The existence of peritrophic membrane in the naiad of *T. aurora* in the form of fragmented layers supports the earlier identical observations on *Pantala flavescens* [16]. These layers probably fused together to form a continuous peritrophic membrane and corroborates observations made on odonate larvae by Voinov [17]. The laminar of peritrophic membrane observed in the present membrane classified by Wigglesworth [18] and Richard and Richards [19]. The naiads of *Aeschna* sp. secreted two such membranes under starvation [20].

The basement membrane conspicuously perceived in the mesenteron of the naiad, similar continuous basement membrane has been identified in the naiad *P. flavescens* [16]. Homogenous eosinophilic granular cytoplasmic texture of epithelial cells in the midgut of immature *T. aurora* corroborates the occurrence of granular cytoplasm in *P. flavescens* [16] and in predaceous *R. elongata* [11]. Gouranton and Thomus [21] have reported protein crystals without membrane in the nuclei of the columnar cells in the various species belonging to different order inclusive *Sympetrum depressiusculum* Sely. The occurrence of such crystals has not been observed in present investigation. The weak old epithelial cells replaced by specialized mass of multinucleate cells accumulated at the base of each epithelial fold is known as nidus are distinctly observed. Parson [10] on the basis of frequent mitotic activity among those cells termed them regenerative units. Snodgrass [22] discussed clusters of such small cells as nidi. The proepithelial cells generated by the nidial cells with cavities in fourth type of epithelium studied in carnivorous coccinellid, *C. septempunctata* by Pradhan [12] have not visualized in the present study. The musculature of mesenteron of the present naiad comprised of an elaborated circular muscles and longitudinal muscle bundles. The circular muscles are 2 to 4 muscles strand thick with striations and nuclei. The longitudinal muscle bundles are located at equal distances outside the circular muscles. A distinct peritoneum finally wrapped the midgut wall.

The continuous forty hours treatment of *T. aurora* naiad under LC₅₀ concentration, 2.69 x 10⁻⁵ and 2.50 x 10⁻³ ppm. of cypermethrin and deltamethrin insecticides respectively has proved highly deleterious and toxic, induced histopathological derangements in midgut tissues. The mesenteron was observed to be prone to present pesticides. High accumulation of cell contents was seen at the apical ends of epithelial cells under cypermethrin constrain, consequently there was intense vacuolation at the basal portion of the epithelial cells. Nuclei were displaced and nuclear wall was observed damaged. The turgid apical portion of epithelial cells was torn off, thus cells expelled their contents forcibly into the midgut lumen. This pesticide damaged the peritrophic membrane and not observed. The nidi showed hyperactivity. The basement membrane remained very thin. The circular and longitudinal muscles were severely affected. The peritoneum was not observed.

The deltamethrin separated latero-apical ends of epithelial folds, leaving them exposed for more pesticidal activity. The cellular contents of epithelial cells were moved towards the apical periphery of epithelial cells and caused vacuolation at the basal, middle and apical portion of epithelial cells. The nuclei were seen displaced. The cellular content was thrust into the midgut lumen.

The intercellular boundaries of epithelial cells were damaged at the portion of epithelial folds. The nidi or regenerative cells were seen shrunken and clamped together. The peritrophic membrane was affected

and damaged completely. The circular and longitudinal muscles affected severely by present pesticide deltamethrin.

CONCLUSION

The three segmented nature viz. pro-, meso-, and metamesenteron of mesenteron of last instar naiad of *T. aurora* was simple having uninucleate columnar epithelial cells constituted epithelial folds. The functional characterization of various epithelial cells, top, lateral, basal and nidal cells have been made during the present study. The musculature of mesenteron of present naiad comprised of an elaborated circular and longitudinal muscle bundles.

A continuous 40 hrs. exposure of naiad to LC₅₀ constrain of cypermethrin and deltamethrin has revealed histopathological derangements in mesenteron, therefore the midgut tissues were affected severely.

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