Histopathological Study on the Intestine of the Fresh Water Oranmental Fish, *Puntius conchonius* (Ham.) Exposed to Sublethal Concentrations of Endosulfan and Fenvalarate Chemicals

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Abstract: Ornamental fish culture is one of the small scale self employment practices by humans. This practice requires unpolluted freshwater in large scale. Agricultural usage of pesticides in the fileds ultimately reach aquatic ecosystem. Pollutants such as endosulfan and fenvalarate are found to be highly toxic to very sensitive fish like Rosy barb, *Puntius conchonius*. In the present study the intestine of this fish exposed to sublethal concentrations of the pesticides endosulfan and Fenvalarate show abnormal histology. The microvilli are hypertrophied and adjacent will have fused together due to erosion of the outer epithelial layer.

***Index Terms – P. conchonius*, Endosulfan, Fenvalarate, Intestine, Microvilli**

I. INTRODUCTION

Fish bioaccumulation poses a serious risk to human health due to its highly vulnerable nature to toxic substances. The behaviors, biochemistry, and physiology of fish are typically impacted by changes in the chemical composition of a natural aquatic environment caused by exposure to hazardous substances such as pesticides, heavy metals, and industrial effluents (Vosyliene and Kazlauskiene, 1999). Fish provide numerous benefits to humans, including those related to fisheries, wildlife, agriculture, urban, industrial, and social development. They are among the most valuable natural resources on the planet. Notwithstanding, the uncontrolled discharge of agricultural chemicals, particularly pesticides, into aquatic environments has resulted in ecological challenges for all taxa within the aquatic ecosystem. Because of the careless use of pesticides, the aquatic ecosystem is at risk of losing biodiversity. A vast array of non-target organisms, including fish and invertebrates living in aquatic environments, are impacted by pesticides in addition to the intended pests (Pandey, 1988). Pesticides are introduced into aquatic environments by agricultural runoff of rain and irrigation water, where they pose serious toxicological risks to local organisms (Kumari and Kumar, 1997). According to Dutta and Maxwell (2003), pesticide pollution has a detrimental effect on aquatic life, which in turn affects all other animals and humans up the food chain. In this work, the guts of *P. conchonius*, a commercially significant ornamental fish that can be easily found in both aquaria and rivers, are examined in detail for the sublethal effects of pesticides. The criteria for the sublethal action of endosulfan and fenvalarate was determined by looking at histological changes in the intestinal tract, which is the organ that is exposed to foodborne contaminants first. Consequently, the aim of this research was to examine the effects of endosulfan and fenvalarate on *P. conchonius*'s gut, both at the lethal and sublethal levels.

MATERIALS AND METHODS

The physico-chemical characters of the water used in the present study were estimated following standard methods described by APHA (1975). Fenvalerate is a commercial grade liquid synthetic pyrethroid (Cyano(3-phenoxyphenyl) methyl 4-chloro-2-(1-methyl-ether) benzeneacetate, fenvalerate, 20% EC) marketed by Isagro (Asia) Agrochemical Pvt.Ltd., Panoli-Mumbai, India as Fenval was used throughout the experiment. Endosulfan is a commercial grade organochlorine pesticide (6, 7, 8, 9, 10-hexachloro-1,5,5a,9a-hexahydro-6,9-methano-2,4,3-benzodi-oxithepin-3-oxide; 35% EC) marketed by Excel Crop Care Limited, Mumbai, India, as Endocel was used throughout the experiment.

Sublethal Concentrations

LC_{50} Values were determined for both the pesticides as prescribed by McLeay (1973). Probit analysis was done to derive mortality values for each pesticide. This would indicate the Log LC_{50} values of the pesticides for the experimental group exposed to a period of 6, 12 ,48 , 72 and 96 hours (Finey, 1971). After this two sublethal concentration of each pesticides namely endosulfan $1/10^{th}$ (1 x 10 -7 ppm) and $1/20^{th}$ (5 x 10 -8 ppm) and fenvalarate $1/10^{th}$ (9 x 10 -7 ppm) and $1/20^{th}$ (4.5 x 10 -7 ppm) were used for this study.

EXPERIMENTAL DESIGN

Healthy *P. conchonius* weighing 2000 ± 200 mg (live weight) were selected from stock tanks and exposed to two sublethal concentrations of each pesticide, namely endosulfan {$1/10$th($1x10^{-7}$ppm) and $1/20^{th}(5\ x10^{-8})$}and fenvalerate {$1/10^{th}$ ($9x10^{-7}$ppm) and $1/20^{th}(4.5x10^{-7})$} dilutions of 96 hours. LC_{50} value was determined as prescribed by Mc Leay (1973). Three replications with ten individuals [healthy *P. conchonius* weighing 2000 ± 200 mg (live weight)] in each trough of 17 l capacity for each sublethal concentration of both the pesticides were maintained for 21 days. Fresh test media were supplied daily. The fish were fed ad libitum with pelleted feed of 35% protein at 10.00 hours everyday. Simultaneously a control group of 10 individuals was maintained throughout the experimental period in well water. The experimental concentrations were prepared using the same well water. After exposure for 21 days five fish from each replication of the sublethal concentrations were sacrificed to obtain the necessary tissues for histopathological studies.

Preparation of Permanent Microscopic Slides

The gill tissues were fixed in Zenker's fluid, dehydrated and embedded in paraffin following the method of Wesner (1968). Sections at 7mm thickness were prepared using rotary microtone. After deparafinizing, the slides for Histological and Histopathological observations were stained using one of the following stains:

1. Ehrlich's hematoxylin used for pathological studies as nuclear stain
2. Aqueous 0.2% Eosin Y as the cytoplasmic stain and
3. Van Gieson with Methylene blue to study connectivity tissues.

Finally the sections were mounted in DPX (Weil, 1945).

RESULTS

Histology of normal Intestine

The inner wall of the intestine is produced into finger shaped folds called villi. In the control fish they appear as short and narrow extensions into the lumen. The mucous lining is composed of columnar epithelial cells which are arranged in a single layer. These cells are rectangular with a broad free end and a narrow base. The nuclei are elliptical with dense chromatin. These cells have terminal bars and striated border. The epithelial cells are interspersed with goblet cells which secrete mucous and have a characteristic vacuole filled with mucous. The nucleus is situated at the base of the cell (Plate: 1). Beneath the mucosal layer is the connective tissue forming the lamina propria. This layer projects into the villi. It contains characteristic capillary bed in villi (Plate: 2). Circular muscles envelope the connective tissue layers as the muscles of the wall. The outermost covering is a layer of longitudinal muscles which are themselves covered by peritoneal epithelium (Plate: 3).

Histopathological changes in Intestine exposed to fenvalerate

($1/10^{th}$ LC_{50})

Plate 4 shows that the T.S. of intestine of *P. conchonius* exposed to this concentration of the toxicant. The intestinal villi have fused together due to erosion of intestinal epithelium lining the outer layer of the villi. In another section, (Plate, 5) the villi appear to be long and their tips have been detached due to toxicant's effect. Plate 6 shows very long villi fused with adjacent ones. The tips of the villi have been corroded and seen as debris near lumen.

($1/20^{th}$ LC_{50})

Plates 7-9 show the T.S. of intestine of the *P. conchonius* exposed to this concentration. The effect of the toxicant at this concentration is not as vigorous as the previous one. In all the three plates intestinal villi appear to be normal. In plate 7 increased blood supply to sub mucosal region is noticed. The intestinal wall appears thick due to toxicity of the chemical. Plate 8 also shows mild haemorrhage in sub mucosal region. However, plate 9 shows very normal histology of intestine.

Histopathological changes in Intestine exposed to endosulfan
($1/10^{th}$ LC_{50})

All the sections obtained from the intestine of *P. conchonius* exposed to this toxicant show similar kind of changes in the architecture of the intestine. Plate 10 shows intestine under low magnification with swollen villi due to edema caused by the toxicant. Plates 11 and 12 are transverse sections of intestine observed under higher magnification. These sections reveal edematic condition of the villi and that resulted in fusion of adjacent villi.

($1/20^{th}$ LC_{50})

Intestinal sections of the *P. conchonius* exposed to this chemical show minor alteration in the structural components of the intestine. Plate 13 is a section of intestine under low magnification, revealing too short and stumpy villi with apparently normal histology. In some regions the villi are not seen. Plate 14 shows a portion of the previous Plate (13) under higher magnification. The mucosal and sub mucosal regions are normal. The connective tissue, circular and longitudinal muscles of the intestinal wall are normal in structure.

DISCUSSION:

The organ of absorption in the vertebrates is the intestine and hence the harmful effects of the constituents of the pesticides may be expected to manifest themselves in the structure and functioning of the intestine (Murugesan, 1988). The most pronounced among the changes is the hypertrophy of the absorptive surface. This increase in the area of absorptive surface of the intestinal mucosal lining is due to a reduction in the efficiency of the epithelial cells in absorption brought about by the pollutant. The hypertrophy and proliferation of the goblet cells result in the secretion of mucous in abundance (Othuman, 1994). Narayanan (1989) in his study on the effect of heavy metals, cadmium, mercury and zinc on the portunid crab *Scylla serrata* has reported similar formation of a hypertrophied structure. Murugesan (1988) has reported the formation of an additional extension of intestine in the form of "caecum – like" evagination to augment absorption in *H. fossilis* treated with higher sublethal concentration of textile mill effluent for a prolonged period. A similar condition has been observed in *A. lineatum* exposed to 3.25% concentration of coconut husk retting pollutant for 30 days (Madasamy, 2001). Haniffa and Sundaravadhanam (1984) have also observed swelling in the luminal portions of intestinal villi in the fish *Barbus stigma* exposed to higher concentration of distillery effluent.

The hypertrophied intestinal villi due to pesticidal effect have fused among themselves resulting in large irregular branched masses protruding into the lumen of the intestine in the test fish as has been recorded earlier by Naidu *et al*. (1982) in *O. mossambicus* when exposed to mercury. Such a condition has also been reported by Murugesan (1988) in effluent exposed *H. fossilis* and Narayanan (1989) in *S. serrata*, when exposed to higher concentrations of heavy metals, cadmium, mercury and zinc for longer durations. Sakthi and Gaikwad (2002) have reported similar findings in *G. affinis* exposed to dimecron and Sultana and Rajan (2007) have reported ruptured villi in *O. mossambicus* due to exposure of heavy metals like Cu, Pb and Zn.

Sublethal dose exposure of phosphamidon in *O. mossambicus* has shown drastic histopathological changes in the intestine of the fish due to pesticide intoxication such as decrease in the number of mucosal villous folds, degeneration of muscle fibres, loosened sub mucosa, rupture of epithelial cell and serosal layers and vacuolation (Indira *et al*.,1999). Similar observations have been made in the intestine of *Puntius sarana* exposed to Malathion and vacuolation of the connective tissue and widening of the lumen when exposed to BHC (Moitra and Lal, 1989). Bakthavatsalam and Rajaratnam (1990) have observed disorganization in the serosa and mucosal folds indicating severe damage, dilation in certain regions of sub mucosa and mucosa and histolysis of columnar and goblet cells in *Anabas testudineus* treated with lindane and atropine sulphate which is similar to the present report on *P. conchonius.*

[Abbrevations: gb - Goblet cell, m-Muscle, vi -Villus, bc - Blood capillary Bc-Blood capillary, d- Debris-cellular, l-Lumen, m-Muscle, mu-Mucous, n-Nucleus, se-Serosa, sm-Submucosa]

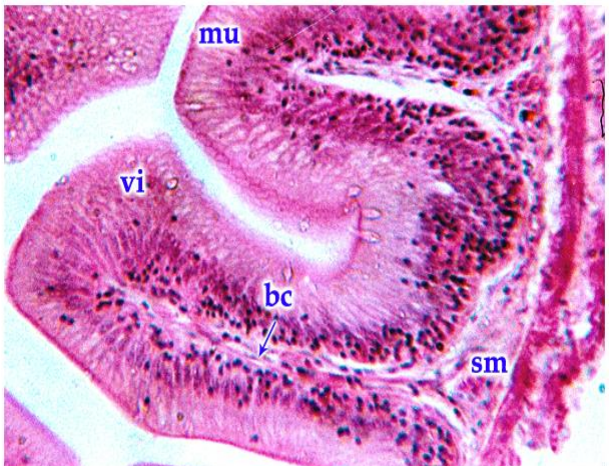


Plate 1

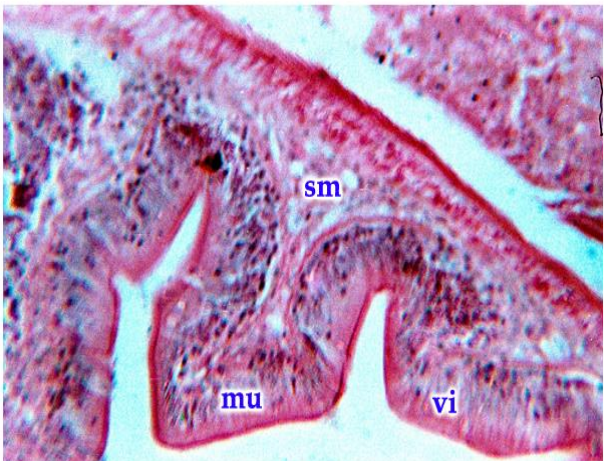


Plate 2

Plate No: 1 A segment of intestine shows normal villi, goblet cells, mucosa, sub mucosa and wall musculature.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 2 Another segment of intestine shows normal villi, goblet cells, mucosa, sub mucosa and wall musculature.
Stain: Ehrlich's hematoxylin and Eosin

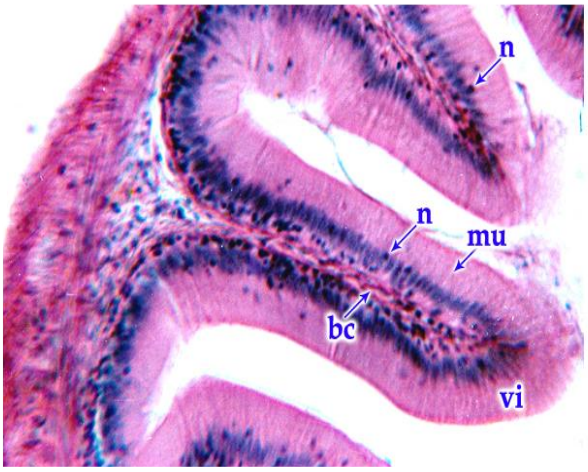


Plate 3

Plate 4

Plate No: 3 T. S. of intestine under low magnification shows damaged and fused villi.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 4 The villi are long in another section of intestine whose tips are detached and deleted.There is mild hemorrhage in submucosa, also.
Stain: Ehrlich's hematoxylin and Eosin

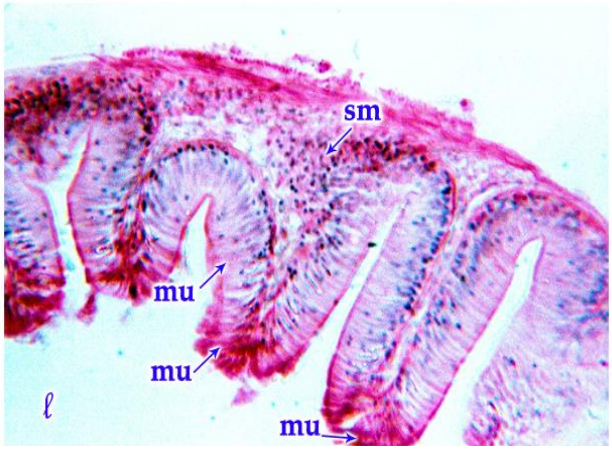


Plate 5

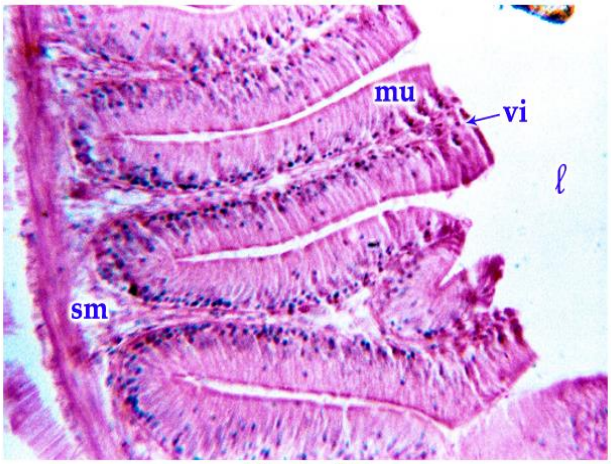


Plate 6

Plate No: 5 The deformities observed in previous plate are noticed in this section from another fish.
Stain: Von Gieson

Plate No: 6 Almost normal villi, but the blood supply to submucosa has slightly increased. The intestinal wall seems thickened.
Stain: Ehrlich's hematoxylin and Eosin

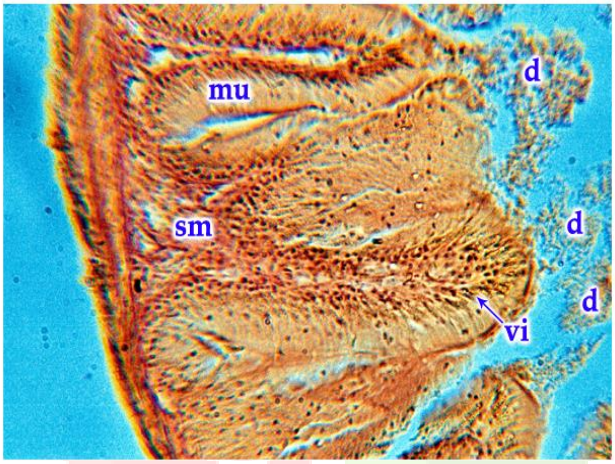


Plate 7

Plate 8

Plate No: 7 Nearly normal villi, but, short in length. There is mild hemorrhage in submucosa.
Stain: Ehrlich's hematoxylin and Eosin

Plate No: 8 Another section: The villi are normal in all details.
Stain: Von Gieson

Plate 9

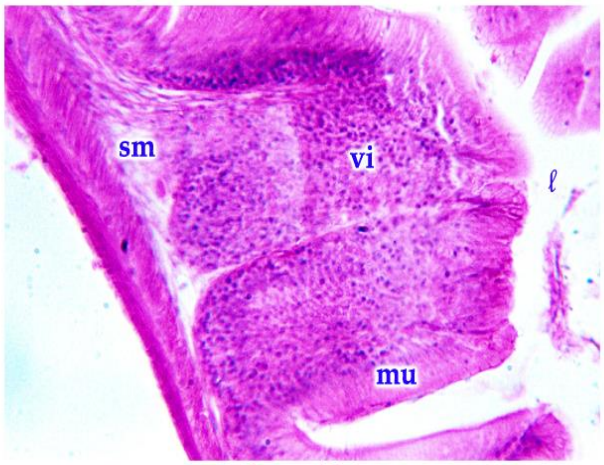
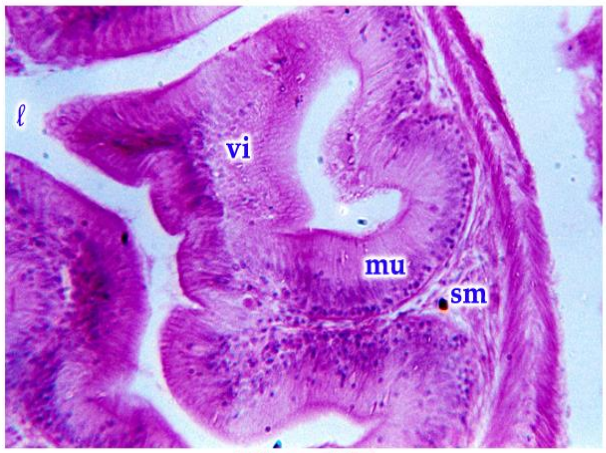


Plate 10

Plate No: 9 T.S. of intestine under low magnification shows swollen villi due to edema.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 10 T.S. of intestine under high magnification shows fused swollen villi due to edema.
Stain: Ehrlich's hematoxylin and Eosin



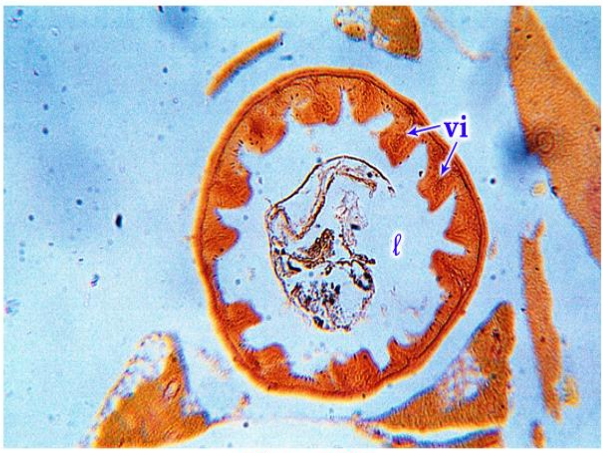
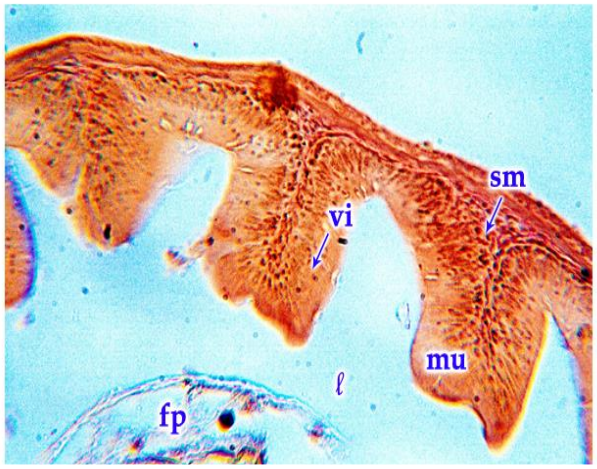


Plate 10 **Plate 11**

Plate No: 11 T.S. of intestine under high magnification shows fused swollen villi due to edema. Similar condition in another area.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 12 Section of intestine under low magnification: Short, very short and stumpy villi with normal histology. The villi are missing in some regions of the section.
Stain: Van Gieson



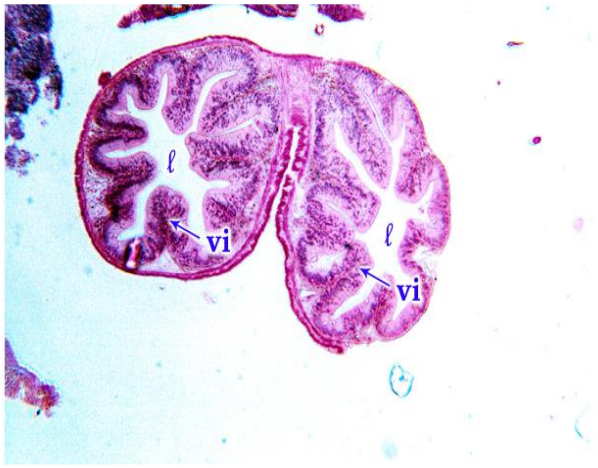


Plate 13 **Plate 14**

Plate No: 13 Section of intestine: A portion of the previous plate under high magnification.
Stain: Van Gieson
Plate No: 14 Two sections of intestine under low magnification showed signs of complete recovery in architecture of mucosa and sub mucosa of villi..
Stain: Ehrlich's hematoxylin and Eosin

CONCLUSION

Agricultural and Live stock are two important practices in India which grow side by side. Usage of Pesticides is un avoidable to protect the interested species from Fish. Nevertheless, a judicial use of chemicals with conscious mind of concentration of species on the earth is to be emphasized.

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