Impact of Organochlorine and Pyrethroid Pesticides on the Stomach of the Fresh Water Oranmental Fish, *Puntius conchonius* (Ham.)

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Abstract : Pesticides enter aquatic ecosystem from agricultural usage through run-off. Freshwater ornamental fishes are very sensitive to water quality parameters. Since aquaculture requires enormous ground water, pesticide contamination in such water source even at sublethal concentrations effect sever damage to the internal organs. In the present study the pesticides fenvalarate and endosulfan effected severe histopathological damage to the stomach of *Puntius conchonius* as it is evident from the Histological studies.

IndexTerms **–** Stomach, Puntius conchonius, endosulfan, fenvalarate

I. INTRODUCTION

Large-scale pesticide applications are made to increase agricultural crop yield production. The purpose of this is to manage agricultural pests. However, pesticides also find their way into lakes, ponds, and rivers through rainfall, which has an adverse effect on aquatic life, primarily fish. Fish are particularly susceptible to harmful substances, and bioaccumulation poses a serious risk to their lives (Ganeshwade 2012) Once pesticides enter a fish's body, they alter various target and non-target organs histopathologically and biochemically (Kumari and Mishra 2015) . Fish organs and tissues have been shown to undergo moderate to severe histopathological changes as a result of long-term exposure to sub-lethal pesticide concentrations (Rebolledo and Vial 1979). Fish growth and development depend on the stomach, one of the main organs of the alimentary canal, to properly digest food that is ingested (Thorat 2001). The stomach wall is made up of four layers, according to histology: the mucosa, submucosa, muscularis, and serosa. In general, the stomach mucosal layer is simpler than that of higher vertebrates. There are, however, only a few studies in the literature that examine the histochemistry of mucosubstances in the fish stomach. Fish gastric glands secrete both pepsinogen and hydrochloric acid. The majority of toxic substances, such as pesticides, affect the alimentary canal and digestive glands after entering the digestive system through food or water. It comes into direct contact with the contaminants dissolved in the water, but it also gets indirectly impacted by blood contact. A review of the literature that is currently available on fish and environmental pollutants shows that most pesticides have sub-lethal doses that can alter fish behavior, cause variable amounts of histopathological injuries to different organs, and alter fish biochemistry. The extent of these effects is typically influenced by the pesticide's type, dosage, and duration of exposure. (Pandey et al., 2014; Cengiz and Unlu, 2006; Ghanbahadur and Ghanbahadur, 2012; Senapati et al., 2013; Oguei et al., 2013; Ullah et al., 2014; Mishra et al., 2006, 2008; Ullah and Zorriehzahra, 2015., Tilak et al.,2005) The current study is set out to examine and understand the sublethal toxic effects of fenvalarate and endosulfan pesticides on the histological changes in the stomach of the freshwater ornamental fish, *Puntinus Conchonius*..

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

LC50 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 1/10th (1 x 10 -7 ppm) and 1/20th (5 x 10 -8 ppm) and fenvalarate 1/10th (9 x 10 -7 ppm) and 1/20th (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 {1/10th($1x10^{-7}$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 Stomach: The stomach wall consists of four distinct layers, namely: 1.Mucosa 2.Submucosa 3.Muscularis externa and 4.Serosa.

The innermost lining of the lumen of stomach is mucosa. This forms a continuous layer and is composed of tall simple columnar cells. The nuclei are elliptical or oval and lie at the base of the cell. The cytoplasm is densely granular and basophilic. These cells secrete mucous (Plate:1).

A dense connective tissue layer outer to mucosa forms the sub mucosa. This layer contains blood vessels, lymph vessels and nerves. Muscularis externa envelopes sub mucosa. This layer consists of circular, longitudinal and oblique muscles. The outermost layer is the serosa. This is a layer of areolar connective tissue continuous with the mesenteries supporting the gut (Plate: 3). The surface of the mucosa is thrown into a number of folds by shallow furrows that run in longitudinal and transverse rows. This results in the subdivision of mucosa into somewhat bulging areas called faveolae gastricaes. The mucous epithelium is clustered as conical glandular projection into the faveolae and the cells are fully loaded with mucous.

HISTOPATHOLOGICAL CHANGES IN STOMACH EXPOSED TO FENVALERATE ($1/10^{th}$ LC_{50})

Plate 4 shows the pathological changes observed in the stomach of the fish, *P. conchonius* treated with 1/10th sublethal concentration of the pesticide, fenvalerate. The gastric folds are abnormal in structure due to heavy toxicity of this chemical. They have been damaged and are irregular in shape. The gastric pits are highly disturbed (Plate, 4).The toxicity has resulted in excessive blood supply to sub mucosa. The mucous cells are seen hypertrophied. Another section (Plate, 5) shows very compactly arranged epithelial cells in the gastric fold and gastric pits. The lumen of the intestine has debris of gastric mucosa which are pinched or peeled off from the original place and are being digested along with food material.

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

The stomach of *P. conchonius* exposed to 1/20th sublethal concentration of this pesticide shows an abnormal histology. The gastric folds are numerous in number, obliterating the lumen of the stomach. The gastric pits are narrowed down. The stomach wall has thickened. In some parts the gastric folds have been distorted. Plate 7 shows section of stomach of a fish exposed to this concentration. There is heavy bleeding in the sub mucosal region of the stomach due to toxicity. As a result of haemorrhage and corrosion of the wall of the gastric fold, fusion of adjacent gastric folds is seen: a transverse section of the stomach of another fish exposed to this concentration when observed under higher magnification shows thickened stomach wall. There is also increased blood supply to the region of sub mucosa (Plate 8). Plate 9 shows delamination of the gastric mucosa from the distal portions of the gastric folds of the stomach of the fish exposed to this concentration. The distorted portions are accumulating in the lumen of the stomach as fragments. The same condition is also noticed in another section of a fish exposed to the same treatment (Plate 10).

Histopathological changes in Stomach exposed to Endosulfan

(1/10th LC_{50})

P. conchonius exposed to this toxicant also shows abnormal histology of stomach. Plate 11 reveals extremely wide and highly branched unusual type of gastric folds. Where as the wall of the stomach is in normal condition. Plate 12 exhibits maintenance of sub mucosa. The wall of the stomach is unusually quite thicker. Plate 13 shows the distal half of gastric folds maintaining normal histology.

(1/20th LC_{50})

Plates 14-16 reveal the histopathology of *P. conchonius* exposed to this lower sublethal concentration. Plate 14 shows the basal region of the gastric fold and gastric pit in a disturbed condition. The mucous epithelium and sub mucosa are flooded with plenty of R.B.Cs. Similar condition, but at the distal part of the stomach is seen in Plate 15. In another fish, the stomach shows extremely branched gastric folds obstructing the lumen (Plate, 16).

DISCUSSION

Toxicants may produce various prolific changes in the alimentary tract as it is one of the routes of penetration for them. Histopathological changes have been widely used as biomarkers for monitoring the effects of pollutants on specific target organs like gills, liver, kidney and gut of fishes that are responsible for vital functions. The alterations found in these organs on exposure to chemicals like pesticides are normally easier to identify than the functional ones and serve as markers of damage to animal health. Histologically the alimentary canal has four layers, serosa, muscularis mucosa, sub mucosa and mucosa. The mucosa is thrown into a number of folds in the lumen of the stomach. The histopathological changes observed in the stomach of fishes treated with both the pesticides, endosulfan and fenvalerate, have shown damaged epithelial cells, hypertrophied mucous cells and abnormal, irregular and damaged gastric folds. The gastric folds have been enlarged to the extent of obliterating the lumen of stomach due to the effect of both the pesticides. Obviously, this has affected the digestive capability of the stomach. The quantum of food in the lumen of stomach was found to be little or none when compared with that of the control fish. These findings of the present study are in agreement with the findings observed in the fish *Aplocheilus lineatum* treated with coconut husk retting effluent (Madasamy, 2001). The Mucous cups of the epithelial cells deepen to secrete copious mucous. The mucous secreted by the hyperactive mucous glands is poured into the gastric lumen. In the present study the hypertrophied sub mucosa shows increased vascularization and large blood vessels engorged with R.B.Cs occur at several places. Such changes in the shape of gastric folds, ulceration in the surface epithelium and lamina propria, pronounced desquamation and damage in the sub mucosal layers in the stomach of *Mystus armatus* exposed to sublethal concentration of paper mill effluent have been noticed by Isaiarasu and Haniffa (1987) and Yadav *et al* 2019)

The following changes have also been noticed in the stomach of *P. conchonius* as observed in *H. fossilis* by Murugesan (1988) and in *A. lineatum* by Madasamy(2001). The connective tissue became small in sub mucosa and the blood vessels had hypertrophied. The latter was due to the enormous increase in the number of erythrocytes that flowed through these vessels. Haemorrhage was a common event and blood was found to collect in the sub mucosa and serosa. Another remarkable phenomenon was the obstruction of the lumen of the stomach by the hypertrophied wall of the stomach and by the debris of cells which had sloughed off from different layers of the stomach wall.

[Abbrevations: Bc-Blood capillary, d- Debris-cellular, cm -Circular muscle, ery-Erythrocyte, fp-Food particles, gf-gastric fold, gepi -Gastric epithelium, gg- Gastric gland, gp-Gastric pit, l-Lumen, m-Muscle, mu-Mucous, n-Nucleus, se-Serosa, sm-Submucosa, lm-Longitudinal muscle]

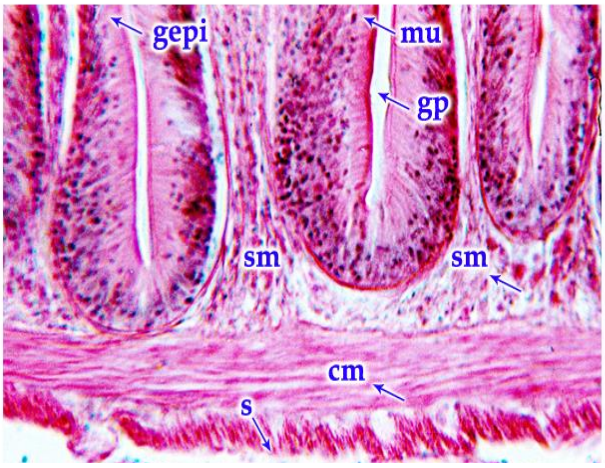

Plate 1

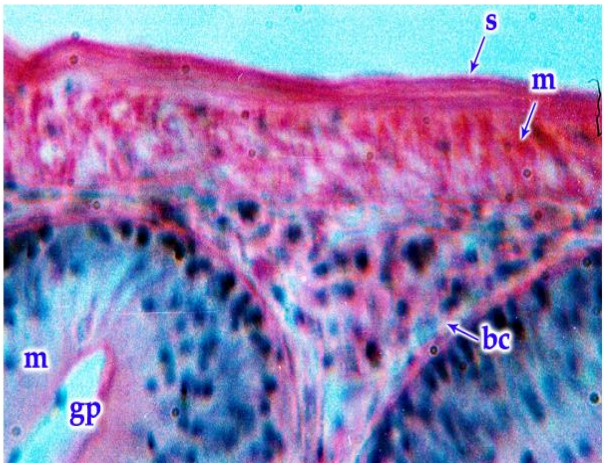

Plate 2

Plate No: 1 Three gastric pits under high magnification: Gastric mucosa with mucous secreting cells, connective tissue, blood capillaries and musculature of stomach wall.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 2 Section of base of a normal gastric fold under high magnification shows gastric mucosa, and sub mucosa with connective tissue, blood vessels and circular and longitudinal muscle fibers of stomach wall.
Stain: Ehrlich's hematoxylin and Eosin

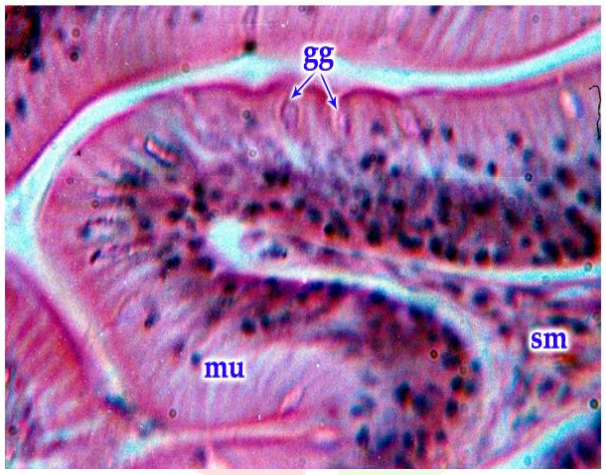

Plate 3

Plate 4

Plate No: 3 Section of distal portion of a gastric fold under high magnification shows gastric mucosa, sub mucosa with connective tissue, blood vessels.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 4 Histology of gastric pit and fold disturbed, blood supply to sub mucosa increased and mucous cells hypertrophied.
Stain: Ehrlich's hematoxylin and Eosin

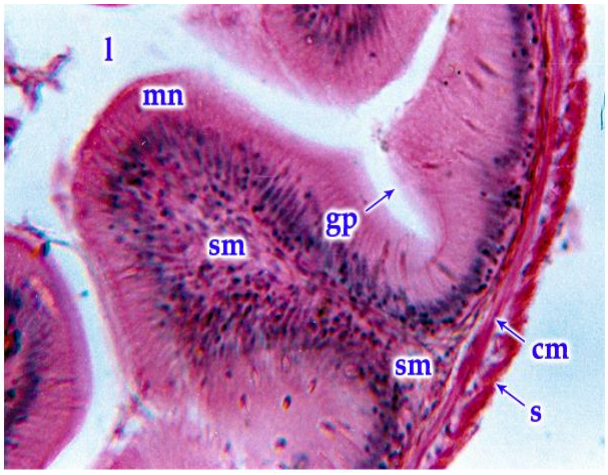

Plate 5

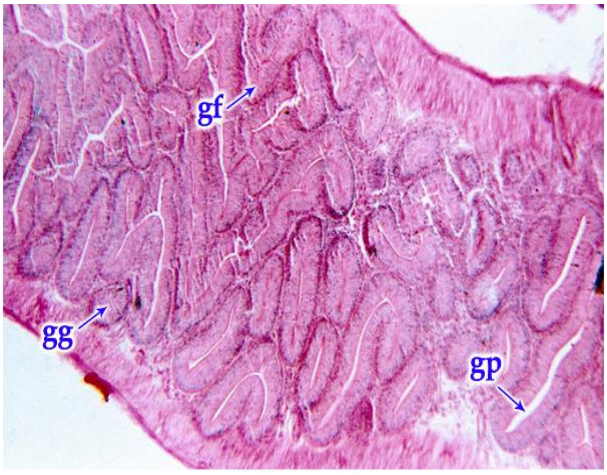

Plate 6

Plate No: 5 Gastric mucosa shows compactly arranged epithelial cells in gastric folds and gastric pits. Pinched and peeled off gastric mucosa is being digested in the lumen along with food.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 6 L S. of stomach shows intimately packed gastric folds.
Stain: Ehrlich's hematoxylin and Eosin

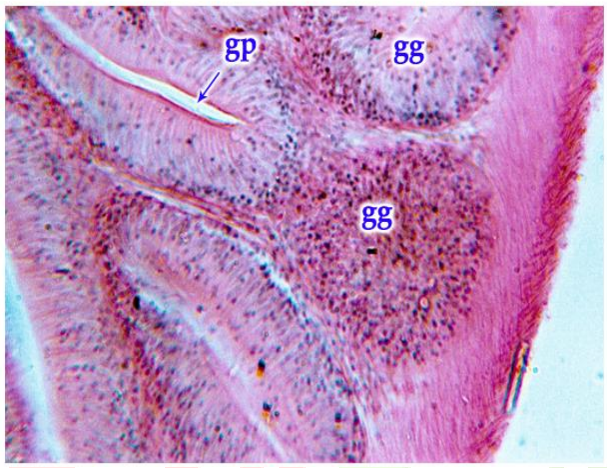

Plate 7

Plate 8

Plate No: 7 T. S. of a segment of stomach under high magnification shows heavy bleeding in sub mucosa and fusion of gastric folds.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 8 T. S. of a segment of stomach under high magnification shows thickened stomach wall and increased blood supply to sub mucosa.
Stain: Ehrlich's hematoxylin and Eosin tissue fragments in the lumen, in another area.

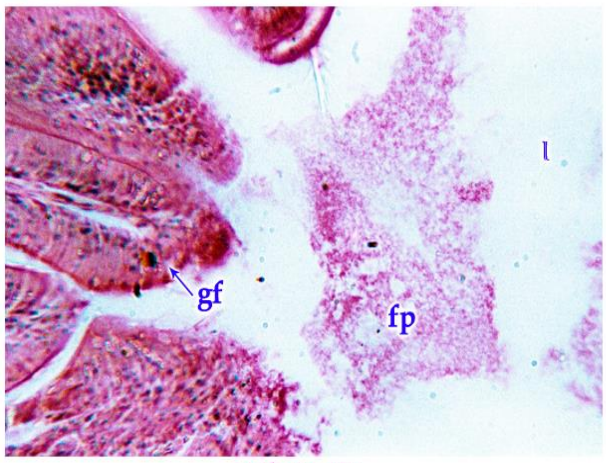

Plate 9

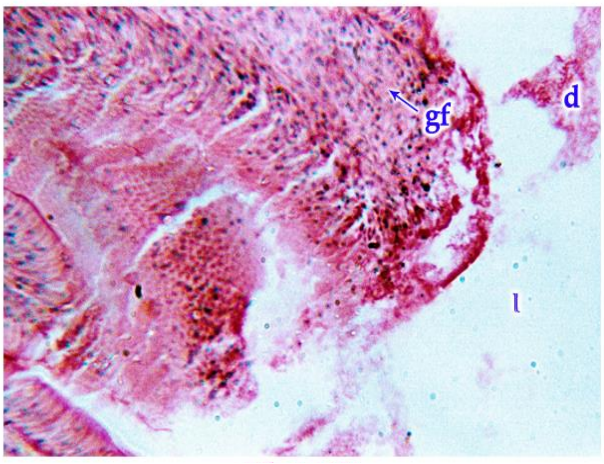

Plate 10

Plate No: 9 Delamination of gastric mucosa from the distal portions of gastric folds and accumulation of tissue fragments in the lumen.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 10 Delamination of gastric mucosa from the distal portions of gastric folds and accumulation of tissue fragments in the lumen, in another section.
Stain: Ehrlich's hematoxylin and Eosin

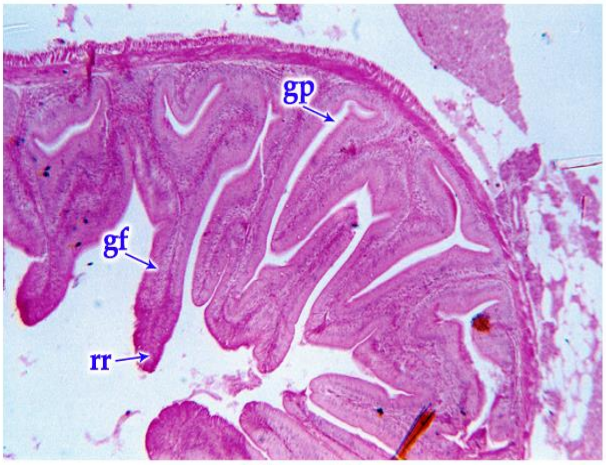

Plate 11

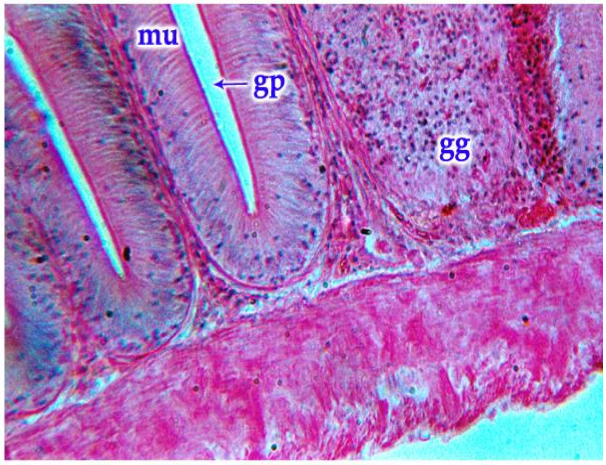

Plate 12

Plate No: 11 Gastric folds are very wide and elongated. The stomach wall is normal.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 12 Gastric folds maintain normal histology, There is an increased blood supply in submucosa.and the stomach wall is thick. .
Stain: Ehrlich's hematoxylin and Eosin

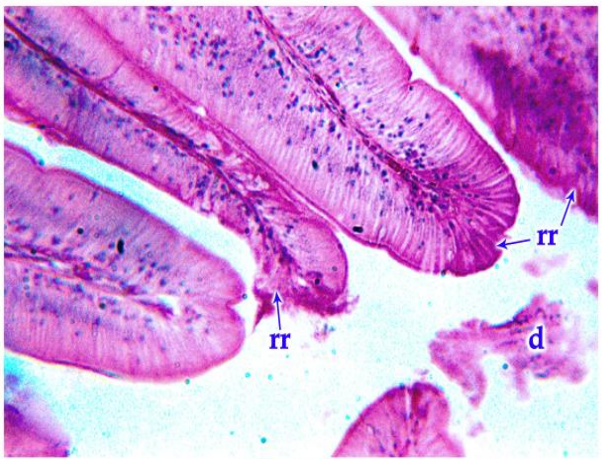

Plate 13

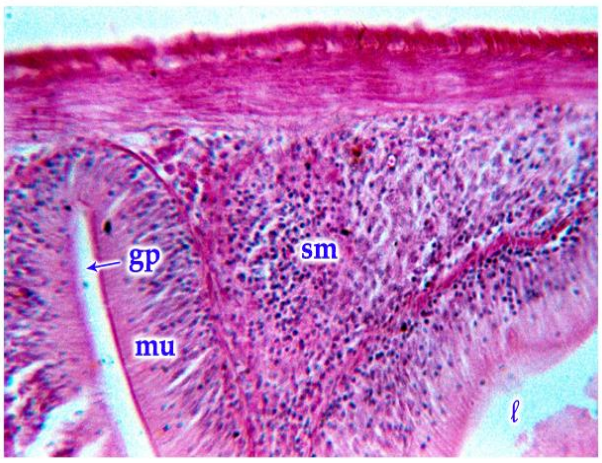

Plate 14

Plate No: 13 Distal halves of gastric folds maintain normal histology.
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 14 Section of stomach under high magnification: Base of a gastric fold and a gastric pit showing disturbed mucous epithelium and sub mucosa with heavy supply of blood indicates by R.B.Cs..
Stain: Ehrlich's hematoxylin and Eosin

Plate 15

Plate 16

Plate No: 15 Another section of stomach under high magnification: Tip of gastric folds showing dissociated mucous epithelial cells and sub mucosa with heavy supply of blood indicated by R.B.Cs..
Stain: Ehrlich's hematoxylin and Eosin
Plate No: 16 Section of stomach under low magnification: Elongated, branched and fused gastric folds.
Stain: Van Gieson

Conclusion:

The Present Study concludes that both the pesticides, Endosulfan and Fenvalarate are equivalently detrimental to the study fish, *Puntius conchonius* even at sublethal concentrations. This study also confirms that the histopathology of the stomach of fish can be taken as a bio-indicatior of aquatic pollutions.

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