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Histopathological alterations in the gills of *Garra mullya* (Sykes) exposed to sub lethal concentrations of bioneem and bifenthrin

Surekha Bansode*, Rajendra Patil
Department of Zoology, A. C. S College, Navapur, Dist- Nandurbarm, Maharashtra, India

Abstract
Histology, the structural science serves to complement anatomy, physiology and pathology as well as functioning of tissues and organs of living organism. The histological structure of gill was found to be badly affected due to pesticides, after acute and chronic exposure. Histopathological changes induced by sublethal (96 hours LC_{50}/10) exposure to Bifenthrin (0.1253 ppm) and Bioneem (16.7455 ppm) on the gills of a fresh water fish *Garra mullya* (Sykes) were studied. Degenerated secondary gill filament, appearance of necrotic tissue, atrophy of secondary lamellae and separation of epithelial cells are the lesions seen in Bifenthrin exposed fishes. While Bioneem though biopesticide, prolonged application resulted in lamellar necrosis and hypertrophy of secondary lamellar cells at the base. Changes are more pronounced in Bifenthrin treated fishes than in Bioneem.

Keywords: bifenthrin, bioneem, *Garra mullya*, gills, histopathology, sublethal conc

Introduction
Histopathological biomarkers provide a rapid sensitive and reliable method to detect effects of pesticides (Johnson *et.al*, 1993) [5]. Chemical contaminant induces number of lesions in different organs of fish after exposure to toxicant (Bucke *et.al*, 1996) [2]. Several pollutants could initiate the formation of a specific enzyme that brings about changes in metabolic activities further leading to cellular intoxication and death of the cell, which manifests as necrosis. This is histopathological biomarker at a tissue level.
Histopathological techniques are found to be rapid, sensitive and reliable. These are comparatively inexpensive tools for the assessment of stress response to pollutants. This finally helps to predict safe concentration of contaminant. Pacheco and Santos (2002) [8], mentioned importance of morphological changes of gills in bio monitoring programs as they are used in the defence mechanism to potential stressors of the aquatic environment. Pesticides used in the present study are Bifenthrin and Bioneem.
Bifenthrin [(2-Methyl-3-phenylphenyl) methyl (1S, 3S)-3-[(Z)-2-chloro-3, 3, 3-trifluoroprop-1-enyl] - 2, 2-dimethylcyclopropane-1-carboxylate] is third-generation synthetic pyrethroid insecticide characterized by and high insecticidal activity and strong environmental persistence (Mokry and Hoagland, 1989) [7]. Bioneem, a biological pesticide used in the present study is extracted from neem seed kernels as an insecticide. It can disturb number of vital physiological processes so that their activity of the insects is strongly affected. (Anisuddin Siddiqui; 2013) [1]
As fish gills are located externally and have large surface area, they are vulnerable to toxicants in aquatic reservoirs. *Garra mullya* (Local name – Molga) is commonly preferred as a food fish by tribal community in the local market of Nandurbar District. Limited histopathological studies have been reported with fresh water fish *Garra mullya*. Therefore the work was undertaken to study histopathological changes in the gill tissue after sublethal exposure of Bifenthrin and Bioneem.

Material and Methods
The experimental medium sized fresh water fishes *Garra mullya* were collected from Bhavare dam, Tal- Navapur, District Nandurbar. They were brought to the laboratory condition in well aerated and dechlorinated water and acclimatized in glass aquarium for one week. The acclimatized fishes *Garra mullya* were exposed to sub lethal (LC_{50}/10 values of 96 hours) concentrations of pesticides i.e. Bifenthrin (0.1253 ppm) and Bioneem (16.7455 ppm). After an interval of 7 days fishes were dissected. Gills were removed and further processed for histopathological study up to 21st days for control and treated groups.
The gills of sacrificed fishes were fixed in alcoholic Bouin's fluid, dehydrated through a graded series of ethyl alcohol. After clearing in xylene gills were infiltrated and then embedded in paraffin. Gill sections (6µ) were then stained with Haematoxylin - Eosin and observed for the lesions.

Result
Structural details of control gill of fresh water fish *Garra mullya* are given in the figure 1. Exposure to sublethal concentration of Bifenthrin (0.1253 ppm, LC_{50}/10 of 96 hours), showed degenerated secondary gill filament, appearance of necrotic tissue, atrophy of secondary lamellae, separation of epithelial cells, atrophy of secondary lamellae and sloughed off secondary lamellar cells were observed along with fusion of secondary gill filaments (figure 2).
Continuous exposure to sublethal concentration of Bioneem (16.7455 ppm) for 21 days revealed lamellar necrosis and hypertrophy of secondary lamellar cells at the base were observed. Increase in the size of mucous cells and swelling of

tips of secondary lamella were also noticed during the exposure period (figre3).

Comparison of different lesions seen in the gill tissue exposed to sublethal concentration of Bifenthrin and Bioneem is shown in Table. 1. Changes are more pronounced in Bifenthrin treated fishes than in Bioneem.

Discussion

Number of workers has reported the histopathological effects of various toxic substances on fish. Carpenter (1927) [3] reported the coagulation of mucous over gills which would impair the respiratory function resulting in death of fish. Histological changes such as epithelial hyperplasia, oedema, lamellar telangiectasis and swelling of cells and deformities of nucleus due to parathion and malathion were reported by Metelev *et.al,* (1971) [6] and Walsh and Ribelin (1975) [10]. The histological changes are of serious nature as these are bound to adversely affect the respiratory physiology of the fish. Hyperplasia of branchial epithelium noticed in this study may be attributed to low oxygen tension faced by fish under the toxic stress of Bifenthrin and Bioneem. Similar opinion has been advocated earlier (Elller, 1975; Roberts, 1978) [9].

Present investigation revealed fusion of secondary lamellae in the gills exposed to Bifenthrin and Bioneem may be a safety measure against toxicant. The damage in the gill epithelium noticed in this study reduced the respiratory area and increased the diffusion distance thereby reducing the respiratory and osmoregulatory efficiency of the fish.

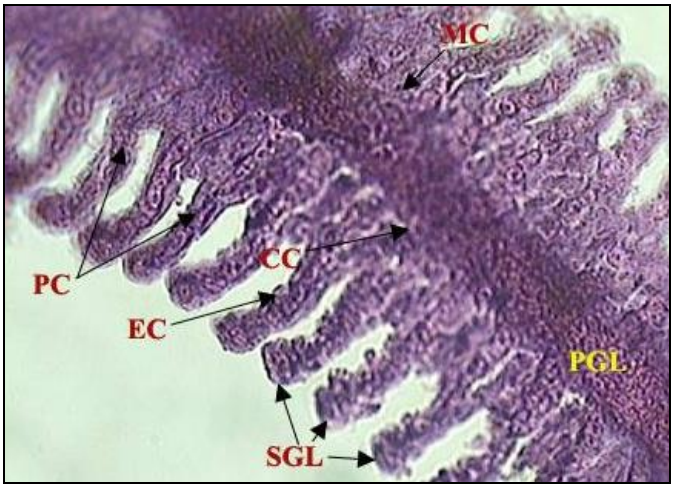
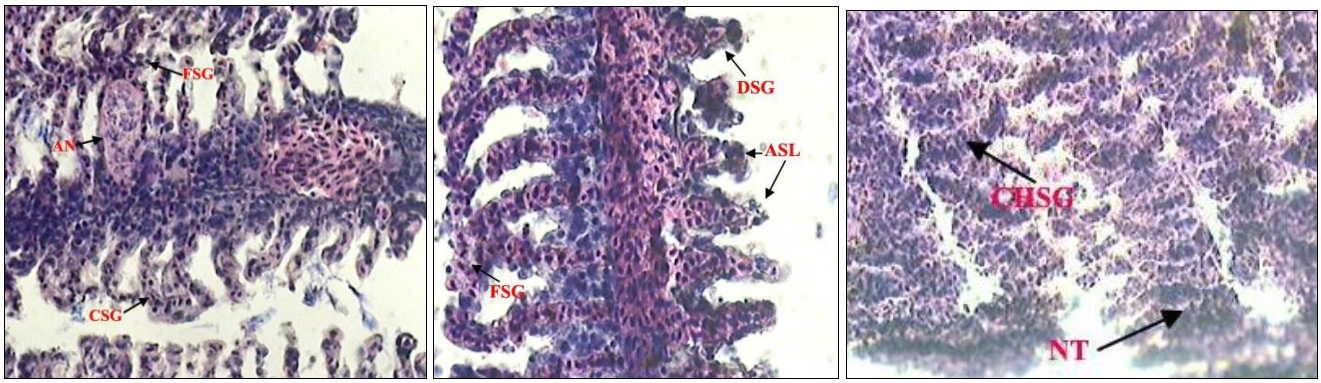
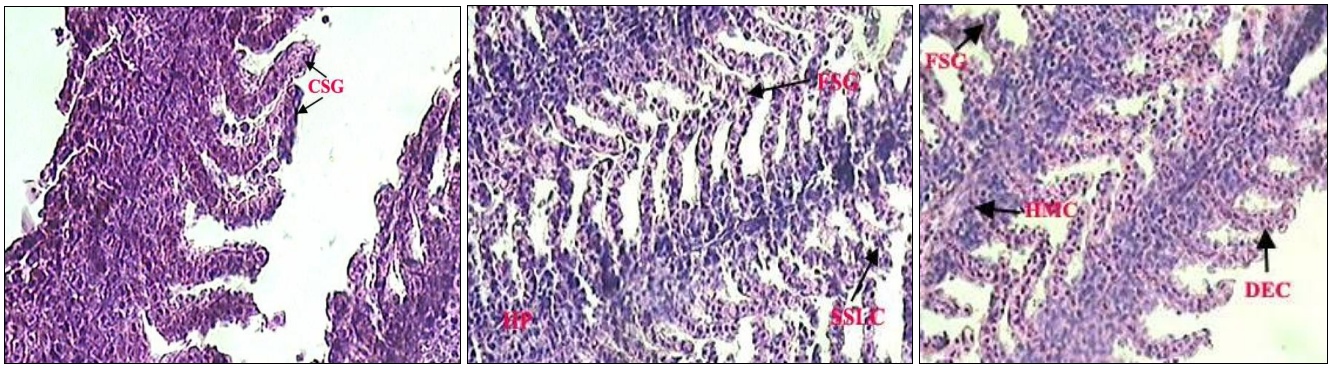


Fig 1: Microphotograph of longitudinal section of Gill of *Garra mullya* (Sykes) Showing Normal architecture (H & E; 400 X) [PGL: Primary Gill Lamella; SGL: Secondary Gill Lamellae; PC: Pillar Cells; EC: Epithelial cells; CC: Chloride cell; MC: Mucous cell]



7 Days exposure 14 Days exposure 21 Days exposure

Fig 2: Exposure to sublethal concentration of bifenthrin



7 Days exposure 14 Days exposure 21 Days exposure

Fig 3: Exposure to sublethal concentration of bioneem

Table 1: Histopathological lesions in gill of *Garra mullya* (Sykes) exposed to sublethal concentration of Bifenthrin and Bioneem

	CSG	HMC	DSG	ASL	CHSG	AN	FSG	SSLC	HP	NT
Control	-	-	-	-	-	-	-	-	-	-
Bifenthrin	+	-	+	+	+	+	+	-	-	+
Bioneem	+	+	-	-	-	-	+	+	+	-

(-): Lesion absent; (+): Lesion present

CSG: Curling of Secondary Gill Filament; SSLC: Sloughed off Secondary Lamellar Cells; HP: Hyperplasia of Primary lamellar Cells; FSG: Fusion of Secondary gill Filaments; HMC: Hypertrophied Mucous cell; NT: Necrotic Tissue; AN: Aneurysm; DSG: Degenerated Secondary Gill; ASL: Atrophy of Secondary lamellae and CHSG: Cellular Hypertrophy of Secondary Gill.

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