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Histopathological analysis of the gills of fresh water fish *Channa gachua* exposed to fungicide Pydiflumetofen

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ABSTRACT

Indiscriminate use of pesticides has become a serious problem among human and ambient environment. The present study is aimed to assess the damage caused to the fish *Channa gachua* (family: Channidae) exposed to fungicide Pydiflumetofen. Respiration in *Channa gachua* mainly occurs through their gills. Any change in the chemical quality of water directly affects the structure and function of gills. Fish gill also helps in osmoregulation and excretion besides respiration. When fish exposed to sub-lethal concentration of Pydiflumetofen pesticide for 10 days, 15 days and 20 days include Curling and fusion in secondary lamellae, Hyperplasia, reduction in the respiratory surface area of filaments, fusion at the tip of the lamellae, sub lamellar and sub epithelial space occur.

KEYWORDS: Histopathology, gills, *channa gachua*, pydiflumetofen.

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INTRODUCTION

Different industries like distilleries, cotton mills, tanneries, paper mills, jute mills, fertilizers plants and chemical plants pass out their effluents in adjoining rivers, ponds, ditches and other water resources. Apart from these, the run-off water from agricultural fields carries a lot of pesticides, herbicides, fungicides and weedicides. All these chemicals threaten the existence of flora and fauna and adversely affect the ecological balance leading to unwanted mortality of aquatic biota including fishes [1,2]. Though, the survey of literature reveals that a lot of work has been carried out on various aspects of toxicity of different chemicals to fishes, there is little information on the toxicity of parathion. In the present study, an attempt has been made to assess the toxicity of an organophosphate insecticide, parathion on a freshwater air-breathing fish, *Channa gachua*.

Channa gachua is one of the important fresh water fish in the tropical region of India belonging to the group of an air breathing teleost. It is commonly called 'Chenga'. It can survive out of water for a considerably long period if the skin remains moist. This adaptability is occurs due to presence of four pairs of gills, a pair of supra-brachial chamber and highly vascular dendritic plates which together constitute the air breathing organ of fish. Fish gills are the dominant site for gas exchange, ion regulation acid-base and nitrogenous waste excretion. Gills are primary site of toxicities due to pesticides, because of the close association between gill and the medium in which the fish lives. When fish

is exposed to environmental pollutants these vital functions are deleteriously affected and the functional impairment of the gill can significantly damage the health of fish [3,4,5]. Many investigators have reported the histopathological changes in the gills of different fish species exposed to pesticides [6-11]. Fish live in intimate contact with the surrounding water through their gills [12] whose surface comprises over half the body surface area [13]. Only a few microns of delicate gill epithelium separate the internal environment of the fish from a continuously flowing external environment [13] thus, it makes the fish very susceptible to aquatic pollutants. Pydiflumetofen, a carboxamide pesticide is extremely toxic to fishes. Pydiflumetofen is succinate dehydrogenase inhibitor. Pydiflumetofen has been in agricultural use over the last three years for controlling pest of soybeans, cereals, vegetables, corn, pranut, potato, grapes.

MATERIAL AND METHODS

Channa gachua were collected from local ponds and paddy fields of Muzaffarpur district and brought to the laboratory. Healthy fish size (16-20cm) and weight (20-25g) were washed with 0.1% KMNO₄ to remove dermal infection. Fishes were acclimatize in glass aquaria for 15 days. The water of aquaria was replaced by fresh water at every 24 hours to remove faces and food remnants. Fishes were fed with chopped earthworm and commercial food. Feeding was stopped a day before the initiation of bio-assay test. The physio-chemical properties of

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water used for the fish bio-assay test were determined according to the procedure described in standard methods [14]. The water quality parameters were as follows: PH=7.2, temperature =270 °C, dissolve oxygen =6.8 mg/l, CO₂ =8 mg/l, hardness in CaCO₃ =56mg/l, alkalinity as HCO₃ =130 mg/l. For the preparation of stock solution, 1 ml of Pydiflumetofen was dissolved in 1 lit. of doubled distilled water. This stock solution was used for preparing different concentration of Pydiflumetofen in water.

It was stored in flask at room temperature in laboratory. The bio-assay test to determine the toxicity of Pydiflumetofen was done following the method described by APHA [14]. Four groups (A = control, B = 10 days, C = 15 days, D = 20 days) of ten fishes in each of four aquaria with 10 lit of water were exposed to 0.06 ppm (1/5th of LC₅₀ for 96 hours). The experimental and control both media were renewed after 24 hours to maintain the effective concentration. At any stage of exposure no mortality was observed. At different exposure period gills were dissected out and fixed into Bouins' fluid and 10 per cent Formalin. After fixation for 24 hours, tissues were dehydrated through a graded series of ethanol, cleared in xylene and block were prepared in Paraffin wax 6 μ m thick section were cut on microtome. These sections were stained with Hematoxylin and alcoholic eosin (dissolved in 70 % alcohol) and mounted in DPX for routine histopathological analysis. The tissue samples were investigated by using a light microscope.

RESULTS AND DISCUSSION

Channa gachua is an air breathing fish having four pair of gill that helps in respiration. The gills can be studied under two parts, first bony part called gill head and second gill filaments. The gill head consists taste buds, connective tissue, gill arch and gill rakers. The gill filament consists gill septum, gill rays, blood vessels, pillar cells mucous cells abductor and adductor muscles joining two hemi branches. In T.S of control gill, the secondary lamellae attached to primary filament. The secondary lamellae of one side hemi-branch alternate with other side of hemi-branch and arranged parallel. The primary and secondary gill filaments separated by a thin septum (Figure 1). After 10 days of exposure to 0.06 ppm concentration of Pydiflumetofen, the gill tissue showed curling of secondary lamellae resulting in distention of the lamellae. The gill also shows fusion of secondary lamellae at some places (Figure 2). After 15 days of exposure to 0.06 ppm concentration of Pydiflumetofen causes Hyperplasia of mucous cells occurred in gill, this reduced the respiratory surface area of some filaments (Figure 3). After 20 days of exposure to 0.06 ppm concentration of Pydiflumetofen, showed fusion at the tip of the lamellae occur. It also showed sub-lamellar space and sub-epithelial space (Figure 4). Gills are generally considered good indicator of water quality. The gills are the primary route for the entry of pesticide. Gills are primary respiratory organs and all metabolic pathways depends upon the efficiency of the gill for their energy and damage to this organ cause a chain of destructive events, which ultimately lead to respiratory distress [15]. If gill would be destroyed due to Xenobiotic [16] or the membrane function are destroyed by a changed permeability [17]. In the initial stage of exposure

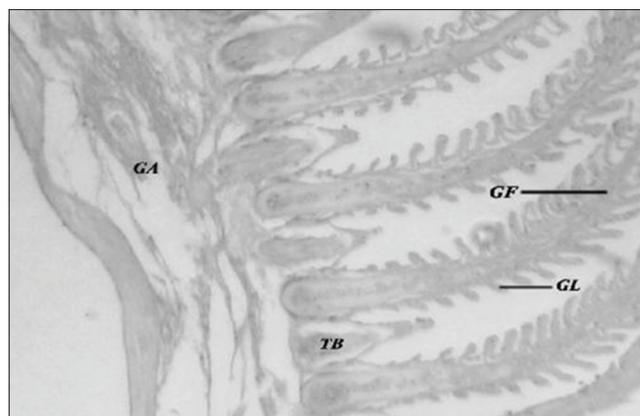


Figure 1: T.S of Normal Gill showing Gill Lamella, Gill Filament, Taste Buds and Gill Arch (H/E 120)

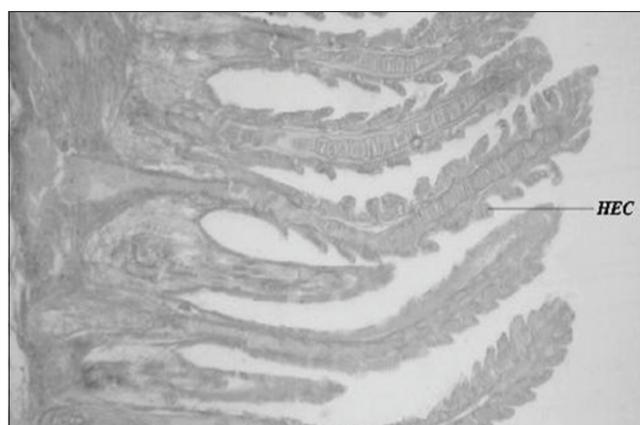


Figure 2: T.S. of Pydiflumetofen Treated Gill (after 10 days) showing curling in secondary lamellae (CSL) and fusion in secondary lamellae (FSL)

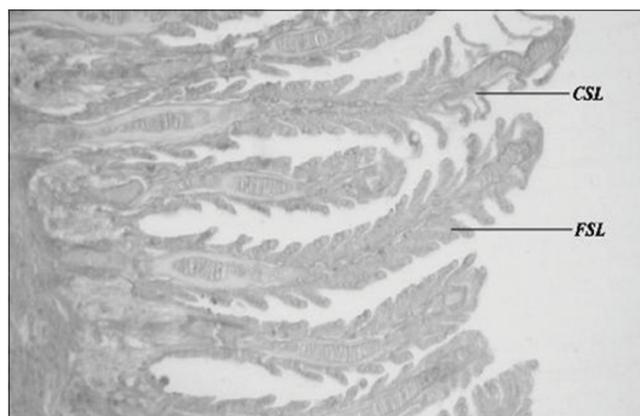


Figure 3: T.S. of Pydiflumetofen Treated Gill (after 15 days) showing Hyperplasia in epithelial cell (HEC)

of Pydiflumetofen showed curling and fusion of secondary lamellae resulting in distention of the lamellae. As a result of fusion lamellar surface may be reduced by 75 per cent. Previous report [18] shows similar types of gill lesions in zinc treated *Heteropneustes fossilis*, mercury treated *Cirrhinus mrigala* and chromium treated *Labeo rohita*. After 15 days of exposure, hyperplasia of mucous cell observed in the gill. The observed

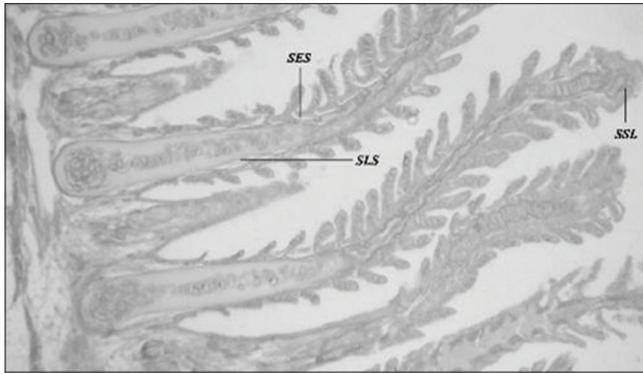


Figure 4: T.S. of Pydiflumetofen treated gill (after 20 days) SSL= swelling at the tip of Lamellae, SES= Sub epithelial space, SLS= sub lamellar space

hyperplasia of the cell epithelium and lamellar fusion could have interfered with the efficiency of the gill resulting in reduce gases exchange.

Hyperplasia of secondary lamellae has been reported in organism exposed to environmental pollutants often associated with the complete fusion of two neighbouring secondary lamellae [19,20]. These observation can compare with pathological lesion induced in gill by mercuric chloride in *Acipenser persicus* fry, by lead and cadmium treatment in *Cyprinus carpio*, *Lates calcarifer* [20], *Brachydanio rerio* and *Salmo gairdneri*. When the exposure period of Pydiflumetofen increased it showed detachment of epithelial layer with reduction of inter-lamellar space, and lamellar swelling at the tip. Similarly epithelial lifting, proliferation of epithelial cells of primary and secondary lamellae, hyperplasia of mucous cells and necrosis of epithelial cells in the gills of *C. nasus* and *L. cephalus* from river Mures. Such changes have also been reported by Dhanapakiam et al. [21] in *Channa punctatus* exposed to industrial effluent. Similar observation have been reported by Ghanbousi et al. [22] in the fish, *Aphanius dispar* exposed to deltamethrin showed fusion of secondary lamellae, lifting of lamellar epithelium and hyperplasia of chloride cells. Deng et al. [23] reported that the gill lesion (Epithelium hyperplasia and Lamellar fusion) observed in the Sacramento splittail larvae. In the present study severe respiratory distress, behavioural changes, rapid opercular movements, leading to the higher amount of toxicant uptake, increased mucus secretion, higher ventilation volume, decreases in oxygen uptake efficiency and engulfing of air through the mouth were observed in *Channa gachua* exposed to the Pydiflumetofen.

CONCLUSION

The respiratory system provides the most extensive surface of a fish with the aquatic environment. The primary aquatic air breathing organ of *Channa gachua* is four pairs of functional gills. Fish gill remains in direct contact with ambient water. It is easily affected with the toxicants like pesticides. Pydiflumetofen as an organophosphate pesticide used as its histopathological effect on the gill of *Channa gachua* fish. The present investigational have revealed that the Pydiflumetofen which is

usually released into the water system through leaching or run off water from agricultural operation have enough potential to cause different cellular alterations and even death to the fish. The pathological alteration in the gill of *Channa gachua* have been observed in laboratory condition after exposing to the sub-lethal concentration of Pydiflumetofen (0.06ppm) during different time period (10days, 15 days and 20 days). The gross pathological changes after Pydiflumetofen exposed includes: Curling and fusion of secondary gill lamellae, hyperplasia of epithelial cells, fusion at the tip of secondary lamellae, sub lamellar space and sub epithelial space. It is advice to use safe concentration of pesticides for conservation of environment.

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