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Biochemical and Histological Impact on Sub-lethal Concentration of Chlorpyrifos on *Channa punctataus* (Bloch) Fish

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ABSTRACT: Chlorpyrifos is an organophosphate pesticide, it is used world wide. It take pivotal part in agricultural field to kill the pests in order to produce the highly verities of crop. The exposure of Chlorpyrifos shows the magnificent changes and alteration in non target organisms like fish. In present work Channa punctatus was used as non target animal. The fishes were acclimatized near about 10 days, dead and infected fished were removed immediately. The sub lethal concentration, 24 hours, 96hours, 7 days, 15 days and 30 days respectively was studied during research work. The work showed, gradual decline of protein as well as histopathylogical change in gill.

Keywords: *Channa punctatus*, Chlorpyrifos, Protein, Gills.

INTRODUCTION

Pollution problem is presently agonies throughout the globe, with the hike of manufacturing, urbanization, excess use of agrochemicals like pesticides, herbicides, fungicides etc. Pesticides are serious threat for aquatic ecosystem like fish. Although fishes are more frequently exposed to the pollutants and may be taken in through gills, skin, contaminated food. It has also great impact on sprayer farmers, most of health issues are caused by pesticides. Although pesticides are used in agro field, it is washed out by rain and ultimately reaches into water bodies like pond and rivers which alters the physicochemical properties of water and shows the toxic alteration in aquatic organisms, and can even cause death of an aquatic animals. The pesticide can enter through the food chain and their consequent bioaccumulation and biotransformation at different levels have catastrophic effect to the ecosystem (Grande *et al*., 1994). The fishes have provide connotation as bio indicators of the aquatic environment called ecological integrity (Faggio *et al*., 2014; Gobi *et al*., 2018; Bartoskova *et al*., 2013). The aquatic animals were basic species in many ecosystem (Lonsdale *et al*., 2009). Fish is one of the most that aquatic organisms which is widely distributed in its ecosystem and reflect the biological effects of environment pollution.

Chlorpyrifos (CPF) [O, O–diethyl–O (3,5,6-trichlor-2-pyridyl) phosphorothioate] is considered second largest selling organophosphorus in India. This organophosphate has various adverse effects on aquatic organisms as well. It can enter inside through a various dramatic ways, the main rout of entry into the body is through inhaling, injection, or dermal contact. The chlorpyrifos is transformed into chlorpyrifosoxon within the body of animal, and is highly toxic than chlorpyrifos (Siddiqua *et al*., 2016).

Histopathological studies plays key role in the study of sensitive and crucial parameters reflect the of toxicants on different organs (Abdel Warith *et al*., 2011). Histopathological studies give an idea of a cost effective tool to determine the health of fish populations. The gills are connected with water breathing. The occur in two rows over the pharyngeal region. They are connected to pharyngeal slits. The gills are supported by skeletal structure known as branchial arches. The gills are mostly used in the estimation as well as the impact of aquatic pollutants in freshwater habitats (Fernandes *et al*., 2007).

MATERIALS AND METHODS

Channa punctatus a fresh water fish was collected was collected from wadali lake Amravati from a fisher man, The wadali lake a near about 7 kilometres away from the GVISH college Amravati state Maharashtra. Fishes were acclimatized to laboratory condition in Fisheries Research lab. Department of Zoology, Government Vidarbha Institute of science and Humanities Amraviti. The infected and dead fishes were removed immediately. After two weeks the fishes were divided into groups and kept in aquaria 30 L.

Physiochemical parameters maintained in aqueous medium.

Parameters	Values
Biological oxygen demand mg/L	4.2
Temperature	26-30
Ph	8.1
Dissolved oxygen	5.9
Total hardness	244.8

The water parameter were constantly maintained throughout the experiment (APHA, 1998). The food pellets for fish were provided ad libitium (Affonso *et al*., 2002). In histochemical analysis protein was examined from Gill tissue of fish by Lowry *et al*. (1951). In histopathology the tissue preparation for gill observation was done. The fish was sacrificed in a Petri dish then gills were extracted during dissected and immediately fixed in Bouins fluid (Gurr, 1962), fixative 48 hours and dehydrated in an alcohol series, then they are cleared in xylene, infiltrated with liquid paraffin at 58 °C, and finally embedded in paraffin blocks. The desired blocks were trimmed and sectioned at 5 µm thick cut on a rotary microtome. The fixed sections were stained with Hematoxylin and Eosin stain, and prepared slide was observed under a light microscope.

The LC_{50} calculation was used for probit analysis tool. The data were presented as mean ± SD. ANOVA was performed as a statistical analysis for using one way analysis of variances.

The statistical analysis was done by manually and cross checked SPSS.

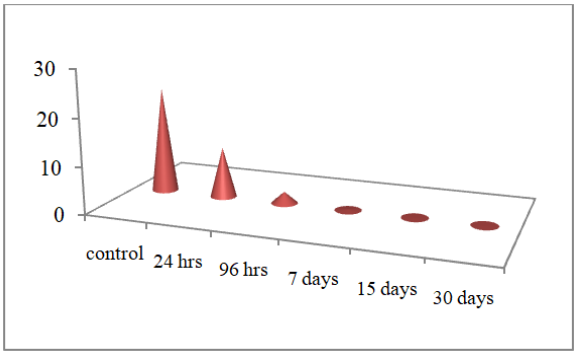
RESULT

Chlorpyrifos, a dreadful agony having adverse effect on all living organisms. The present study shows the significant decrease in protein due to sub lethal concentration exposure of chlorpyrifos in kidney. Below, the table shows gradual change and difference or significant change between control and 24 hrs, 96 hrs, 7 days, 15 days, 30 days in the gills of fresh water fish *Channa punctatus*.

Changes in Total Protein level in gills of fresh water fish Channa punctatus exposed to organophosphate pesticide Chlorpyrifos at experimental exposure period (mg/ 100 mg wet wt. tissue).

Control	24 hrs	96 hrs	7 days	15 days	30 days
22.60±1.77	10.78±1.15**	2.49±0.72**	0.23±0.12**	0.06±0.01*	0.03±0.01*

Values in mean ±S.E. (standard deviation) n=5,*P<0.05, **P<0.01, ***P<0.001 when compared with control, ns = non signification.



Changes in protein content (mg/g wet wt of tissue) in Gill of fresh water fish Channa punctatus, exposed to sublethal concentration of glyphosate for 24 hrs, 96 hrs, 7 days, 15 days and 30 days.

HISTOPATHOLOGICAL ALTERATION

Aquatic respiration includes respiratory organs like gills. Gills are plate like or filamentous, highly vasculated and sacculated outgrowths which are bathed with water for exchange of gases. In fishes gills are connected with water breathing. The gill occurs in two rows over a pharyngeal region. They are connected to pharyngeal slits. The gills are supported by skeletal structure called branchial arches. The gills are covered by operculum in bony fishes and uncovered in cartilaginous fishes. Each gill consists of two rows of primary lamella and each primary lamella having numerous secondary lamella. The counter current exchange of gasses occur in secondary lamellae between blood and water current. The exposure of chlorpyrifos on gills showed the various modification and alteration. The gills in control showed normal cellular orientation, while the gills of the groups exposed to different concentration of chlorpyrifos for 96 hrs to 30 days showed general cellular disorientation. Detached cuticle, Lamellar Fusion, Swelling of secondary lamellae, Hyperplasia, Curling of secondary gill lamellae. Degeneration of epithelium in secondary gill lamella, Necrosis, Hyperplasia, Infiltration of haemocytes, Rapture of capillaries.

Gills of freshwater fish *Channa punctatus* treated at control.

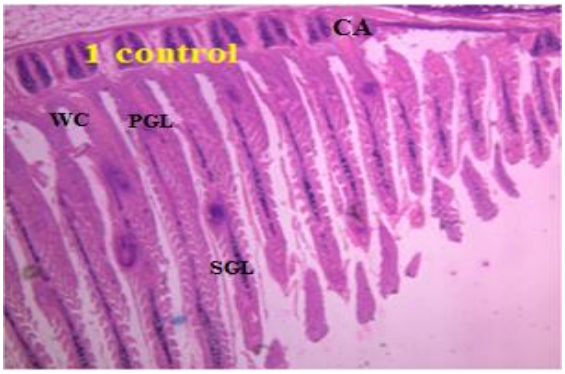


Fig. A. Control, gills: WC= Water canal, PGL=Primary gill lamella, SGL= Secondary gill lamella, CA= corn axis

Gills of freshwater fish *Channa punctatus* treated with Chlorpyrifos.

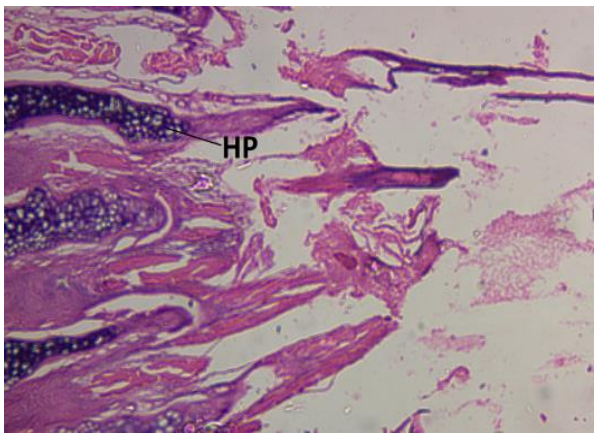


Fig. B. 24 hrs exposure of chlorpyrifos, HP = Hyperplasia.

Gills of freshwater fish *Channa punctatus* 96 Hours exposure of Chlo.

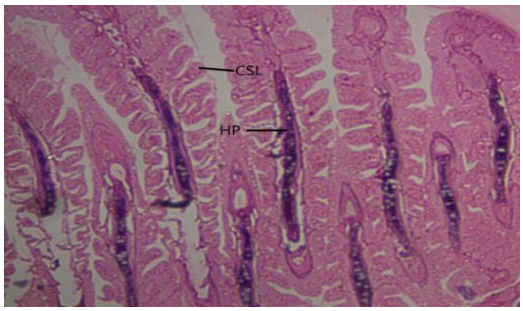


Fig. C. 96 hrs. days exposure on gills: HP= Hyperplasia, CSL= Curling of secondary gill lamella

Gills of freshwater fish *Channa punctatus* 7 days exposure of chlo

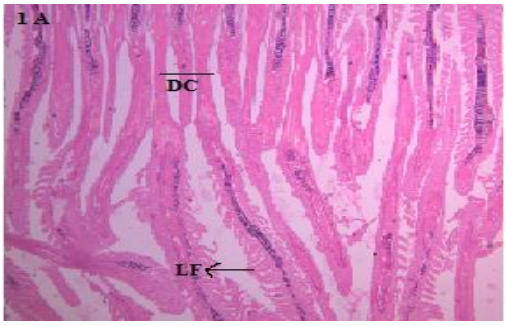


Fig. D. 7 days exposure on gills:DC=Detached cuticle, LF= Lamellar Fusion

Gills of *Channa punctatus* 15 days exposure of Chlo.

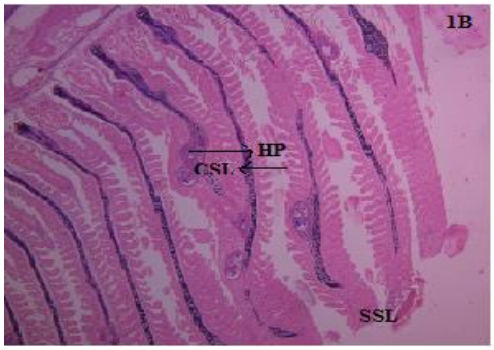


Fig. E. 15 days exposure on gills : SSL=Swelling of secondary lamellae, HP= Hyperplasia, CSL= Curling of secondary, gill lamella

Gills of freshwater fish *Channa punctatus* 30 days exposure of Chlor.

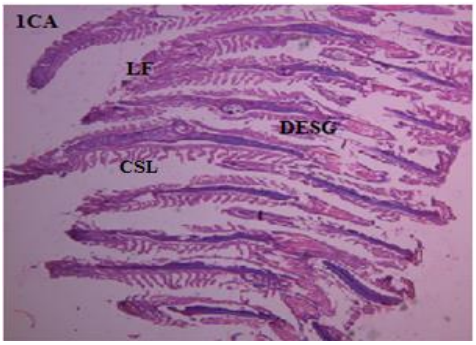


Fig. F. 30 days exposure on gills: CSL= Curling of secondary lamella, DESG= Degeneration of epithelium in secondary gill lamella.

DISCUSSION

In the present study, the effect of sublethal concentration of chlorpyrifos has been studied in gills of fresh water fish *Channa punctatus.* The comparative analysis of the value of protein in fish exposed to sublethal concentration of pesticides gives evidence that there is decrease in protein content of fish in gills highly fluctuation were found due to the exposure and reduction was observed by chlorpyrifos. Significantly decrease in protein level of liver, muscle, intestine, gills and blood of fresh water fish *Channa punctatus* up on the exposure of oleondrin and Cyprinus carpio to endosulfan have been observed (Jenkins *et al*, 2003; Tiwari and Singh 2009).

Chlorpyrifos exposure shows various histological alterations in the gills lamellae, swollen tip of secondary lamellae, necrotic lamellae, lifting of lamellae epithelium, degeneration of epithelium in secondary gill lamella, Curling of secondary lalella, Lamellar fusion, Hyperplasia proliferation of chloride cells in fresh water fish *Channa punctatus*. Zahran *et al*. (2018), observed the histopathological changes in the gills of Nile tilapia (*Oreochromis niloticus*) on exposure to different doses of chlorpyrifos. Fifteen grams per liter of chlorpyrifos can cause damage in the histoarchitecture of secondary lamellae such as decreased interlamellar space, curved and fused tips of secondary lamellae, epithelial hyperplasia at the base of secondary lamellae, mucous accumulation inside secondary lamellae, and necrosis severity at the tip of primary lamellae. Devi and Mishra (2013), reported the damages in the gills of *Channa punctatus*, included the hypertrophy and proliferation in the erythrocytes of cartilaginous core, whole destruction of the secondary lamellae, and lifting of epithelial cells for a period of 7 days exposure. Karmakar *et al*. (2015) reported fusion of primary and secondary lamellae, curling of secondary lamellae, epithelial hyperplasia, and decrease of inter lamellar cell mass with increasing malathion concentration (10, 50, and 100 µg/L) in gills of fish *Labeo rohita*. In present study LC_{50} of chlorpyrifos was 0.55ppm/l, in which the 50% of fishes were dead.

CONCLUSIONS

The pesticide like organochlorine in which chlorpyrifos as a topic of interest was considered very harmful for both flora and fauna in an aquatic medium. The chlorpyrifos is mainly used in various orchids and agriculture purposes. As, is washed out through the rain and ultimately reaches to the nearby river or lakes. And ultimately affects directly or indirectly on the fauna organisms which in turn affect the human health by food chain.

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