

Biochemical and histological impact on sub-lethal concentration of Glyphosate on *Channa punctata* (Bloch) fish

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Abstract

Herbicides are mainly used to protect the crops, and plays vital role in management practice for the benefit of agricultural productivity,. It take pivotal part in agricultural field to kill the pests in order to produce the highly verities of crop. Glyphosate in the surrounding, has payed a lot of attention towards the sub-lethal effects of chronic exposures on Non- target organisms. 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, 96 hours, 7 days, 15 days and 30 days respectively was studied during research work. The work showed, gradual decline of protein as well as histopathological change in gill.

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

Introduction

Pesticides are used in agricultural fields in order to control pests which are highly toxic to non target organisms like fish and affect the fish health through metabolism, ultimately leads to mortality (Shankar *et al.*, 2013). Due to the increase in human population and rapid pace of industrialization can cause problem of disposal of waste waters. The domestic wastes and industrial effluents, supplemented with pollutants such as heavy metals, many organic compounds, and pesticides are equally toxic also leads to massive fish death of aquatic ecosystems (Dhasarathan *et al.*, 2000). Proteins are important component and organic substances which are required in tissue building and repair. Upon acute stress conditions, protein suppliers energy in the biochemical reactions metabolic pathways (Winer, 1971). The proteins are primary and the most importance in the living world as well as also they appear to confer their biological specificity among different types of cells (Jha and Verma, 2002). As the proteins are organic substances, plays vital role in tissue building and energy metabolism in organisms (Yeragi, 2003). Rohankar *et al.*, (2012), states that, under the exposure of phosphamidon, shows gradual significant decrease in both soluble and insoluble proteins in muscle, liver, kidney and tissues of the fish *Channa punctatus*. Herbicides are mainly used to protect the crops, and plays vital role in management practice for the benefit of agricultural productivity, therefore it is one of the beneficial method for us. Economy in Indian is largely based on agricultural production, therefore, crop production and crop protection during the growing season while transport of harvested crops is of chief importance. As the large variety of weeds are usually encountered in crop fields causing 33% losses in crop yield (Majumdar, 2001). Near about 6000 tons of herbicides were used for weed control in India, in which irrigated crops (about 77% on rice and wheat) and on plantations (about 10%) in the onset of this century (Bhat and Chopra, 2006). India is gaining the use of agrochemicals at a very rapid rate, 25 to 30% per annum, which maintains a paramount status in the world

(Majumdar, 2001). Human beings are exposed to toxic herbicides or pesticides either directly or indirectly due to occupation or as residue while enters through water, air or food chains.

In histopathological, Langiano and Martinez (2008) observed that the degeneration of hepatocytes, nuclear pyknosis and nuclear vacuolization in *P. lineatus* caused due to the toxicity of an herbicide based glyphosate. 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.

Materials and Methods

A fresh water fish *Channa punctatus* was collected from fish market and wadali lake Amravati. Fishes were acclimatized to laboratory condition in Fisheries Research lab. Department of zoology, Government Vidarbha Institute of science and Humanities Amravati. The infected and dead fishes were removed immediately. After two weeks the fishes were divided into groups and kept in aquaria 30 L. The water parameter were constantly maintained throughout the experiment (APHA 1998).

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

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 LC₅₀ 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.

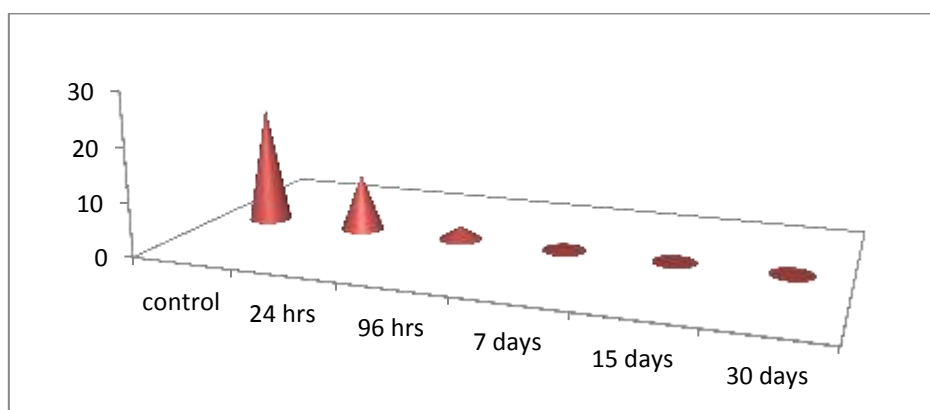
Result

Herbicides are utilized mostly in conjunction with pre emergence weed killers to burn down established annual weed. Herbicides are generally used in agriculture purposes. In present study the outcome result was significant decrease in protein due to sub lethal concentration exposure of Glyphosate in Gill. Below, the table shows gradual change between control and 24 hrs, 96 hrs, 7days, 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 Glyphosate at experimental exposure period (mg/100 mg wet wt. tissue).

Control	24 hrs	96 hrs	7 hrs	15 hrs	30 hrs
Gills	22.60±1.77	15.75±1.39* (-30.30)	1.21±0.64** (-94.64)	0.56±0.11** (-99.97)	0.19±0.02*** (-99.99)

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

In an aquatic mode of respiration where the respiratory organs are 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 occur in two rows over a pharyngeal region. They are connected to pharyngeal slits. Histologically gills of *Channa punctatus* are have numerous secondary lamellae which are lined up along the both sides of the primary gill lamellae, swelling of secondary lamellae, lamellar fusion, curling of secondary lamella, necrosis, hyperplasia, degeneration of epithelium in secondary gill lamella, infiltration of haemocytes, rapture of capillaries. Primary lamellar epithelium was provided with numerous mucous cells and chloride cells as well.. The gills in control showed normal cellular orientation, while the gills of the groups exposed to different concentration of Glyphosate for 96 hrs to 30 days showed general cellular disorientation.

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

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

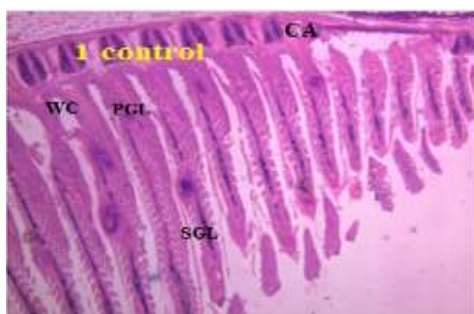


Fig. A. Control, gills: WC= Water canal, PGL=Primary gill lamella, SGL= Secondary gill lamella, CA= corn axis

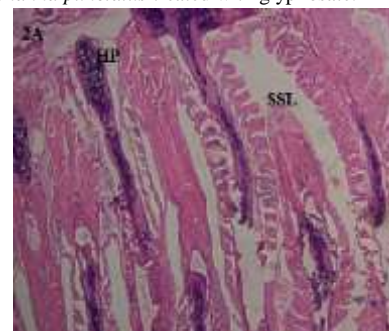


fig. B. 7 days exposure SSL = Sloughing of secondary lamellae PH = Hyperplasia

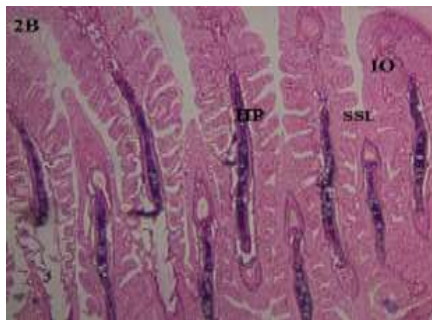
Gills of freshwater fish *Channa punctatus* 15 days exposure of glyph

Fig. C. 15 days exposure

HP= Hyperplasia,

IO= Intraepithelial oedema,

SSL = Sloughing of secondary lamellae

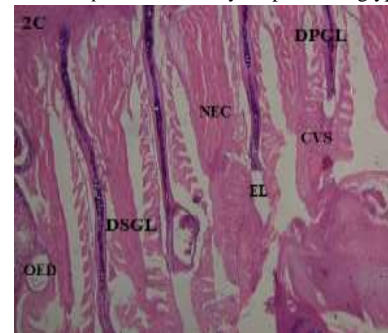
Gills of freshwater fish *Channa punctatus* 30 days exposure of glyph

Fig. D. 30 days exposure

DPGL= Degenerated primary gill lamella,

NEC= Necrosis, EL= Ephitel lifting,

CVS= Haemorage in the Central venous sinus,

DSGL= Degenerative secondary gill lamellae

OED= Oedematous

Discussion

In the present study, the effect of sublethal concentration of Glyphosate 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 glyphosate. Herbicides are pesticide which are commonly used to kill unwanted plants in the medium. Herbicides kill the plants, by affecting their biochemical mechanisms implicated in photosynthesis, respiration, growth, cell and nuclear division or synthesis of protein, carotenoides or lipids (Ecobichon, 1991). The use of herbicides can also effect on non target species and on environmental quality.

Fish gills are also recognized to be the first organ that directly contacts aquatic environmental pollutants due to their wide surface, anatomic location, and direct and continuous contact with the external environment and the fish gill is easy to be injured (Baxter et al., 2011), which often results in impairment of their physiological function, which results in poor growth and even mass mortality in fish (Peters et al., 2021).

Conclusion

It was concluded that the herbicide, Glyphosate as a topic of interest and is considered very harmful for both flora and fauna in an aquatic medium. The Glyphosate has less effect than organochlorine like chlorpyrifos as previously discussed. It is widely used in research field. Although it is used in orchid fields in order to control the pests. As, it 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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