IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(P): 2347-4580; ISSN(E): 2321-8851
Vol. 4, Issue 11, Nov 2016, 65-70

EFFECTS OF CHLORPYRIPHOS (20% EC), AN ORGANOPHOSPHATE PESTICIDE ON GILL AND LIVER OF A CATFISH *HETEROPNEUSTES FOSSILIS*, (BLOCH, 1794)

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ABSTRACT

Organ-histopathology appears to be an effective and sensitive monitoring tool to measure and analyse the health condition and level of pollution in aquatic ecosystem. The present study is an attempt to evaluate the toxic effects of Chlorpyrifos, an organophosphate pesticide on the gill and liver of a cat fish *Heteropneustes fossilis.* A long term experiment was performed for 60 days by using $1/10^{th}$ and$1/20^{th}$ of 96 hrs. LC_{50} value of Chlorpyrifos as sub-lethal concentrations, which were 0.178 mg/l and 0.089 mg/l respectively for *H. fossilis*. Simultaneously a control (exclusively toxicant free medium) group was also conducted in each species for comparison. The gills of exposed fishes showed alterations like degeneration of secondary gill lamellae, massive vacuolization, blood congestions and dilation of blood vessels etc., whereas liver of exposed fish exhibited changes like cytoplasmic degeneration, degeneration of hepatocytes, vacuolization and haemorrhages.

KEYWORDS: Organ-Histopathology, Heteropneustes Fossilis, Organophosphate, Chlorpyrifos

INTRODUCTION

Pesticides have brought remarkable benefits to mankind by increasing food production and controlling the vectors of man and animal diseases. At the same time use of these pollutants has posed potential health hazards to the life of aquatic organisms. According to Joseph and Raj (2011) modern agricultural practices result in indiscriminate use of various agrochemicals, which usually enter into the aquatic environment. The use of agrochemicals in the field has the potential to change the aquatic medium, affecting the tolerance limit of aquatic fauna and flora, as well as these chemicals adversely affect the non-target organisms, especially plankton and fish. Rand and Petrocelli (1984) have shown that the amount of pesticide which is applied to the area of operation, only 0.1% of them reaches the specific target. According to Singh *et. al.*, (2009), among different cases of pesticides, organophosphates are more frequently used, because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment. The Organophosphate pesticides are considered to create a greater human health hazard than the other families of pesticides. Histopathology of vital organs of fish is increasingly being utilised as good biomarkers of xenobiotic experiments. So the present work was conducted to measure the toxic effects of Chlorpyrifos (an organophosphate pesticide) on histopathology of gill and liver of an air-breathing catfish *Heteropneustes fossilis.*

METERIALS & METHODS

Fingerlings of *Heteropneustes fossilis* (mean length 9.7±0.9 cm and mean weight 13.0±1.2 g) were collected from local hatchery and carefully brought into the laboratory. Fishes were then treated with 0.05% $KMnO_4$ solution for disinfection and transferred to the cemented vats (mean diameter 62 cm, mean depth 30 cm and each vat holds approximately 80 L of water) for chronic toxicity tests. The vats were conditioned prior to experiment by keeping them water filled in outdoor condition for 30 days. The fishes were fed with commercial pelleted food, 2-3 times per day during the acclimatization period. The water was renewed daily and the faecal matter, dead fish were siphoned off in regular interval.

The mean temperature of the vat water was 28.02 ±0.51 °C, pH 7.27±0.03, Dissolved Oxygen 4.18±0.50 mg/l and alkalinity was 76.91±2.09 mg/l. The water quality parameters were analysed following the standard method of APHA (1995) and Trivedi and Goel (1984).

For this study, the 96 hrs. LC_{50} value (1.776 mg/l) of chlorpyrifos (PYRIFEX 20% EC, SAFEX chemicals, INDIA ltd.) on the test fishes were determined by the use of Finney's probit analysis LC_{50} determination method (1971) and SPSS, version 17.0. The long term experiment was performed for 60 days by using $1/10^{th}$ and$1/20^{th}$ of 96 hrs. LC_{50} value of Chlorpyrifos as sub-lethal concentrations, which were 0.178 mg/l and 0.089 mg/l respectively for *H. fossilis*. Simultaneously a control (exclusively toxicant free medium) group was also conducted in each species for comparison.

For the histopathological examination, fishes were randomly selected after the end of exposure period of 60 days. The tissues of gill and liver were dissected out from both the control and experimented fishes. The isolated fish tissues were rinsed with 0.85% NaCl solution. Then the tissues were fixed in aqueous Bouin's solution for 48 hrs, and were processed by passing it through different grades of alcohol. After that the tissues were cleared in xylene and finally embedded in paraffin. The double embedding technique was administered in case of gills. The microtome sections were cut at the thickness of 6µ and after that the slides were stained with Ehrlich Haematoxylin/Eosin stain (dissolved in 70% alcohol) (Humason, 1972). Finally the slides were mounted in Canada balsam. The stained slides were examined with the help of Olympus light microscope and the images were captured through a digital camera (Model: Sony DSC-H70).

RESULTS & DISCUSSIONS

Effects on Gill

Under light microscope, gill tissues of control fishes appeared to be normal. The primary lamellae of gills were arranged in double rows and secondary lamellae were formed from these primary filaments. The gill lamellae are separated by Inter lamellar regions (Figure: 1). Marked histopathological changes were observed in experimental group of fishes. Those fishes showed variations in their histological structure of the gills from the control gill. During the sublethal exposure (0.089 mg/l) of chlorpyrifos in *H. fossilis*, the degeneration of secondary gill lamellae, fusion of secondary gill filaments and blood congestions were seen (Figure: 2). Moreover degeneration of secondary gill lamellae, massive vacuolization, blood congestions and dilation of blood vessels were also observed during the sublethal exposure (0.178 mg/l) of chlorpyrifos (Figure: 3). The gill lamellae were shortened and secondary gill lamellar cell walls were disappeared.

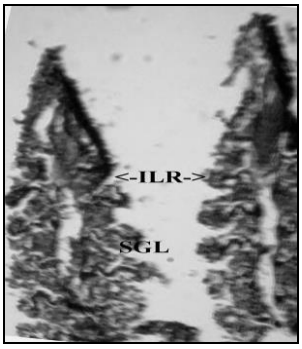
Effects on Liver

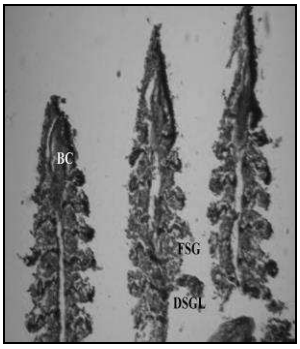
The liver is a vital organ of fishes, because it performs a number of important functions. Under light microscope the control liver of *H. fossilis* showed large polygonal hepatocytes (liver cells). Each hepatocyte possesses a distinct round central nucleus and granular cytoplasm. These hepatocytes are separated by the blood sinusoids (Figure: 4). Massive vacuole formation and appearance of irregular shaped cells were detected in sublethal exposure (0.089 mg/l) of chlorpyrifos in *H. Fossilis* (Figure: 5). Moreover cytoplasmic degeneration, degeneration of hepatocytes, vacuolization and haemorrhages were also observed during the sublethal exposure (0.178 mg/l) of chlorpyrifos (Figure: 6).

As the pesticides cause major abnormalities and malfunctions in the organs of aquatic animals such as fishes, so it is necessary to study the histopathological changes in fish tissues associated with this toxicity. The histopathological changes in affected fishes are more or less dose dependent. Considerable interest has been shown in recent years in histopathological study while conducting sub-lethal tests in fish (Girija *et. al.,* 2014). The gills are one of the most delicate and vulnerable organs of the body of aquatic organisms mainly in fishes. It performs many vital functions like respiration, osmoregulation, acid-base balance, excretion *etc.* As the gills are located externally to their body, they are liable to immediate contact with the pollutants and are susceptible to be damaged by any irritant material, which may be dissolved or suspended in water (Sahoo *et. al.,* 2003). The degeneration and erosion of primary and secondary gill lamellae, necrosis, swelling and curling of secondary gill epithelium, fusion of secondary gill filaments, destruction of inter lamellar region and dilation of blood vessels were observed in the present study, which might be due to the stressful behaviour in test fishes leading to respiratory impairments and the swelling of secondary gill epithelium was happened mainly due to the lamellar oedema and hypertrophy of epithelial cells. The results of the present study correlate with the study conducted by Girija *et. al.,* 2014.

Liver is one of the most vital glandular organs of fish body. The most well-known functions of liver include the breakdown of fats during digestion process, production of bile which helps to remove many waste products and it can detoxify chemical compounds. Due to its position, function and blood supply it is also one of the most affected organ by contaminants in water (Camargo and Martinez, 2007). A number of works have been conducted to measure the histopathological alterations in fish liver due to pesticide pollution. So from the results of present study it can be concluded that chlorpyrifos is a highly hepatotoxic compound. The changes in fish liver such as formation of vacuole or vacuolization, degenerative changes, appearance of blood streaks, formation of indistinguishable hepatocytes with mesh like appearance were probably due to the disintegration of lattice fibres, which support the hepatocytes. The results of the present study correlate with the study conducted by Susan *et. al.,* 2012.

So it can be inferred that chlorpyrifos acts as a toxicant in a similar fashion as any other heavy metal, pesticides, effluent or a pollutant of chemical origin. Furthermore it can also be inferred that organ-histopathology in aquatic organisms are good biomarkers of aquatic pollution.





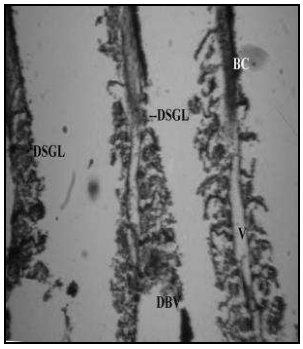
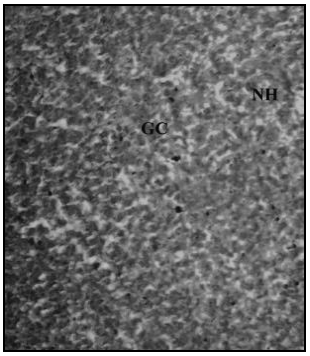
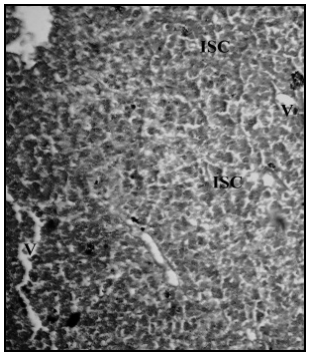


Figure 1 **Figure 2** **Figure 3**

Figure (1): Photomicrograph of gill (control) of *H. fossilis*, H/E Stain (×200), showing SGL: Secondary gill lamella; ILR: Inter lamellar region. **(2):** Photomicrograph of gill of *H. fossilis*, exposed to Chlorpyrifos ($1/20^{th}$ of LC_{50}) for 60 days, H/E Stain (×200), showing FSG: Fusion of secondary gill filaments, BC: Blood congestion, DSGL: Degenerated secondary gill lamella. **(3):** Photomicrograph of gill of *H. fossilis*, exposed Chlorpyrifos ($1/10^{th}$ of LC_{50}) for 60 days, H/E Stain (×200), showing DSGL: Degenerated secondary gill lamella, BC: Blood congestion, V: Vacuolization, DBV: Dilation of blood vessels.





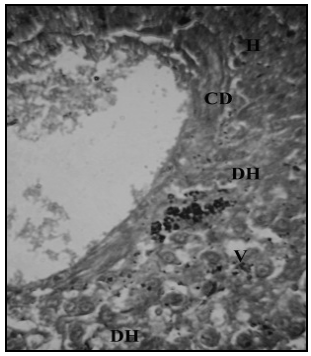


Figure 4 **Figure 5** **Figure 6**

Figure (4): Photomicrograph of liver (control) of *H. fossilis*, H/E Stain (×200), showing NH: Normal hepatocytes; GC: Granular cytoplasm. **(5):** Photomicrograph of liver of *H. fossilis*, exposed to Chlorpyrifos ($1/20^{th}$ of LC_{50}) for 60 days, H/E Stain (×200), showing ISC: Irregular shaped cells and V: Vacuolization. **(6):** Photomicrograph of liver of *H. fossilis*, exposed Chlorpyrifos ($1/10^{th}$ of LC_{50}) for 60 days, H/E Stain (×200), showing DH: Degeneration of hepatocytes, V: Vacuolization, CD: Cytoplasmic degeneration and H: Haemorrhage.

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