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Research Article

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Histopathological changes in Liver of fish *Channa punctatus* exposed to sub lethal concentration of Hybrid Pesticide

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Abstract: Water pollution has its own importance and concern with human life, as water is the main component of living organisms and is in bottom of our everyday life activities like cleaning, drinking, washing, agricultural purpose, sea food and fish food. The harmful effect of any pollutant which entered into water bodies can be assessed by investigating health of aquatic fauna. In the present investigation, an attempt was made to examine the sub-lethal toxic effect of Chlorpyriphos (50%) and Cypermethrin (5%) on the histology of liver of *Channa punctatus* after short term chronic exposure. In the fish exposed to hybrid pesticides, irregular shape of nuclei, nuclear hypertrophy, necrosis, space formation and vacuolation were observed in the liver.

Keywords: *Channa punctatus*, Chlorpyriphos, Cypermethrin, Liver, Organophosphate pesticide.

INTRODUCTION

Industrial effluents, agricultural and domestic wastes generally contain a wide variety of organic and inorganic pollutants, such as oils, heavy metals; pesticides, fertilizers, and suspended solids. These are discharged into small rivers and streams without proper treatment. Such contaminants change water quality and may cause several problems to fish including diseases and structural alterations. The aquatic ecosystem is the greater part of natural environment which is facing the threat of shrinking genetic base and biodiversity due to indiscriminate use of these pesticides (Rahman *et al*., 2002). The maintenance of healthy aquatic ecosystem is required for ecological balance and agriculture and widespread biodiversity (Verma, 2017, 2018a, 2018b) which depends on good physico-chemical properties of water. Insecticides play havoc to fish production as well as quality; it is widely used in agriculture and is commonly detected in surface water and ground water. Increased used of these pesticides in most tropical countries has been resulted in severe toxicities and bio accumulation (Palmer, 1972). The largest problem is the accumulation of heavy metals and pesticides in fish tissues, which cause damage to basic histological structure of different organs. The accumulation of heavy metals and pesticides becomes hazardous to the aquatic organisms and to surrounding human population because of fishes are the most important factors of food chain which have great nutritive value in the environment (Singh *et al.*, 2010). The polluted water may lead to the destruction of the beneficial species either directly by affecting the aquatic forms of life or indirectly through breaking the biological food chains such as fish and their habitat and behavioral pattern (Verma and Prakash, 2019).

Synthetic pyrethroids, insecticide combined with organophosphate are widely used in north Bihar to increase the production of crops. It is also used in public health application to control insects such as cockroaches, mosquitoes, ticks, and flies which may act as a disease vector. Therefore, the present work was undertaken to study the histopathological changes in liver of snake headed air

breathing fish, *Channa punctatus* (Bloch) exposed to sub-lethal concentration of hybrid pesticides (Chlorpyriphos and Cypermethrin).

MATERIALS AND METHODS

Live fresh water fish *Channa punctatus* of size ranging from 15 to 20 cm and weight 120-200 g were collected from local fish pond of Muzaffarpur, Bihar. They were transported to laboratory and transferred in to fish tank. Fishes were treated with 0.1% $KMnO_4$ to free from any dermal infections and were allowed to acclimatize to the laboratory conditions for 15 days. In the period of acclimatization fishes were fed alternatively with the pieces of pila after removing alimentary canal and soya bean chunks. The average physico-chemical conditions were maintained during the acclimatization period. Water of the tank was changed every alternate day to maintain the contamination as well as physico-chemical characteristic of the water. All the necessary precautions for maintaining the fish were laid down as per the recommendation of APHA, 2005.

To study the toxicity, LC_{50} of the hybrid pesticide was observed. The static renewal bioassay test method was followed. For conducting LC_{50} experiment, 5 glass jars were set up having 10 liters of water. A sum of 10 fishes were transferred in each Jar and left for 24 hours for stability and acclimatization. The fresh water Murrel, *Channa punctatus* were exposed to various concentration of the pesticide till 96 hours. No feed was given during test period. Based on the mortality observed at different concentrations during 96 hours, LC_{50} value was estimated for different periods *i.e.* 24 hours, 48 hours, 72 hours and 96 hours using straight line graphical interpolation method. After the estimation of LC_{50} value at different hours, sub-lethal concentration was chosen for exposing the fishes for short term exposure (the sub-lethal concentration as taken 1/10 of the LC_{50} value of, 96 hours). Then acclimatized fishes were exposed to this sub-lethal concentration for 30 days. After 30 days, fishes from the normal as well as treated tank were dissected and their liver was isolated and rinsed in physiological saline solutions (0.58% NaCl) then fixed in 2.5% gluteraldehyde for 24 hours. After 24 hours, liver was washed in 50% alcohol for 5 minutes, then tissues were transferred in 70% alcohol for 30 minutes in 2 changes, after 2 changes of 70% tissues were transferred in 90% alcohol for 30 minutes in 2 changes. After 90% alcohol tissues were transferred in absolute alcohol for 30 minutes in 2 changes. After that 3 grade of alcohol and amyl acetate were prepared which were 3:1, 2:2, 1:3 respectively. In alcohol and amyl acetate grade tissues were kept in 30 minutes in each grade. After alcohol and amyl acetate grade tissues were kept in amyl acetate for 30 minutes. After that CPD (critical point drying) was done that takes 1 hour and 30 minutes. After CPD tissues were dried then arranged on small stabs. When tissues were fixed on stab then gold coating of tissues done. After gold coating tissues of stabs were observed under scanning electron microscope (SEM).

RESULTS AND DISCUSSION

Liver is responsible for digestion, filtration and storage of food energy in the form of glycogen. It is the vital organ for detoxification and biotransformation process of unwanted and toxic substances. Fish liver is susceptible to chemical damaged due to slow blood flow and lower rate of bile flow. As the liver has multiple metabolic functions, such damage can have serious effects on the metabolism (Srivastava and Prakash, 2019).

The SEM studies of normal liver of fish, *Channa punctatus* revealed a clear histological structure of hepatocytes, liver cells, other cells along with hepatic portal vein (Fig 1 and 2). The fish exposed to the sublethal concentration of hybrid pesticides showed highly degenerated liver cells, hypertrophy in hepatocytes and damaged hepatic portal vein (Fig. 3 and 4). The study therefore showed a direct effect of hybrid pesticide exposure on the liver cells of the fish studied.

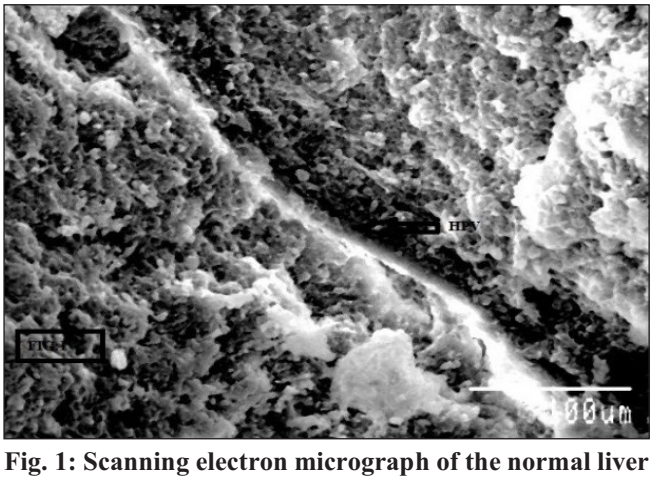

Fig. 1: Scanning electron micrograph of the normal liver showing hepatocytes and hepatic portal vein (HPV) (300X).

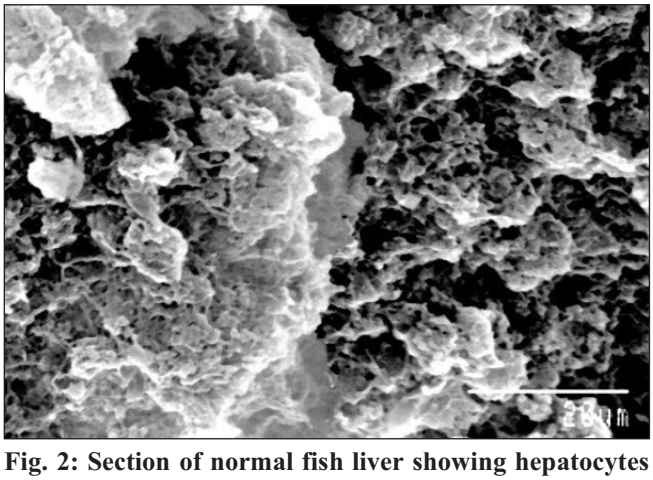

Fig. 2: Section of normal fish liver showing hepatocytes (500X).

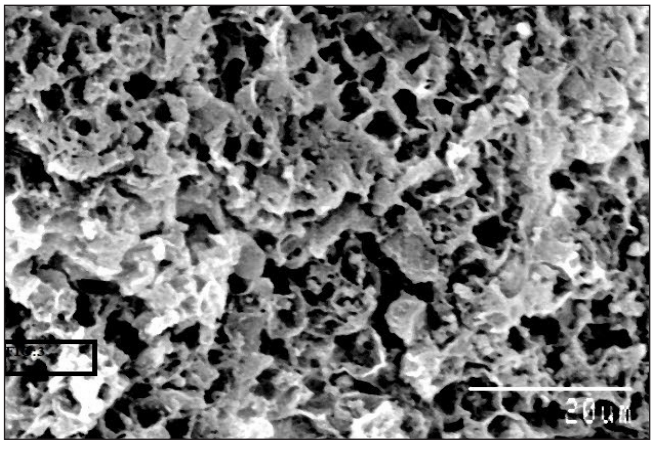

Fig. 3: Section of treated fish liver showing damaged hepatic architecture and empty Spaces (1500X).

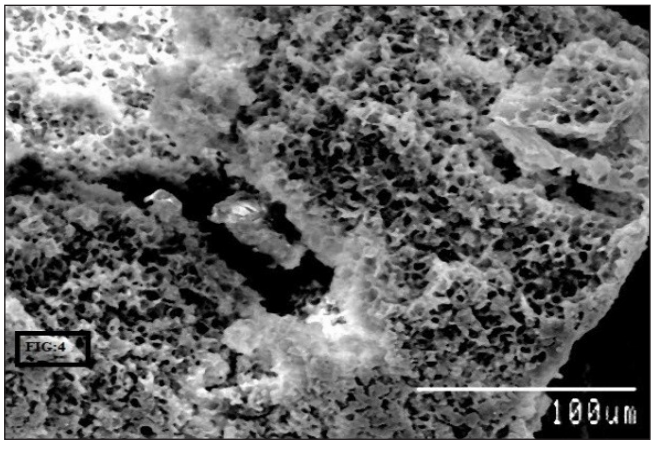

Fig. 4: Section of treated fish showing lots of empty spaces due to degenerations of hepatocytes (3000X).

Irregular hepatocytes nucleus, cytoplasmic vacuolation, were observed in organophosphate pesticides exposed *Siluriform corydoras* (Fanta *et al*., 2003). Srivastava and Prakash (2019) have suggested that the severe destruction of hepatocytes and formation of intercellular spaces could possibly due to sudden withdrawal and utilization of stored glycogen from the liver cells to meet the energy demands during toxicant stress in fish. The results of the present observation in *Channa punctatus* exposed to hybrid pesticides were in agreement with those of the earlier workers especially in the vacuolization, pyknotic nuclei and necrosis in hepatocytes. Intracellular vacuolization, necrosis and pyknotic nuclei were also apparent in the present study in the pesticides exposed *Channa punctatus*.

Vacuoles in the cytoplasm of the hepatocytes can contain lipids and glycogen, which are related to the normal metabolic function of the liver. Depletion of the glycogen in the hepatocytes is usually found in the stressed animals because the glycogen acts as a reserve of glucose to supply the higher energetic demand in such situations (Panepucci *et al*., 2001). Pacheco and Santos (2002) described increased vacuolization of the hepatocytes as a signal of degenerative process that suggests metabolic damage possibly related to exposure to contaminated water. Evidences proved that the histopathological changes in the liver cause metabolic problems as well, bile stagnations in the liver of most fish studied. This lesion characterized by the remains of the bile in the form of brownish yellow granules in the cytoplasm of the hepatocytes (Pacheco and Santos, 2002), indicates that the bile is not being released by the liver. This accumulation of bile indicates possible damage to the hepatic metabolism (Fanta *et al*., 2003). Olarinmoye *et al*. (2009) observed severe damage in liver cells such as vacuolar hepatocellular degeneration and necrosis and pancreatic necrosis, architectural disruption.

Mohanta *et al*. (2010) observed parenchymal vacuolation and focal coagulative necrosis in the liver tissue treated with effluents after 29 days. Vacuolation in the cytoplasm with the moderate degeneration of hepatic mass were noticed in the liver cells. Hydropic degeneration and fatty infiltrations in the liver tissue of *Channa punctatus* were also detected. Full congestion of central vein, diffusion of hepatic cells and dilation of sinusoids were observed. Bruno and Ellis (1988) reported similar results of vacuolations in the hepatocytes of the Atlantic salmon after exposure to tributyltin. Narain and Singh (1991) observed extensive degeneration of cytoplasm with pyknosis of nuclei and loss of glycogen in liver tissue *Heteropneusteus fossilis* while subjecting them to acute thioden toxicity. In general, hybrid pesticide creates manifold disturbances in the target tissues. Fishes are therefore particularly sensitive to environmental contamination of the water and pollutants like heavy metal may cause significant impairments of certain physiological and biochemical processes which can result in serious tissue damage.

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