A Study on the Effect of Organophosphorous Pesticide Profenfos on Hepato - Renal Organs of *Labeo Rohita (*Hamilton 1822)

Swapna Mishra[1], Rashmi Sao[2]

[1]Assistant Professor, Department of Zoology, Govt. Jajwalyadev Naveen Girls College, Janjgir (C. G.)
Corresponding Author Email: *swapnamishra1969[at]gmail.com*

[2]Professor and Head, Department of Zoology, Govt. E. Raghavendra Rao Science College, Bilaspur (C. G.)

Abstract: ***Organophosphorous pesticides are extensively used to control pests of different food (Vegetable, Fruits tea etc.) and non - food (Tobacco, cotton etc.) crops due to their rapid biodegradability and non - persistent nature but their broad spectrum of harmful effects extends far beyond the pests. Profenfos is an important widely used organophosphorous pesticide but its toxic effects on Labeo rohita are not well studied. The main objective of this paper is to carry out an empirical study to investigate the effect of different Sub - lethal concentrations of Profenfos on the hepato - renal organ of Labeo rohita. The pre - determinated LC_{50} value of Profenfos 0.1 mg/L was used for this study. The freshwater Teleost Labeo rohita (Hamilton) was exposed to a sub - lethal concentration of Profenfos (10%, 20% and 50% of LC_{50} Value) for 96 hours. The vital tissue liver and kidney were isolated and studied for the changes in the histology of these organs. The histopathological examination of the liver showed hepatic necrosis, haemorrhage vacuolation, glycogen vacuolation, and cellular swelling. The kidney showed different changes like degeneration of cell membrane, damage of renal tubules and hypertrophy of nuclei. The current study clearly revealed Profenfos as a potent hepato - renal toxic pesticide; therefore, the injudicious, indiscriminate, and extensive use of Profenfos should be prohibited or at least reduced and strictly monitored.***

Keywords: *Labeo rohita*, Hepato - renal, Profenfos, Organophosphorous pesticide

1. Introduction

Pharmaceutical and chemical industries introduce thousands of chemicals every year without having detailed information about them, and they create air, water, and land pollution as well as hazards not only for plants but also for animals including human beings. The aquatic life is continuously disturbed by toxic chemicals from industrial effluents, domestic activities, and agrochemicals. Pesticides are widely used to increase crop yield with low labour and less time. Different types (insecticides, rodenticides, fungicides, herbicides, bactericides etc.) and classes like organochlorinate, organophosphate, Pyrithroid, etc. of pesticides have been used in agricultural practices, domestic purposes, communities, and industries for a very long, based on the target species and their efficiency against them (Ullah *et al*., 2018). The runoff from treated areas enters the river and aquaculture ponds that are supplied by the river and contaminate them (Begum, 2004). After introducing the Green Revolution programme in India, many remarkable technological advancements were seen in the field of agriculture. In that time food, shelter, education and medical facilities are not provided to our countrymen due to the problem of population explosion.

Food is one of the basic requirements and the country's developmental assessment starts from the situation of food supply in the country. Pests are the main enemy for better yield of crops. Fertilizers, pesticides, and industrial effluents are directly or indirectly discharged into the various water bodies and deteriorate the water quality to a devastating extent. This deteriorated water affects not only aquatic life but also human beings. Various diseases and incidents of mass death of fish are reported and other aquatic animals are also affected.

Thus, it has become a direct need to evaluate the toxic effect of pesticides on aquatic life. Different types of scientific studies have been taken up by many researchers from different fields. In the present study, the author has studied the toxicological effects of Profenfos on histopathological and biochemical aspects of *Labeo rohita* (Hamilton).

2. Materials and Methods

The following materials and methods were used for the current study.

2.1. Test Pesticide: Profenfos

Profenfos is non - systemic but has excellent translaminar action and broad - spectrum organophosphate pesticide widely used for agricultural and household purposes in India (Rao *et al*., 2006; Ganguly *et al*., 2010; Chatterjee *et al*., 2021). It is effective against a wide range of chewing and sucking insects and mites, for controlling creepy crawlies in cotton and paddy plants (Chakra Reddy and Venkateswara, 2008). The half - life period of Profenfos is about one week in soil, it has been recognized as a highly persistent and toxic pesticide even at low concentrations. Profenfos is extremely toxic to fish and macroinvertebrates.

2.2. Chemicals

Analytical grade chemicals viz., Profenfos, Potassium Permanganate, Acetone, Saline Solution, Canada Balsam, and Xylene were used.

2.2.1. Preparation of stock solution of pesticide

For the preparation of the stock solution, 0.05 ml of Profenfos is added with 50 ml of Acetone. No toxic effect of acetone was observed.

2.2.2. Fixative

Neutral buffered formalin was used as a fixative agent.

2.2.3. Stain

Haematoxylin and eosin were used as stains.

2.3 Test Organism: Labeo *rohita*

Rohu, *Labeo rohita* (Hamilton, 1822) is freshwater Teleost, widely distributed in Asian countries i. e. India, Pakistan, Sri Lanka, Bangladesh, Nepal, and Myanmar. Rohu is a bottom - feeder vegetarian, but young fry consumes zooplankton.

2.4 Fish Handling and Acclimatization

Healthy and uniform - sized (9.1 ±1.2cm length and 7.2 ±8.34g weight) freshwater *Labeo rohita* were purchased from the local fish market and transported to the laboratory in aerated polythene bags to avoid any damage to the fish. Before starting the experiment, fish were washed with 0.1% $KMnO_4$ solution for 15 minutes to avoid any bacterial infection and acclimatized the fish for 15 days to the laboratory condition. During this period, fish were fed a 35% basal protein diet in small dried pellets twice daily.

Table 1: Properties of Profenfos

S. N.	Characteristics	Description
1.	Classification	Organophosphate, Acetyl cholinesterase inhibitor
2.	Empirical Formula	$C_{11}H_{15}BrClO_3PS$
3.	IUPAC Name	(O - (4 - bromo - 2 Chloro phenyl) - O - ethyl S - propyl phosphorothioate)
4.	CAS Number	41198 - 08 - 7
5.	Molecular Weight	373.63 g/mol
6.	Solubility in Water	20 ppm (20 mg/L)
7.	Appearance	Pale yellow
8.	Odour	Garlic like

Figure 1: Profenfos

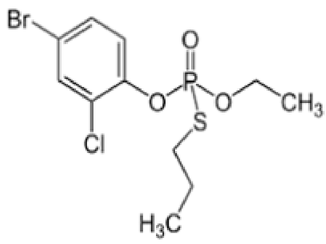

Figure 2: Structural formula of Profenfos

2.5. Maintenance of Aquarium

The aquarium was fitted with aerating stones for a continued supply of air. During the experimental period, the exchange of water daily and feed remains and wastes were siphoned off to avoid stress to the fish. During the experimental period sustaining photoperiod of 12: 12.

2.6 Water quality parameters

Water quality parameters including pH, hardness, dissolved oxygen, and temperature checked daily. An attempt was made to keep within the optimum range by removing the dead fish and wastes instantly to avoid any changes in the physicochemical parameters of the water.

2.7 Experimental Design

The current experiment was conducted in a triplicate and semi - static closed system. Even - sized healthy fish were distributed into 6.0 aquaria, 3.0 as a control group and 3.0 as treated. LC_{50} value of Profenfos is determined as 0.1mg/L. Fish were sacrificed with sub - lethal concentrations of Profenfos (10% (0.01 mg/L), 20% (0.02 mg/L) and 50% (0.05 mg/L) of LC_{50} value of Profenfos). After 1, 2, 3 and 4 days, for histopathological study, 6 fish were captured from each aquarium before changing the water and restoring the Profenfos concentration. The liver and kidney were dissected from control and exposed fish and transferred into a physiological saline solution (0.75% NaCl) for rinsing and cleaning the tissue. Tissues were fixed in Neutral buffer formalin (NBF).

2.8 Histopathological Examination

Six fish from each aquarium were captured and their morph metric and anatomical parameters were recorded like body length, weight, and skin colour. After dissecting the fish, the liver samples were weighted and transferred into NBF. The tissues were preserved and slides were prepared by following Rosety *et al.* (2005). The slides were stained with eosin and haematoxylin, mounted with Canada balsam, having coverslips. Extra Canada balsam was removed by xylene. The slides were studied and photomicrographs were procured under a digital camera - fit light microscope.

3. Observations and Discussions

Temperature ranged between 25.5 ±1.5ºC, dissolved oxygen ranged from 6.0 to 7.5 mg/L, pH ranged between 6.5 to 7.5, Total hardness ranged from 162 to 175 mg/L and conductivity ranged from 240 to 290 µS/cm in water. Histopathology is a reliable parameter for toxicological and safety assessment studies. Tissue - specific toxicity evaluated by histopathological study. Hence, to evaluate the toxicity of Profenfos on the liver and kidney of *Labeo rohita*, slides were prepared and general morphometry of fish was observed. Non - significant difference was observed for anatomical and morph metric parameters between control and treated groups. The results are analogous to previous studies of Ullah *et al.* (2018) that exposed *Labeo rohita* to different acute concentrations of Malathion and didn't observe any significant changes in the morphometric and anatomical changes in control and treated groups. The same observations were seen when Hasan *et al*. (2015) exposed grass carp to different acute concentrations of endosulfan and didn't see any remarkable changes in the body length, weight, depth, and liver weight (anatomical and morphometric parameters).

3.1 General histology of the liver

The liver is composed of a parenchyma (hepatic cell) covered by a delicate and thin capsule (serosa membrane), which is composed of a layer of simple squamous epithelial cells and a thin layer of loose connective tissue containing blood vessels, fibrocytes and fibres. Hepatic cells are polygonal in shape with granulated cytoplasm and spherical nuclei. Hepatocytes contain large quantities of lipid glycogen granules. Hepatocytes play an important role in the metabolism of carbohydrates, protein lipids and secret bile juice. Hepatocytes are storage sites for many nutrients and detoxify some chemicals.

3.2 Pathology of liver tissue under exposure to Profenfos

Profenfos has induced discrete pathological changes in the liver tissue of the fish. These changes included hepatic necrosis (HN), cellular swelling, haemorrhage vacuolation (HV), fatty infiltration (FI), glycogen vacuolation (GV), and congestion (C). The liver detoxified chemicals and metabolites of administrated substances. Hepatocytes were damaged due to the breakdown of toxic material carried out by the endoplasmic reticulum of hepatic cells. Pesticide toxicity concerning a histopathological point of view has been studied by different authors on different fish species like Moniruzzaman *et al.* (2017) on the silver barb, Ullah *et al.* (2018) on *Labeo rohita*, Hasan *et al*. (2015) on *Ctenopharyngodon idella*, Mostakim *et al.* (2015), Nannu *et al.* (2015). The current results are in congruence with previous research studies revealing some histopathological changes induced by different pesticides in the liver of different fish species including *Ctenopharyngodon idella* when exposed to technical and sublethal concentrations of 20% EC of Fenvalerate. The tissue damage necrosis, vacuolar degeneration and atrophy were observed (Tilak and Yacobu, 2002). When *Channa punctatus* was treated with endrin, similar changes were noticed by Shastri and Sharma (1978). The effect of pesticides on the liver of fish can be regarded as an index for the identification of pollution stress on fish (Cough, 1975).

Table 2: Summarized histopathological effects in the Liver of *Labeo rohita* exposed to Profenfos and control fish

Concentration of Profenfos (ppm)	Nuclear degeneration	Cytoplasmic degeneration	Vacuole formation	Melanomacrophase aggregation
Control 0ppm	_	_	_	_
0.01 ppm	_	+	_	_
0.02 ppm	+	++	+	_
0.05 ppm	++	+++	+++	++

(-) None; (+) Mild; (++) Moderate; (+++) Severe

3.4 General histology of fish kidney

Teleostean kidney consists of renal corpuscles, Bowman's capsule, and glomerulus. The anterior portion is the head, which consists of lymphoid tissue. The body is composed of many nephrons and interstitial lymphoid tissue. Each nephron consists of two parts, the glomerulus and the urinary tubules namely proximal tubule, distal tubule, intermediate segments and collecting duct. The glomerulus capsule consists of an inner and outer layer of single flattened epithelia. The renal tubule consists of a single layer of epithelial cells. The proximal convoluted tubule is divided into two parts, I and II. The renal tubules are composed of cuboidal epithelial cells with densely arranged microvilli in the tubular lumen. In the distal convoluted segment, epithelial cells have no microvilli. The cells of this segment are stained with eosin more faintly than those of the proximal convoluted segment. Thus, it is easily distinguished between proximal and distal convoluted segments under a light microscope (Oguri, 1982).

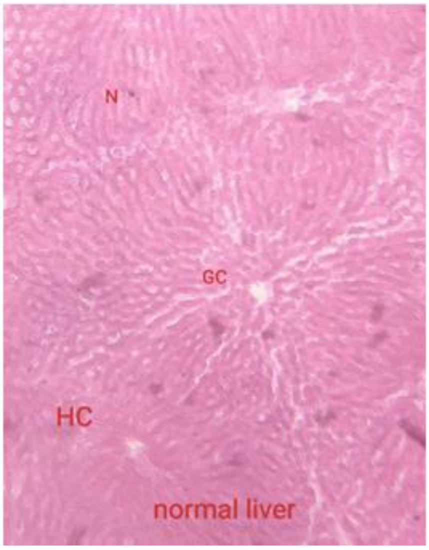

Figure 3 (a)

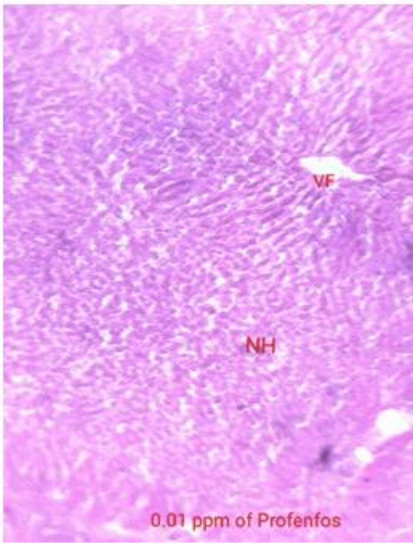

Figure 3 (b)

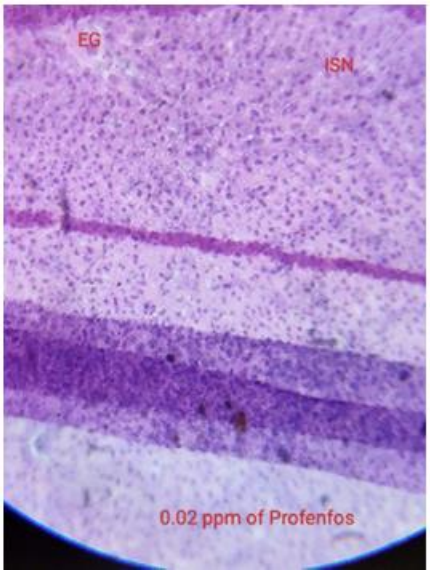

Figure 3 (c)

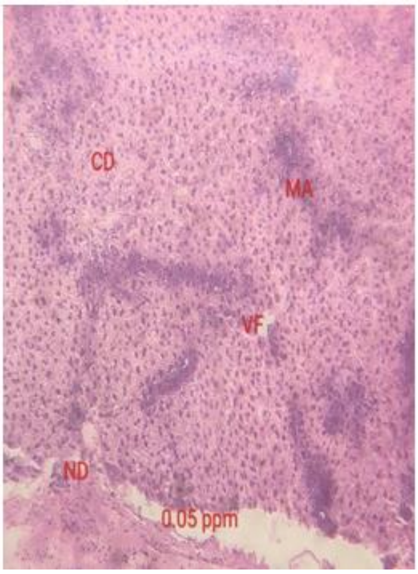

Figure 3 (d)

Figure 3: Photomicrograph of histological changes of liver of *Labeo rohita* (Hamilton 1822) upon Organophosphorous pesticide Profenfos

Figure 3 (a): Photomicrograph of T. S. of the liver of untreated *Labeo rohita* after 4 days;
Figure 3 (b): Photomicrograph of T. S. of the liver of *Labeo rohita* after 4 days exposure to 0.01 ppm of Profenfos for 4 days;
Figure 3 (c): Photomicrograph of T. S. of the liver after 4 days exposure to 0.02 ppm of Profenfos for 4 days;
Figure 3 (d): Photomicrograph of T. S. of the liver after 4 days of exposure to 0.05 ppm of Profenfos for 4 days
N: Nucleus; **HC:** Hepatic Cell; **GC:** Granular Cytoplasm
NH: Nuclear Hypertrophy; **BS:** Bile Stagnation, **VF:** Vacuole Formation
ISN: Irregular Shaped Nucleus; **VF:** Vacuole Formation; **EG:** Eosinophilic Granules
ND: Nuclear Degeneration; **CD:** Cytoplasmic Degeneration; **VF:** Vacuole Formation; **MA:** Melanomacrophase Aggregate

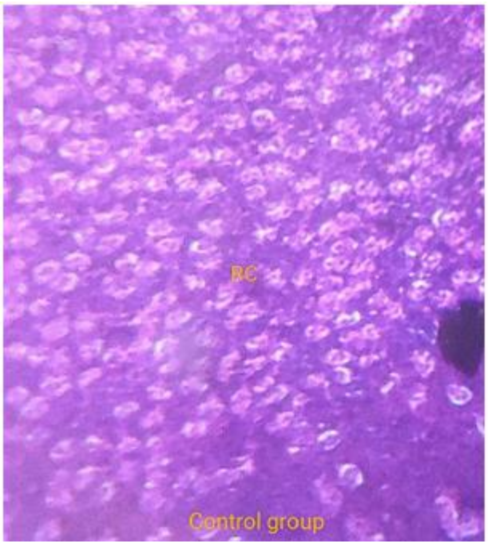

Figure 3 (a):

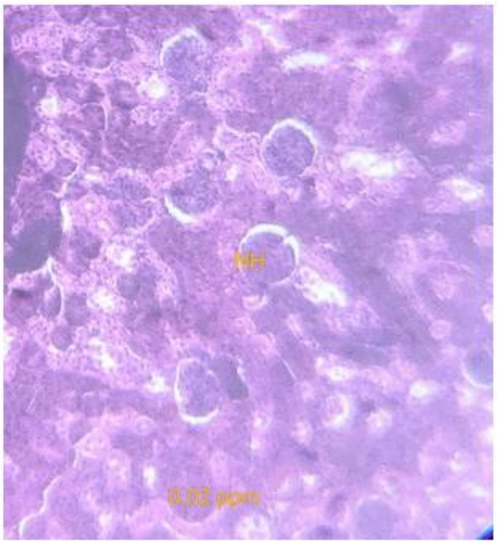

Figure 3 (b)

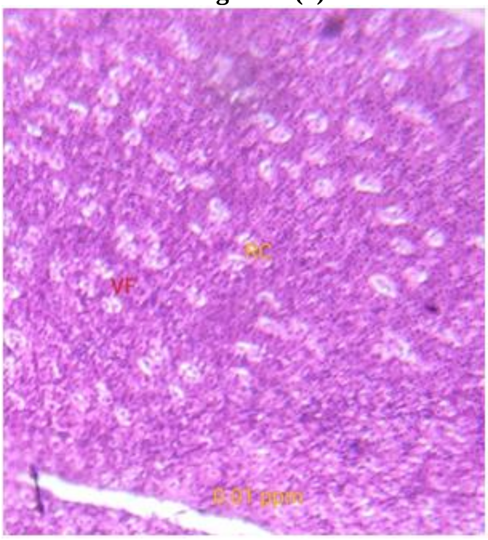

Figure 3 (c)

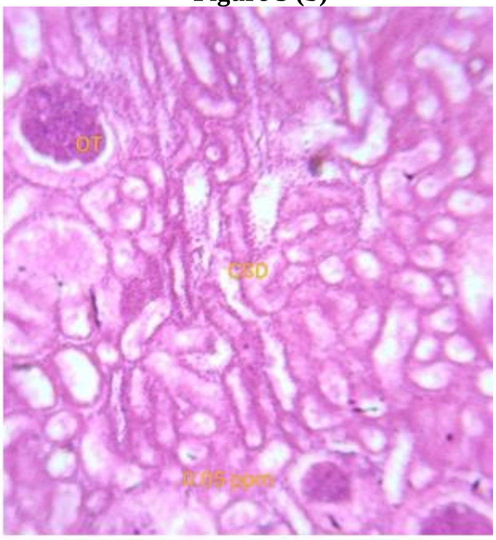

Figure 3 (d)

Figure 3: Photomicrograph of histological changes of Kidney of *Labeo rohita* (Hamilton 1822) upon Organophosphorous pesticide Profenfos

Figure 3 (a): Photomicrograph of T. S. of the kidney of untreated Labeo rohita after 4 days;
Figure 3 (b): Photomicrograph of T. S. of the kidney of *Labeo rohita* after 4 days exposure to 0.01 ppm of Profenfos for 4 days;
Figure 3 (c): Photomicrograph of T. S. of the kidney after 4 days exposure to 0.02 ppm of Profenfos for 4 days;
Figure 3 (d): Photomicrograph of T. S. of the kidney after 4 days exposure to 0.05 ppm of Profenfos for 4 days
PT: Proximal Tubules; **DT:** Distal Tubules
NH: Nuclear Hypertrophy; **VF:** Vacuole Formation
RC: Renal capsule showing glomerulus and bowmen's space; **VF:** Vacuole Formation
OT: Occlusion of Tubular lumen; **CSD:** Cloudy Swelling Degeneration; **HDD:** Hyaline Droplets Degeneration

Table 3: Summarized histopathological effects in the kidney of *Labeo rohita* exposed to Profenfos and control fish

Concentration of Profenfos (ppm)	Nuclear hypertrophy	Occlusion of Tubular lumen	Vacuole formation	Glomerular expansion and absence of bowman's space
Control (0 ppm)	-	-	-	-
0.01 ppm	-	-	-	-
0.02 ppm	+	+	+	-
0.05 ppm	++	++	++	+++

(-) None; (+) Mild; (++) Moderate; (+++) Severe

3.5 Pathology of Kidney tissue under Profenfos toxicity

The number of renal cells reduced in the proximal and distal collecting tubules, the resulting narrowness of the lumen. Necrosis of cells, cloudy swelling in renal tubules, cellular hypertrophy and granular cytoplasm are seen on this duration. The interstitial renal tissue was less affected and showed the formation of vacuoles and cellular contours were not visibly distinguished. Similar results were observed in goldfish exposed to DDT (Rudd and Linelly, 1956). Heptachor produced abnormalities in kidney tubules in *Labeo rohita* (Konar, 1970). Veeraiah, 2001 observed some degenerative changes in haemopoietic tissue like severe necrosis, cloudy swelling in renal tubules, cellular hypertrophy, and granular cytoplasm when *Labeo rohita* was exposed to Cypermetrin. The effect of pesticides on fish tissues was directly proportional to the amount of Profenfos and duration of exposure.

4. Conclusion

Labeo rohita is cultured commonly in the Chhattisgarh region and is heavily affected by the huge amount of pesticide intoxication through leaching from the nearby agricultural fields and irrigation canals. These chemicals accumulate in adipose tissue absorb different tissues of fish and bring about some physiological and anatomical changes in fish. As a result, ultimately fish became dying in certain cases. Contaminated fish enters the food chain and creates health problems for consumers. Therefore, for the prevention of fish mortality and contamination, the amount of pesticides used in the field should be monitored and the government should make strict rules to limit the use of pesticides.

References

[1] **Begum, G (2004).** Carbofuran insecticide induced biochemical alternations in liver and muscle tissue of the fish *Clarias batrachus* (linn.) and recovery response. *Aquat. Toxicol*., 66, 83 - 92.

[2] **Chatterjee, A., Bhattacharya, R., Chatterj, ES. & Saha, N. C. (2021).** Acute toxicity of organophosphate pesticide Profenfos, pyrethroid pesticide gama cyhalothrin and biopesticide azadirachtin and their sublethal effects on growth and oxidative stress enzymes in benthic oligochaete worm, *Tubifex tubifex*, Comparative Biochemistry and physiology part C: *Toxicology &* Pharmacology, 242, 10.

[3] **Cough, J. A. (1975).** Histopathological effects of pesticides and related chemicals on the liver of fishes, from 'pathology of fishes" (eds.) W. E. Reblin and Mugaki. The university of Wisconson press, Madison.

[4] **Ganguly, S., Bhattacharya, S., Mandi, S. & Tarafdar, J. (2010).** Biological detection and analysis of toxicity of organophosphate and azzadirachtin - based insecticides in *Lathyrus satiyus* L. *Ecotoxicology*, 19, 85 - 95.

[5] **Hassan, Z., Ghayyur, S., Hassan, Z. U. & Rafique, S. (2015).** Histomorphometric and haematological profile of grass carp (*Ctenopharyngodon idella*) during acute endosulfan toxicity. *Pak. Vet. Jour*., 35, 23 - 27.

[6] **Konar, S. K. (1970).** Toxicity of Heptachlor to aquatic life. *J. Water pollut. Contaim.*, 42 (8), 299 - 303.

[7] **Mostakim, G. M., Zahangir, M. M., Mishu, M. M., Rahman, M. K. & Islam, M. S. (2015).** Alteration of blood parameters and histoarchittecture of liver and kidney of silver barb after chronic exposure to quinalphos. *Journal of Toxicology*. http: //dx. doi. org/10.1155/2015/415984

[8] **Nannu, M. T. A., Mostakin, G. M., Khatun, M. H., Rahman, M. K., Sadiqual, M. I. (2015).** Hematological and histo - architectural damages in the kidney and liver of Nile Tilapia on exposure to kinalux. *Progressive Agriculture*, 26, 173 - 178.

[9] **Ogueji, E. O., Baba, I., Usman, A. J. (2013).** Histopathology of liver and gill of *C. gariepinus* (Burchell 1822) with swollen abdomen following exposure to acute and sub - lethal concentrations of Chlorpyrifos - ethyl. *International Journal of Basic and Applied Sciences*, 2 (3), 223 - 229.

[10] **Rao, V. J., Begum, G., Jakka, NM., Srikanth, K. & Rao, N. (2006).** Sublethal effects of Profenfos on locomotor behaviour and gill architecture of the mosquito fish, *Gambusia affinis*. *Drug Chem. Toxicol*.29, 255 - 267.

[11] **Rosety, M., Rosety - Rodriguez, M., Ordones, F. J. & Rosety, L. (2005).** Time course variations of antioxidant enzyme activities and histopathology of gilthead seabream gills exposed to Malathion. *Histol. Histopathol*., 20, 1017 - 1020.

[12] **Rudd, R. L. & Richard, E. G. (1956).** Pesticides: Their Use and Toxicity in Relation to Wildlife. Special Collections, USDA National Agricultural Library, 7 - 309. https: //www.nal. usda. gov/exhibits/speccoll/items/show/4561.

[13] **Sastry, K. V. & Sharma, S. K. (1979).** Toxic effects of endrin on liver and kidney of Teleost fish. Proc. Symp. *Enviro. Bio*., 337 - 342.

[14] **Tilak, Y. (2002).** Toxicity and effect of fenvaerate on fish *Ctenophargodon idella. Journal of Ecotoxicological Environment Monitoring*, 12 (1), 09 - 15.

[15] **Ullah, S., Zuberi, A., Alagawany, M., Farag, M. R., Dadar, M., Karthik, K., Tiwari, R., Dhama, K. & Iqbal, H. M. N. (2018).** Cypermethrin induced toxicity in fish and adverse health outcome: its prevention and control measure adaptation. *J. Environ. Manag*., 206, 863 - 871.

[16] **Veeraiah, K. (2001).** Cypermethrin toxicity and its impact on histochemical and histological changes in the Indian Major carp *Labeo rohita* (Hamilton). Ph. D. Thesis, Nagarjuna University, Guntur, India.