Original Research Article

Effect of Organophosphorous pesticide stress on Biochemical parameters in freshwater fish *Labeo rohita* (Hamilton)

Abstract

Background: India is an agrarian country and has an agriculture-based economy. Agricultural practices increased crop yield, but indiscriminate use of agrochemicals creates problems like air, water, and soil pollution and affects non-target organisms like fish. Nowadays, organophosphorous pesticides have been widely used to control pests. The main objective of this study was to investigate the acute effect of Organophosphorous pesticides Profenofos and Chlorpyrifos on the biochemical content of protein and glycogen in the liver and kidney tissue of *Labeo rohita* (Hamilton).

Materials and Methods: The study was carried out with freshwater fish *Labeo rohita* (9.1±1.2 cm length & 7.2±1.2 g weight) exposed in sub-lethal concentrations (LC_{50}/4 mg/L & LC_{50}/2 mg/L) of Organophosphorous pesticides Profenofos and Chlorpyrifos for an acute time period of Organophosphorous pesticides Profenofos and Chlorpyrifos for acute time period 48 hours and 96 hours. The physico-chemical analysis of water was carried out by following APHA methods. Biochemical content of protein and Glycogen in the tissue of the liver and kidney was determined.

Results: After acute exposure of *Labeo rohita* in Profenofos and Chlorpyrifos, the LC_{50} Value was determined 0.31mg/L & 0.53mg/L respectively. During acute treatment of pesticides, there were no changes shown in the control group. The amount of protein and glycogen content in the tissue of the liver and kidney gradually decreases in the treated group. This disrupts a normal metabolic pathway, which leads to an increase in the rate of mortality in the fish population.

Conclusion: The finding contributes to understanding the development of safer pesticide formulation and improved aquatic system management. The right amount and proper use of pesticides are very important to keep up the environment clean and maintain the ecosystem.

Keywords: *Organophosphorous pesticides, Labeo rohita, liver, kidney, Profenofos, Chlorpyrifos*

INTRODUCTION

Pesticides are used to protect crops from pests, but indiscriminate use of pesticides in the field has a very harmful effect on the environment (Mercy *et al.*, 2000). Balance of ecosystem disturbed due to long-term use of pesticides because it directly or indirectly affects the aquatic ecosystem, including fish (Bhatnagar *et al.*, 1992; Rajalakshmi and Mohandas, 1998). In the modern era of industrialization, urbanization, developed and developing countries, increased yield of crop production, increased technology, and industries, but released hazardous chemicals into the atmosphere (Mehmood et al., 2024).

Pesticides enter the water bodies either through direct application or erosion from treated areas. It is a serious matter of great concern for the whole world, how to reduce the harmful effects of pesticides on aquatic life (Toumi *et al.*, 2014). In the agricultural land and domestic use of pesticides, it led to creating awareness about toxicity in aquatic life (Nemcsók and Benedeezky, 1990). Organophosphate pesticides are neurotoxins, inhibit the activity of acetyl cholinesterase and damage, weaken the mechanism concerned leading to physiological, pathological and biochemical disorders (Arasta *et al.*, 1999). Profenofos is used for many purposes, indoor pest control, structural pest control, food crops, turf and ornamental plants, and pet collars (Kushwaha et al., 2016). Profenfos is extremely toxic to fish and macro vertebrates (Nataraj et al., 2017). The World Health Organization (WHO) considered Profenfos as a moderately hazardous (Toxicity class II) pesticide and it has a moderate order of acute toxicity following an oral and dermal administration (McAbee et al., 2018). The acute toxicity of Profenfos is due to inhibition of acetylcholinesterase activity resulting in neurotoxicity and oxidative pressure to aquatic vertebrates and human (Rusha et al, 2013; Bacchetta *et al.*, 2014; Pamanji *et al.*, 2016; Rahman *et al.*, 2020). It can accumulate in food and enter the food chain, thus more concerns for public health. In many countries, such as the European Union, Asia, etc., this pesticide is restricted and banned. Chlorpyrifos used in India since 1965. It is a non-systemic broad-spectrum pesticide that affects neuronal development (Weis et al., 2021). Chlorpyrifos is the 2nd largest selling organophosphorous pesticide in India. According to the US Environmental Protection Agency, approximately 800 registered products contain Chlorpyrifos. This pesticide has exhibited reproductive-, neuro-, and geno-toxicity in humans (Wołejko et al., 2022) and causes sublethal toxicities in aquatic animals by inducing morphological, neuro-behavioural, biochemical and histopathological alterations in fish (Sunanda et al., 2016). Hence, organophosphorous pesticides Profenofos and Chlorpyrifos were selected for the present study. Fish act as ideal sentinel and susceptible to any alteration in the physico-chemical characteristics of the habitat (Sadiq Bukhari *et al.*, 2012). Cultivation of fish is very important for human health because of its proteins with obligatory amino acids and lipids with omega fatty acids, which are very common nutrients for human nourishment. Various agrochemicals are used in farms and houses, such as organophosphate, organochlorinate, Carbamate, pyrethroid, and so on, have been reported to cause toxicity stress on fish fauna and show physiological, pathological, biochemical, and histological modifications in it. Biochemical parameters like glucose, protein, and glycogen, play an important role in physiological activities in fish life, so any alteration in these parameters is widely used to identify the target organs of toxicity (Sarvanan and Ramesh, 2013). Biochemical parameter acts as biomarkers and as a diagnostic tool for monitoring toxicity in aquatic life. (Bernet *et al.*, 1991; Baskaran, 1991; El-Sayed and Ei-Bahr, 2007; Li *et al.*, 2010). The freshwater fish *L. rohita* is selected for the study because it is readily available throughout the year, all over India, edible, commercially and economically valuable fish, and easily adapted in laboratory conditions, and their varying degree of sensitivity to the toxicant, to evaluate the toxic effects of insecticides Profenofos and Chlorpyrifos. *L. rohita* is cultivated in many natural freshwater systems in India and the most preferred species among the Indian major carps.

MATERIAL AND METHODS

The present research was done to examine the effect of organophosphorous pesticide stress on biochemical parameters in freshwater fish *L. rohita* (~~Hamilton).~~

Fish handling and Acclimatization

Fresh water fish *L. rohita* (mean length 9.1±1.2 cm and weight 7.2±1.2 g) was procured from a local fish market. Fish were acclimatized to laboratory conditions in an ~~Aquarium~~ (size

60×30×30 cm) in 40 L of dechlorinated tap water for 15 days before exposure. The fish were fed with a basal protein diet in the form of small pellets twice daily according to 5% body weight.

Maintenance of Aquarium water

The water was removed daily for removal of excretory waste. Dead fish are removed quickly for the maintenance of the quality of water. The physico-chemical properties of water were estimated (APHA 1998) (Table-1). Photoperiod of the study was 12:12.

Test Chemical

The Profenfos commercial name ‘Celcron’ and Chlorpyrifos commercial name ‘Radar’ were used as the test pesticides for the present course of investigation. All the chemicals used were of analytical grade. The instruments and apparatus were calibrated and standardized before use as per the instructions provided in the manuals.

Toxicity Bioassay

The toxicity bioassay for *L. rohita* against Chlorpyrifos and Profenofos was examined.

Determination of Median lethal concentration (LC_{50}) value of Chlorpyrifos, Profenofos Method (Probit Analysis)

Semi-static laboratory system was utilized to carry out acute toxicity bioassay. A Glass aquarium of size 60 × 30 × 30 cm (40 L capacity) was used for the determination of LC_{50} value. The aquarium water was replaced every 24 hours. Fish were divided into groups (of 10 individuals). Fishes were exposed to different concentrations of commercial formulation of insecticide Chlorpyrifos (trade name ‘Radar’ 20 % EC) and Profenofos (trade name Celcron 50% EC). Eight different concentrations of radar were selected, which were based on available literature (equivalent to 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mg/L). A separate group of fish (10 individuals) in an aquarium without insecticide was considered as the control. The experiments were performed in triplicate. Probit Analysis is frequently used for the determination of median lethal concentration values in toxicology.

Biochemical Estimation

Six individual animals from each group (Control and treated) were assessed for biochemical estimation. Total protein content in the tissue of the liver and the kidney was estimated according to Lowry *et al.* (1951). Glycogen was estimated from tissues of the Liver and Kidney, by Carroll, Longley and Roe's method (1955).

Statistical Analysis:

The data was subjected to one-way analysis of variance (ANOVA) using Microsoft Excel-2007 and the significance difference was set up at $p< 0.05$. These values were expressed as mean ±SD for all parameters in the experiment.

RESULTS AND DISCUSSION

The present research was carried out to assess the effect of organophosphorous pesticide stress on biochemical parameters in freshwater fish *L. rohita* (Hamilton) and the observations are systematically presented.

Physico-chemical Parameters of water

Table 1: Analysis of physico-chemical parameters of aquarium water under control and treated conditions

S.N.	Parameters	Unit	Control (± SD)	Profenofos (± SD)	Control (± SD)	Chlorpyrifos (± SD)
1	pH	-	6.7 ±0.3	6.8 ±0.4	6.7 ±0.3	6.7±0.2
2	Temperature	º C	27 ±0.4	27 ±0.4	27 ±0.4	27 ±0.4
3	Electrical Conductivity	µs/cm	410.33 ±0.9	411.34 ±0.4	410.33 ±0.9	410.12 ±0.3
4	Dissolved Oxygen	mg/L	7.19 ±0.92	7.02 ±0.34	7.19 ±0.92	7.10 ±0.52
5	Free CO_2	mg/L	0.62 ±0.06	0.60 ±0.05	0.62 ±0.06	0.61 ±0.04
6	Total Dissolved Solid	mg/L	301 ±4.90	302 ±3.58	301 ±4.90	301 ±4.53
7	Total Hardness	mg/L	190.02 ±8.12	188.04 ±7.23	190.02 ±8.12	189.03 ±7.00
8	Calcium	mg/L	130.14 ±3.25	128.06 ±2.89	130.14 ±3.25	129.32 ±3.45

	Hardness					
9	Magnesium Hardness	mg/L	59.88 ±4.87	59.98 ±4.37	59.88 ±4.87	59.71 ±3.55
10	Alkalinity	mg/L	298 ±16.32	299 ±14.52	298 ±16.32	299± 10.39
11	Sulphate	mg/L	14.25 ±0.47	12.57 ±0.85	14.25 ±0.47	13.45 ±0.67
12	Nitrate	mg/L	0.44 ±0.047	0.42 ±0.053	0.44 ±0.047	0.43 ±0.062
13	Phosphate	mg/L	0.12 ±0.004	0.09 ±0.001	0.12 ±0.004	0.10 ±0.002
14	Chloride	mg/ml	8.72 ±0.07	8.34 ±0.05	8.72 ±0.07	8.21 ±0.06
15	Ammonical Nitrogen	mg/L	2.12 ±0.36	2.54 ±0.59	2.12 ±0.36	2.43 ±0.31

Toxicity Evaluation

L. rohita was sensitive to the organophosphorous pesticides. Toxicity test conducted for acute period (96 hours). The results of the present study have been given in the Table 2 and Fig. 1 (Chlorpyrifos) and Table 3 and Fig. 2 (Profenofos). The LC_{50} value of Chlorpyrifos was obtained as 0.59 mg/L for 48 hours, respectively, and the LC_{50} value of Chlorpyrifos was obtained as 0.53 mg/L for 96 hours, respectively. The LC_{50} Value of Profenofos was obtained as 0.51 mg/L for 48 hours. The LC_{50} Value of Profenofos was obtained as 0.31 mg/L for 96 hours. The percentage mortality of medium-sized groups increased progressively up to 96 hours in all concentrations. The LC_{50} value of Profenofos is lower as compared to Chlorpyrifos.

Table 2. Mortality Data Exposed to Chlorpyrifos (96 hours)

S.N.	**Conc. In mg/L**	**Log of Conc. (X)**	**No. of animal Exposed**	**Mortality**	**% Mortality P=r/n×100**	**Empirical Probit**	**Expected Probit**
1	0.1	-1	10	1	10	3.72	3.7
2	0.2	-0.69897	10	2	20	4.16	4.2
3	0.3	-0.52288	10	2	20	4.16	4.2
4	0.4	-0.39794	10	2	20	4.16	4.2

5	0.5	-0.30103	10	3	30	4.48	4.5
6	0.6	-0.22185	10	4	40	4.75	4.7
7	0.7	-0.1549	10	7	70	5.52	5.5
8	0.8	-0.09691	10	9	90	6.28	6.3
LC_{50} Value = 0.53 mg/L							

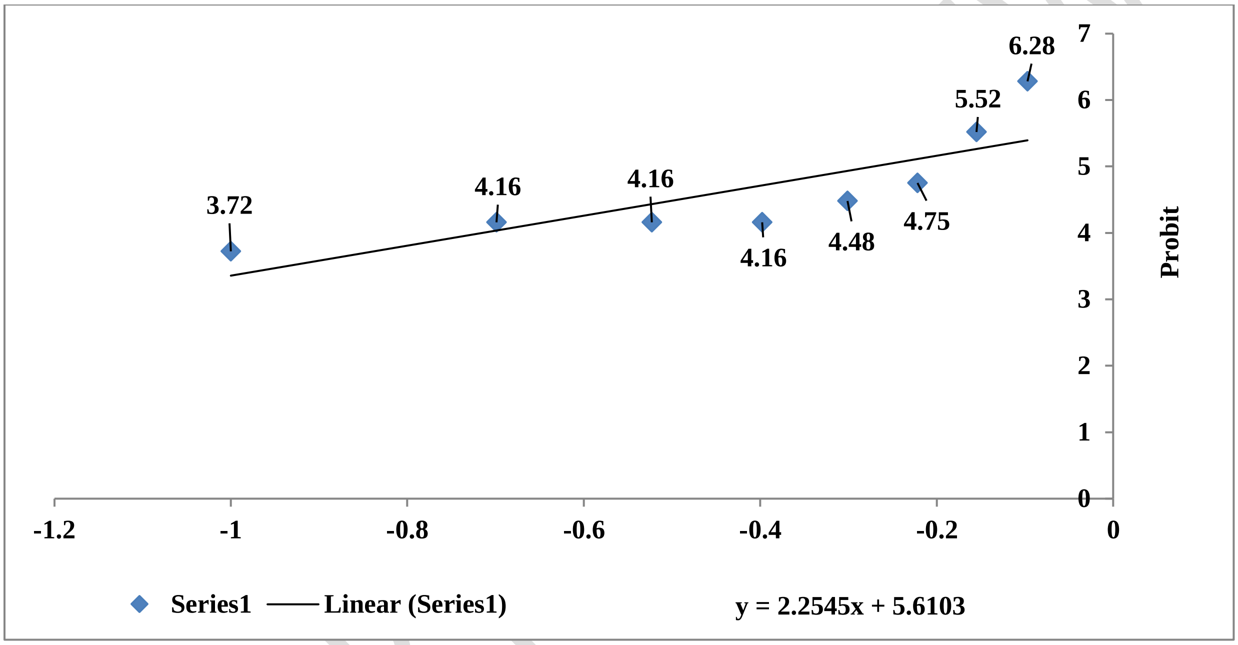

Fig. 1. Probit analysis of Chlorpyrifos (96 hours)

Table 3 Mortality Data Exposed to Profenofos (96 hours)

S.N.	Conc. In mg/L	Log of Conc. (X)	No. Of animal Exposed	Mortality	% Mortality P=r/n×100	Empirical Probit	Expected Probit
1	0.1	-1	10	1	10	3.72	3.7
2	0.2	-0.69897	10	3	30	4.48	4.5
3	0.3	-0.52288	10	3	30	4.48	4.5
4	0.4	-0.39794	10	5	50	5	5
5	0.5	-0.30103	10	6	60	5.25	5.2
6	0.6	-0.22185	10	7	70	5.52	5.5
7	0.7	-0.1549	10	8	80	5.84	5.8

8	0.8	-0.09691	10	10	100	8.09	8
LC_{50} Value = 0.31 mg/L							

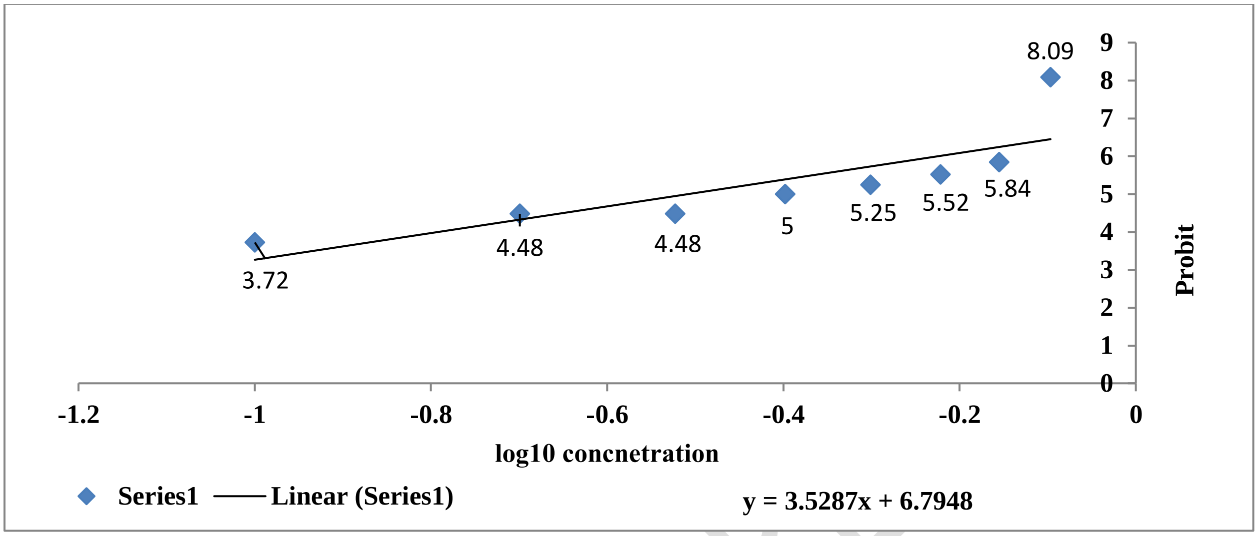

Fig. 2. Probit analysis of Profenofos (96 hours)

Biochemical Evaluations

When *L. rohita* was exposed to acute treatment of Profenofos and Chlorpyrifos, some changes in biochemical composition of the liver and kidney were studied along with the control animal.

The protein content of the liver and kidney was found to be decreased after acute exposure to Profenofos and Chlorpyrifos. Decrease percentage of protein in the liver tissue when *L. rohita* exposed to (LC_{50}/4) concentration of Profenofos after 48 and 96 hours were 25.93% and 36.43% respectively. Total depletion of protein content in the liver of *L. rohita* after 48- and 96-hours exposure of Profenofos (LC_{50}/2), were 45.69% and 59.29% respectively (Fig 1). A significant depletion in protein content of kidney tissue of *L. rohita*, exposed to (LC_{50}/4) of Profenofos exposed to 48 and 96 hours were 23.17% and 25.87% respectively. The decrease of protein content in the kidney, after 48 and 96 hours of treatment with (LC_{50}/2) conc. of Profenofos were 42.88% and 62.66% respectively (Fig. 3).

The glycogen content of the liver of *L. rohita* was found to decrease after acute exposure to Profenofos. Depletion of Glycogen content in liver tissue was seen after 48- and 96-hours exposure of *L. rohita* to Profenofos (LC_{50}/4) 17.36% and 28.10% respectively (Fig 3). Decrease percentage of Glycogen content in the liver of *L. rohita* at 48- and 96-hour exposure of Profenofos (LC_{50}/2) were 46.34% and 55% respectively (Fig 3). Decrease percentage of protein in the liver tissue when *L. rohita* exposed to (LC_{50}/4) concentration of Chlorpyrifos after 48 and 96 hours were 3.53% and 3.37% respectively. Total depletion of protein content in the liver of *L. rohita* after acute exposure of Chlorpyrifos (LC_{50}/2) 48 and 96 hours were 26.58% and 34.13% respectively (Fig. 4).

A significant depletion in protein content of kidney tissue of *L. rohita*, exposed to (LC_{50}/4) of Chlorpyrifos, exposed to 48 and 96 hours, were 2.56% and 3.45% respectively. The decrease of protein content in the kidney, after 48 and 96 hours of treatment with (LC_{50}/2) conc. of Chlorpyrifos were 26.605% and 45.09% respectively (Fig 3). Depletion of Glycogen content in liver tissue was seen after 48- and 96-hour exposure of *L. rohita* to Chlorpyrifos (LC_{50}/4) 1.87% and 25.82% respectively (Fig 5). The glycogen decreases in the kidney tissue with 48- and 96-hour exposure of Chlorpyrifos (LC_{50}/2) were 434.73% and 55.72% respectively (Fig 6). The depletion of glycogen content after exposure to pesticides depends upon the time of exposure and the concentration of insecticides. Profenofos-treated fish is more affected and depletion percentage is higher than Chlorpyrifos.

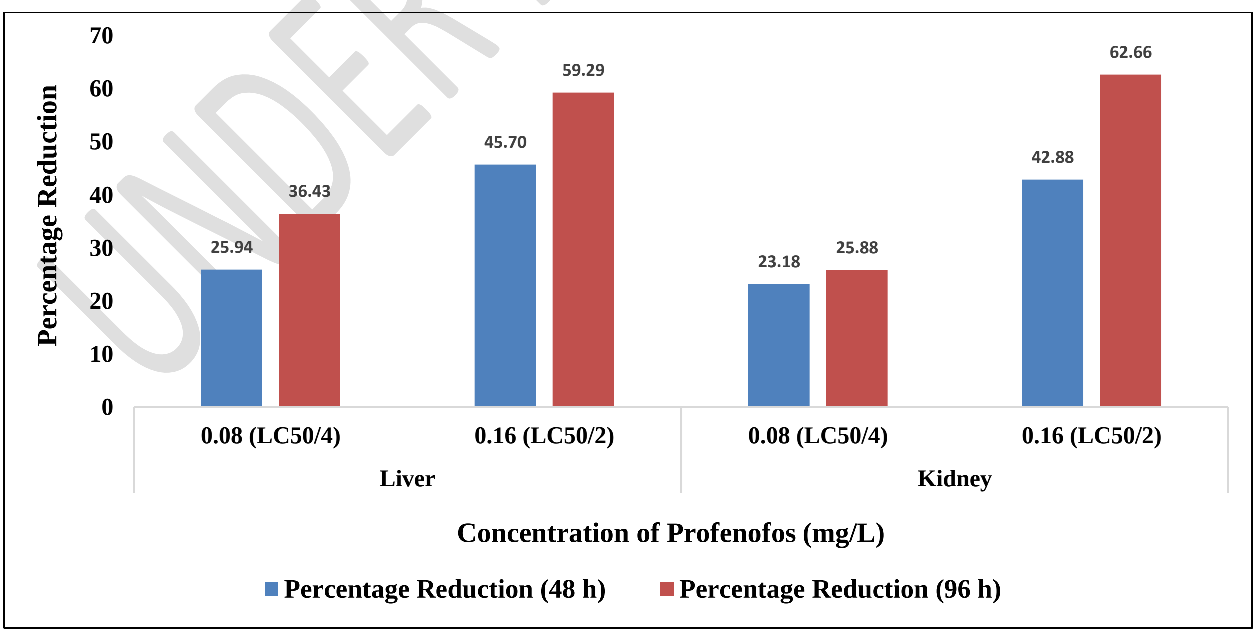

Fig. 3: Total protein content (mg/g) in the liver and kidney tissues of *L. rohita* exposed to different concentrations of insecticide Profenofos

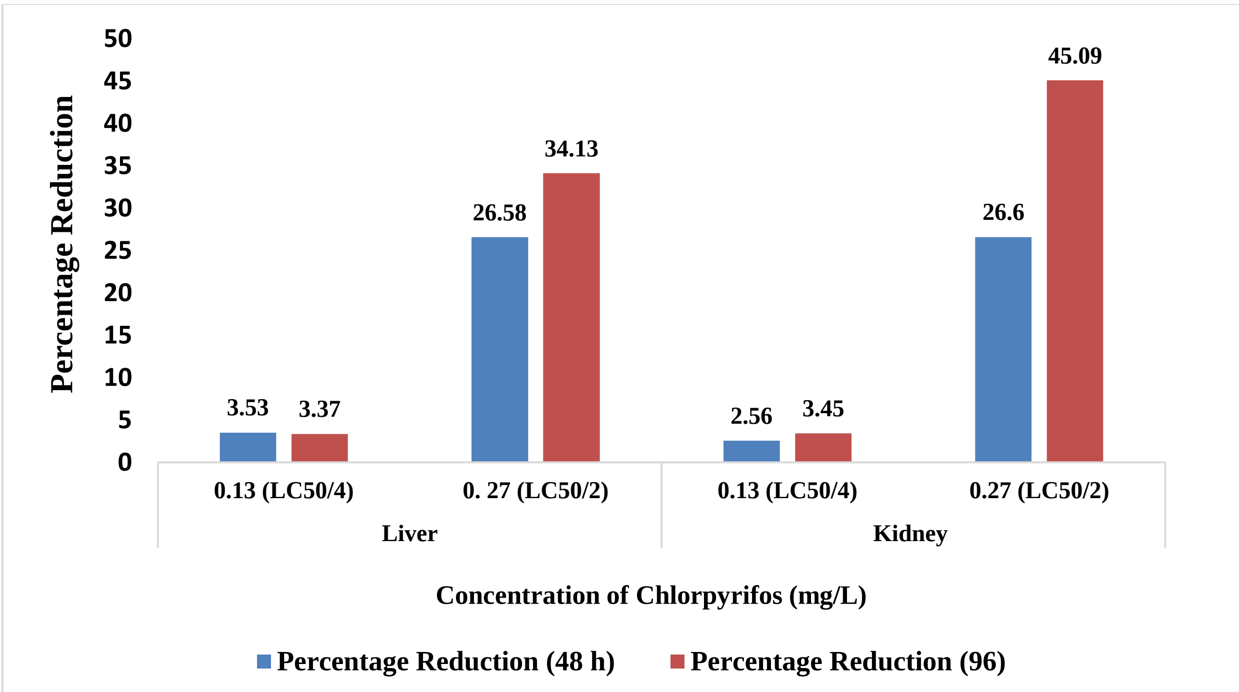

Fig. 4: Total protein content (mg/g) in the liver and kidney tissues of *L. rohita* exposed to different concentrations of insecticide Chlorpyrifos

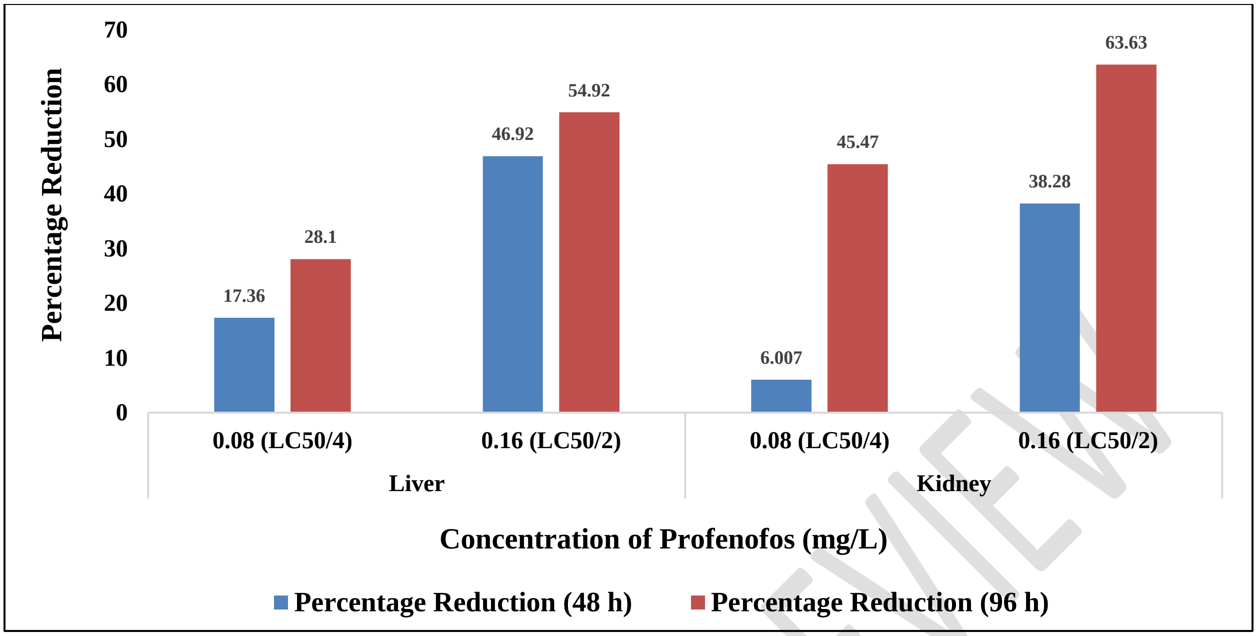

Fig. 5: Glycogen content (mg/g) in the liver and kidney tissues of *L rohita* exposed to different concentrations of insecticide Profenofos

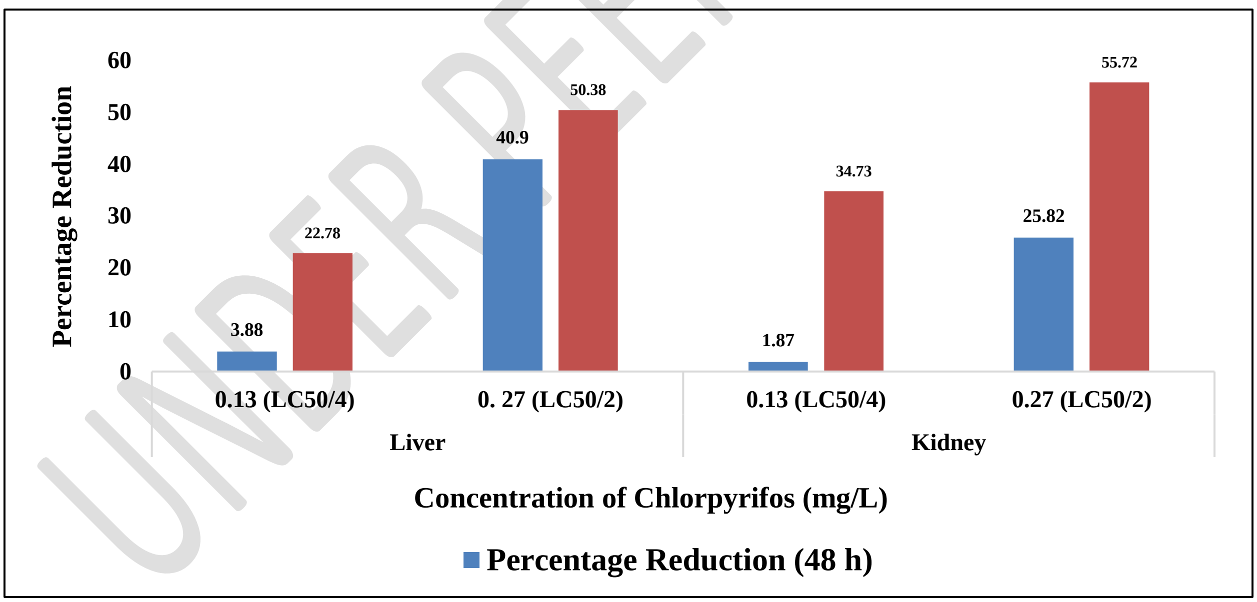

Fig. 6: Glycogen content (mg/g) in the liver and kidney tissues of *L. rohita* exposed to different concentrations of insecticide Chlorpyrifos

The food value of *L. rohita* is very high because it fulfils the required nutritional values in the diet. But, water pollution and environmental degradation continuously degrade the nutritional value and decrease the number of fish in the aquatic system. Fish respond quickly against toxicants, even at deficient concentrations (Cavas, 2011). Aquatic contamination can be easily detected with the use of fish as a biomarker (Van der Oost *et al.*, 2003). In the present investigation, the toxicity evaluation of Profenofos and Chlorpyrifos on *Labeo rohita* was studied, and LC_{50} value was calculated as 0.31mg/L and 0.53 mg/L, respectively. The results clearly show that *L. rohita* is more sensitive to Profenofos. Several factors govern the toxicity of particular chemicals, such as period of exposure, temperature, dissolved carbon dioxide and oxygen contents in water bodies (Macek *et al.*, 1968), the weight of the animal (Pickering *et al.*, 1968), hardness and pH of water (Pakhira, *et al.*, 2015), and developmental stage of animal (Bharathi, 1994). According to Cope (1963), the toxicity of pollutants differed from species to species and different modes of action of pesticides. The acute toxicity of profenofos was studied on many species like *Catla catla* 0.0 69 mg/L (Ghazala et al, 2016), *Labeo rohita* 1.2 mg/L (Bantu *et al.*, 2013). During the present course of investigation, the result showed, after acute exposure of sub-lethal concentrations of insecticides Profenofos and Chlorpyrifos in *L. rohita*, the protein and glycogen content of the liver and kidney was decreased as compared to the control.

The depletion of protein and glycogen level in vital organs of *L. rohita* could be the possible impact on the enzyme-mediated bio-defence mechanism of fish (Jerald et al., 20204), which poses serious threats to the aquatic environment as well as human life through the food chain. Glucose and glycogen are important sources of energy and liver glycogen helps in export of hexose units for maintenance of blood glucose (Nawaz et al., 2020). Depletion of glycogen indicates its rapid utilization to overcome energy demand in toxicological environment through glycolysis or hexose monophosphate pathway. Many investigators reported depletion of glycogen in *L. rohita* after exposure of different pesticides i.e. Veeraiah et al. (1998) exposed fish into Cypermethrin, Muley et al, (2007) exposed fish into tannery effluents, Bantu et al. (2013) after exposure of carbosulfan, Binukumari *et al.* (2013) after exposure of dimethoate, and Mariya Dasu (2013) exposed fish into thiodicarb.

Some literature has already reported a similar depletion of protein content in the tissue of fish, i.e., Suneetha *et al.* (2010) in *L. rohita* after Fenevalerate exposure and Singh (1988) in Clarias batrachus after Malathion exposure, protein was depleted. Patil and David (2009) showed a similar report on *L. rohita* after exposure to Malathion**.** To face the toxicity of insecticides, fish need lots of energy provided by reserved food materials like protein, carbohydrates and lipids. Christobher *et al.* (2016) reported decreased total protein content in the liver tissue of *L. rohita* when exposed to 1.0 ppm concentration of Phosphamidon in 15 days (Control group 2.12 ±0.37 gm/dL and Experimental group 0.95±0.19 gm/dL). The degradation of protein increases the proteolysis activities and products used for metabolic purposes and causes damage to the tissue (Mastan and Rammayya, 2010). After acute 48 and 96 hours of exposure to sub-lethal concentrations I (LC_{50}/4), II (LC_{50}/2) of Profenofos and Chlorpyrifos, showed significant depletion and histopathological alterations were noticed. During acute treatment of pesticides, the amount of protein and glycogen content in the tissue of the liver and kidney gradually decreases. This disrupts a normal metabolic pathway, which leads to an increase in the rate of mortality in the fish population. Additionally, the present research suggested that alternative sources need to be used instead of Organophosphorous pesticides like Profenofos and Chlorpyrifos, such as genetic engineering methods and biopesticides, and also sustainable strategies should be developed to restore the contaminated environment.

Conclusion

The research provides valuable insights into the effects of Profenofos and Chlorpyrifos in *L. rohita*, highlighting the importance of responsible pesticide use and environmental stewardship. In the present investigation, the biochemical alterations in the glycogen and protein in the liver and kidney tissue of *L. Rohita* were studied. After acute 48 and 96 hours of exposure to sub-lethal concentrations I (LC_{50}/4), II (LC_{50}/2) of Profenofos and Chlorpyrifos, showed significant depletion and histopathological alterations were also noticed. The finding contributes to understanding the mechanism of pesticide toxicity in fish, emphasizing the need for environmental monitoring and regulation of pesticide use. Pesticide policy varies considerably across different countries. In India, regulation of pesticides was governed by two bodies viz., Central Insecticides Board and Registration Committee (CIBRC) and Food Safety and Standards Authority of India (FSSAI). International coordination of pesticide law must be adaptable for proper regulation, including pesticide manufacturers, consumers, farm workers and regulators. Developing countries could not adopt this law quickly because of farmers' lack of knowledge and investment, which created a barrier to applying regulations in those countries. Local groups have more involvement in the process of establishing national regulations, they may be more compliant with these as opposed to those set internationally. This study concludes that a very low amount of pesticide seriously affects the liver tissue of *L. rohita* and poses a danger to the survivability of fish in its habitat. There is a need for complete knowledge of using pesticides in agricultural farms, which eventually ends up in the aquatic life. The government should make strict rules for the use of these harmful agrochemicals.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Reference:

- APHA (1998). Standard Methods for the examination of water and wastewater. 20th ed. Washington, DC: American Public Health Association.

- Bantu, N., Vakita, V. R., & Karra, S. (2013). Effect of chlorantraniliprole on biochemical and certain biomarkers in various tissues of freshwater fish Labeo rohita(Hamilton). Environment and Ecology Research, 1(4), 205–215. https://doi.org/10.13189/eer.2013.010403
- Baskaran P. (1991). Use of biochemical parameters on biomonitoring of pesticide pollution in some freshwater fishes. *Ecotoxicology and Environmental Safety*, 1(2), 104-109.

- Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., & Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. Journal of Fish Diseases, 22(1), 25–34. https://doi.org/10.1046/j.1365-2761.1999.00134.x

- Bharathi, C. (1994). Toxicity of insecticides and effects on the behavior of the blood clamAnadara granosa. Water, Air, and Soil Pollution, 75(1–2), 87–91. https://doi.org/10.1007/bf01100401

- Bhatnagar, M.C., Bana, A.K., Tyagi, M. (1992). Respiratory distress to *Clarias batrachus* (Linn) exposed to endosulfan a histological approach. *Journal of Environmental Biology*, 13, 227-231.

- Binukumari, S. and Vasanthi, J. (2013). Changes in protein content of the freshwater fish, *Labeo rohita* due to the effect of an insecticide 'Encounter' (herbal plant extract). *International Journal of Current Microbiology and Applied Sciences*, 2(10), 150-153. https://www.ijcmas.com/vol-2-10/S.%20Binukumari%20and%20J.%20Vasanthi.pdf

- Cavas T. (2011). In vivo genotoxicity evaluation of atrazine and atrazine-based herbicide on fish Carassius auratus using the micronucleus test and the comet assay. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, *49*(6), 1431–1435. https://doi.org/10.1016/j.fct.2011.03.038

- Christober, S., Periyasamy, M., Suganthi, P., Saravanan, T.S. (2016). Effect of Phosphamidon on Haematological and Biochemical parameters in freshwater fish *Labeo rohita*. *International Journal of Fauna and Biological Studies*. 3(1), 114-116. https://www.faunajournal.com/archives/2016/vol3issue1/PartB/2-6-18.pdf

- Cope, O.B. (1963). Spot fishery investigations: pesticides- wildlife studies. A Review of fish & wildlife service investigations during the Calendar year. U.S. Department of the Interior, Fish & Wildlife Services, Circular 199, 29-43. https://spo.nmfs.noaa.gov/sites/default/files/legacy-pdfs/CIRC199.pdf

- El-Sayed, Y. S., Saad, T. T., & El-Bahr, S. M. (2007). Acute intoxication of deltamethrin in monosex Nile tilapia, Oreochromis niloticus with special reference to the clinical, biochemical and haematological effects. *Environmental toxicology and pharmacology*, *24*(3), 212–217. https://doi.org/10.1016/j.etap.2007.05.006

- Li, Z. H., Velisek, J., Zlabek, V., Grabic, R., Machova, J., Kolarova, J., & Randak, T. (2010). Hepatic antioxidant status and hematological parameters in rainbow trout, Oncorhynchus mykiss, after chronic exposure to carbamazepine. *Chemico-biological interactions*, *183*(1), 98–104. https://doi.org/10.1016/j.cbi.2009.09.009

- Macek, K. J., Hutchinson, C., & Cope, O. B. (1969). The effects of temperature on the susceptibility of bluegills and rainbow trout to selected pesticides. *Bulletin of environmental contamination and toxicology*, *4*(3), 174–183. https://doi.org/10.1007/BF01560960

- Mariya Dasu, P., Vivek, C.K., Venkata, R.V. (2013), Thiodicarb (Larvin 75% wp) toxicity and its impact on biochemical changes and oxygen consumption of freshwater fish, *Labeo rohita* (Hamilton). *International Journal of Recent Scientific Research*, 4(12), 2143-2148.

- Mastan, S.A., Rammayya, P.J. (2010). Biochemical profile of *Channa gachuna*(Ham) exposed to sublethal doses of Dichlorvas (DDVP). *The internet journal of toxicology*, 8, 27-32. https://ispub.com/IJTO/8/1/11029

- Mercy, T.V.A., Kurup, B.M., Nair, JR., Sulckha, B.T. (2000). Lethal toxicity of Phosphamidon on the juveniles of *Anahas testudineus* (Bloch) and *Etroplus maculates* (Bloch). *Indian Journal of Fisheries*, 47(1), 87-90.

- Muley, D. V., Karanjkar, D. M., & Maske, S. V. (2007). Impact of industrial effluents on the biochemical composition of fresh water fish *Labeo rohita*. *Journal of environmental biology*, *28*(2), 245–249.

- Nemcsók, J., & Benedeczky, I. (1990). Effect of sublethal concentrations of phenol on some enzyme activities and blood sugar level of carp (Cyprinus carpio L.). *Environmental monitoring and assessment*, *14*(2-3), 377–383. https://doi.org/10.1007/BF00677929

- Pakhira, C., Nagesh, T. S., Abraham, T. J., Dash, G., & Behera, S. (2015). Stress responses in rohu, Labeo rohita transported at different densities. Aquaculture Reports, 2, 39–45. https://doi.org/10.1016/j.aqrep.2015.06.002
- Patil, V.K. and David, M. (2009). Hepatotoxic potential of Malathion in the freshwater Teleost, *Labeo rohita* (Hamilton). *Veterinarski Arhiv*, 79(2), 179-188. https://wwwi.vef.hr/vetarhiv/papers/2009-79-2-8.pdf
- Pickering, Q. H., Henderson, C., & Lemke, A. E. (1962). The toxicity of organic phosphorus insecticides to different species of warmwater fishes. Transactions of the American Fisheries Society, 91(2), 175–184. https://doi.org/10.1577/1548-8659(1962)91[175:ttoopi]2.0.co;2
- Rajalakshmi P. and Mohandas, A. (1998). Acute effects of pesticide stress on the rate of oxygen uptake in the freshwater mussel, L. *amellidans corrianus* (L.ea). *Proceedings of Academy of environmental Biology*, 7(1), 45-49.
- Sadiq Bukhari, A., Syed Mohamed, H. E., Broos, K. V., Stalin, A., Singhal, R. K., & Venubabu, P. (2012). Histological variations in liver of freshwater fish Oreochromis mossambicus exposed to 60Co gamma irradiation. *Journal of environmental radioactivity*, *113*, 57–62. https://doi.org/10.1016/j.jenvrad.2012.04.011
- Saravanan, M., & Ramesh, M. (2013). Short and long-term effects of clofibric acid and diclofenac on certain biochemical and ionoregulatory responses in an Indian major carp, Cirrhinus mrigala. *Chemosphere*, *93*(2), 388–396. https://doi.org/10.1016/j.chemosphere.2013.05.015
- Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental cell research*, *175*(1), 184–191. https://doi.org/10.1016/0014-4827(88)90265-0
- Suneetha, K., Kumar, K.G., Veeraiah, K. (2010). Changes in protein subunits induced by endosulfan and fenvalerate in fresh water fish Labeo rohita through SDS-PAGE. *Journal of Environmental Biology*, 31 (5), 59-763.

https://www.scopus.com/record/display.uri?eid=2-s2.0-77956443138&origin=inward&txGid=cb27693602e01ce0cc22496b585b33fa

- Toumi, H., Boumaiza, M., Immel, F., Sohm, B., Felten, V., & Férard, J. F. (2014). Effect of deltamethrin (pyrethroid insecticide) on two clones of Daphnia magna (Crustacea, Cladocera): a proteomic investigation. *Aquatic toxicology (Amsterdam, Netherlands)*, *148*, 40–47. https://doi.org/10.1016/j.aquatox.2013.12.022

- Veeraiah, K. and Durga, P.M.K. (1998). Study on the toxic effects of Cypermethrin on organic constituents of freshwater fish, *Labeo rohita* (Hamilton). *Proceedings of Academy of environmental Biology*, 7(2), 143-148.

- Mehmood, S., Zaman, K., Khan, S., Ali, Z., & Khan, H. ur R. (2024). The role of green industrial transformation in mitigating carbon emissions: Exploring the channels of technological innovation and environmental regulation. *Energy and Built Environment*, *5*(3), 464–479. https://doi.org/10.1016/j.enbenv.2023.03.001
- McAbee, K. E., Pendergraft, S. S., Atala, A., Bishop, C., & Sadri-Ardekani, H. (2018). Effect of environmental toxins on spermatogonial stem cells. In *Bioenvironmental Issues Affecting Men's Reproductive and Sexual Health* (pp. 53–70). Elsevier. https://doi.org/10.1016/B978-0-12-801299-4.00004-9
- Wołejko, E., Łozowicka, B., Jabłońska-Trypuć, A., Pietruszyńska, M., & Wydro, U. (2022). Chlorpyrifos Occurrence and Toxicological Risk Assessment: A Review. *International journal of environmental research and public health*, *19*(19), 12209. https://doi.org/10.3390/ijerph191912209
- Sunanda, M., Rao, J. C.S., Neelima, P., Rao, K.G., Simhachalam, G. (2016). Effects of Chlorpyrifos (an Organophosphate Pesticide) in Fish. *Int. J. Pharm. Sci. Rev. Res*., 39(1), 299-305.
- Nataraj, B., Hemalatha, D., Rangasamy, B., Maharajan, K., & Ramesh, M. (2017). Hepatic oxidative stress, genotoxicity and histopathological alteration in fresh water fish *Labeo rohita* exposed to organophosphorus pesticide profenofos. Biocatalysis and Agricultural Biotechnology, 12, 185–190. https://doi.org/10.1016/j.bcab.2017.09.006

- Kushwaha, M., Verma, S., & Chatterjee, S. (2016). Profenofos, an acetylcholinesterase-inhibiting organophosphorus pesticide: A short review of its usage, toxicity, and biodegradation. Journal of Environmental Quality, 45(5), 1478–1489. https://doi.org/10.2134/jeq2016.03.0100
- Weis, G. C. C., Assmann, C. E., Mostardeiro, V. B., Alves, A. de O., da Rosa, J. R., Pillat, M. M., de Andrade, C. M., Schetinger, M. R. C., Morsch, V. M. M., da Cruz, I. B. M., & Costabeber, I. H. (2021). Chlorpyrifos pesticide promotes oxidative stress and increases inflammatory states in BV-2 microglial cells: A role in neuroinflammation. *Chemosphere*, *278*(130417), 130417. https://doi.org/10.1016/j.chemosphere.2021.130417
- Nawaz, A., Zhang, P., Li, E., Gilbert, R. G., & Sullivan, M. A. (2020). The importance of glycogen molecular structure for blood glucose control. *iScience*, *24*(1), 101953. https://doi.org/10.1016/j.isci.2020.101953
- Jerald, I., Ravindran, J., & Babu, M. M. (2024). Fish in focus: Navigating organ damage assessment through analytical avenues - A comprehensive review. *Toxicology reports*, *13*, 101841. https://doi.org/10.1016/j.toxrep.2024.101841
- Christobher, S., Periyasamy, M., Suganthi, P., Saravanan, T.S. (2016). Effect of Phosphamidon on Haematological and Biochemical parameters in freshwater fish *Labeo rohita*. *International Journal of Fauna and Biological Studies*, 3(1), 114-116. https://www.faunajournal.com/archives/2016/vol3issue1/PartB/2-6-18.pdf
- Ghazala, G., Mahboob, S., Al-Ghanim, K. A., Sultana, S., Sultana, T., Al-Misned, F., Ahmed, L., Shahid, T., & Ahmad, Z. (2016). A study on chronic effect of profenofos on acetylcholinesterase and butyrylcholinesterase activities and protein contents in various tissues in major carps. African Journal of Traditional, Complementary, and Alternative Medicines, 13(3), 54. https://doi.org/10.4314/ajtcam.v13i3.7
- Van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology, 13(2), 57–149. https://doi.org/10.1016/s1382-6689(02)00126-6