A Comparative Study On The Effect Of Common Organophosphorous Pesticides Stress On Behavioural Response Of Freshwater Fish *Labeorohita* (Hamilton, 1822)

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Abstract
Agrochemicals, such as fungicides, insecticides, rodenticides, and molluscicides, are all collectively called pesticides which are very harmful to aquatic life and human health. The accumulation of pesticides on soil and water enters into the food chain and affects human health. The present study aims to compare the effects of some common organophosphorus pesticides Chlorpyrifos, and Profenfos on behavioral aspects of *Labeorohita* (Hamilton). 70 healthy, equal-sized Rohu fish were divided into seven equal groups, one control group, and 6 other treated groups three groups were exposed to Profenfos and another three groups exposed to Chlorpyrifos. Behaviour of fish was observed at intervals of 5, 10, and 15 days. Various behavioural responses were measured qualitatively in terms of normal and abnormal (mild, moderate and severe) behaviour. It is concluded that the toxicity of Profenfos is more than Chlorpyrifos on the behavioural response and morphological deformities of *Labeorohita* and indiscriminate use of agrochemicals creates lots of environmental problems and harms aquatic ecosystems and human health.

Keywords: Agrochemicals, *Labeo rohita*, Profenfos, Chlorpyrifos.

INTRODUCTION

Morden era is based on industrialization, urbanization, and technological advancement with the use of huge amounts of chemicals such as agrochemicals, food additives, and halogenated polycyclic hydrocarbons (Ibeto and Okoye, 2010). Contamination of water bodies through agrochemicals has become a worldwide concern. Agricultural products were protected from pest attacks through the most efficient weapons used by farmers; these are pesticides (EI-Houseiny *et al.*, 2022). Indiscriminate use of pesticides in fields enters into water bodies through water runoff and contaminates water, which affects nontarget organisms like fish and finally via the food chain threatening the biodiversity of nature and irregular the ecological equilibrium (Dar *et al.*, 2016). Pesticide enters aquatic organisms and bioaccumulates in the tissues (Maurya and Malik, 2016; Yadav *et al.*, 2018). Agricultural sources pollute about 50% of the total water surface (Gavrilescu *et al.*, 2015).

Profenfos is an organophosphate insecticide, used against Cabbage caterpillars, Wheat and Cabbage aphids, Mealybug, *Patella xylostella*, and *Asparagus caterpillars* (United State Environmental Protection Agency, 2006). Dichlorvos is widely used in animal husbandry, agriculture, food storage, horticulture, etc, and enters into the aquatic system via runoff. Chlorpyrifos is the second largest selling insecticide in India and is used to control pests on paddy fields, cotton, vegetable crops, etc (Rao *et al.*, 2003).

Fish is the best bioindicator for aquatic toxicology because of very sensitive to the presence of pollutants in water. Fish is a good source of food for humans, so represents a risk to human health also (Abdel-Moneim *et al.*, 2012). Toxic substance accumulates easily in fish tissues (Aghoghovwia*et al.*, 2016; Izah and Angaya, 2016). Since Rohu is the economic backbone of the aquaculture industry in India, the present paper is oriented towards studying the toxic effect of some organophosphorus pesticides on the behaviour of Rohu fish *Labeo rohita* in laboratory conditions. The present study aims to dose-dependent and time-dependent negative effects of Chlorpyrifos, and Profenfos exposure on the behavioural response of freshwater fish *Labeo rohita*.

MATERIALS AND METHODS

A brief description of the materials and methods are described under the following heads:

Collection of the test organism

Seventy healthy freshwater fish *Labeorohita* having an average weight of 7.0 ±1.5 g and nearly equal sized 6.0 ±0.8 cm in length were collected from the local fish market. Fish were transported from the fish market to the lab within well-packed polythene bags containing aerated water.

Fish acclimatization

Fish washed with $KMnO_4$ to protect it from any bacterial infections. The fish were acclimatized in the laboratory condition for 15 days before the experiment. Fish were fed twice a day with commercial fish food. During the experiment, if the rate of mortality exceeded more than 5%, the entire batch of fish was discarded.

Maintenance of aquarium

Seven aquariums, connected with a constant aeration system with a 12:12 light-dark cycle, were used for the experiment. Physico-chemical parameters of water were regularly checked out like temperature, pH, dissolved oxygen concentration etc. The hydrographical conditions of water used for acclimatization are as follows. Dechlorinated tap water is used in aquariums whose physicochemical parameter of water is maintained as pH 7.0±7.2, temperature 25.0 ±2, dissolved oxygen 6.45 mg/L, hardness 23.3±3, and specific gravity 1.000 kg/$m^{3,}$. The aquarium water was renewed after 24 hours in order to reduce the metabolic waste contamination and dead fish if any. During the experimental period, provide optimal conditions for fish to avoid mortality.

Test chemicals

Two different formula grade organophosphorous pesticides Profenfos (Tread name- Celcron) and Chlorpyrifos (Tread name-Pota) were used for the present study procured from the local Agro-chemical market of Bilaspur (C.G.) Pesticides were purchased after checking their expiry date.

Experimental Design

The acclimatized fish was randomly divided into seven groups, each having 10 fish and kept in 30 L of water. One aquarium served as a control group, other six were treated groups. During the experiment period, the control group maintained under normal conditions of control water.

Time-Dependent: The treated group were exposed to Profenfos, and Chlorpyrifos, respectively and experiment were conducted for a period of 15 days with 5 days sampling frequency.

Dose-Dependent: On the basis of previous research (Nagarjuna *et al*., 2013), the estimated 96 h LC_{50} value of Profenfos for *Labeorohita* was 0.1 mg/L. Based on previous literature (Samajdar*et al*., 2015). LC_{50} Value of Chlorpyrifos was 0.109 mg/L. During this course of investigation, the fish were exposed to 50%, 20%, and 10% of the LC_{50} value of respective insecticides and observed behavioural response of fish.

Table 1: Laboratory experimental design

S.N.	Group Name	Type of the group	Exposure time (in days)	Concentration of Profenfos (mg/L)	Concentration of Chlorpyrifos (mg/L)
1.	A	Control	5, 10, 15	None	None
2.	B	Treated	5, 10,15	0.01	
3.	C	Treated	5, 10, 15	0.02	
4.	D	Treated	5, 10, 15	0.04	
5.	E	Treated	5, 10, 15		0.05
6.	F	Treated	5,10, 15		0.02
7.	G	Treated	5, 10, 15		0.01

RESULTS AND DISCUSSION

The present research work was carried out to explore the comparative account of the effect of common Organophosphorous pesticide stress on the behavioural response of freshwater fish *Labeo rohita.*

Results

The systematic observations are mentioned below.

Water analysis: During the experiment, the physico-chemical properties of water were examined at regular time intervals. The recorded values of the properties of water are given below in Table 2.

Table 2: Physico-chemical properties of water:

S.N.	Properties	Value
1.	Temperature	28 ºC
2.	Turbidity	8 silica units
3.	pH	7.2 ±0.5
5.	Electrical Conductivity	816.0 µΩ/cm
6.	Total Hardness (As $CaCO_3$)	23.3 ±3 mg/L
7.	Dissolved Oxygen	6.45 mg/L

BehaviouralResponse of *Labeo rohita*

The changes in behavior patterns in *Labeorohita* were seen when exposed to Profenfos, and Chlorpyrifos organophosphorus pesticides. At the low concentration of Profenfos and Chlorpyrifos, there was no variation placed in treated groups. When fish were exposed to high concentrations many behavioural changes were seen.

In the present study, when the concentration of insecticides increased, fish showed abnormal behaviour like hyperexcitability, jerky movement, loss of scales, opening mouth for grasping air, loss of balance, etc. In the middle duration of exposure, fish exposed to Profenfos showed rapid, erratic semi-circular swimming behaviour, rapid gulping of water, knocking the wall of the aquarium, then the fish showed loss of equilibrium and showed vertical position with head down. In the later stage of exposure, fish lay down at the bottom of the aquarium, the rate of swimming negligible, and sink the bottom of the aquarium. At the end of the exposure period, lethargy is extreme. At post-mortem, the fish showed any abnormal signs such as loss of scales, excessive mucus secretion, shined skin colour, and inflamed organs like kidneys, gills, and liver.

The control group fish with well-synchronized movements and was very active for feeding. When Rohu was exposed to different concentrations of Chlorpyrifos, their behavioural pattern was disrupted. First of all disrupts schooling behaviour and spreads out in swimming. Fish move at the corner of the aquarium so avoidance behavior of the fish to Chlorpyrifos. Fish showed irregular erratic movement, hanging vertically on the water's surface. Fish slowly lethargic, and restless and showed a surfacing phenomenon to gulping the air. The impact of organophosphorous pesticide stress on the behaviour of *Labeorohita* is mentioned in Table 3.

Table 3: Impact of organophosphorous pesticides stress on behaviour of *Labeo rohita*

S.N.	Parameters	Insecticide concentration (% of LC_{50} Value)	Control Group	Profenfos	Chlorpyrifos
1.	Convulsions	10	-	+	-
		20		+	+
		50		+	+
2.	Hyperexcitability	10	-		
		20		++	+
		50			
3.	Loss of Balance	10	-	+	+
		20		++	++
		50		+++	++
4.	Rate of Opercular activities	10	-	++	+
		20		++	+
		50		+	+
5.	Rate of swimming	10	-	-	-
		20		+	+
		50		++	+
6.	Body colour	10	-	-	-
		20		++	+
		50		++	++
7.	Surfacing activities	10	-	+	-
		20		+	+
		50		++	++
8.	Lethargy	10	-	-	-
		20		+	+
		50		+++	++
Normal behaviour of fish (-) ; Rate of abnormality: mild ≤10% (+); modrate 10 to 50% (++); Severe ≤ 50% (+++)					

Discussion

This study was conducted to understand the toxic impact of selected organophosphorus pesticide stress on the behavioural patterns of freshwater fish *Labeo rohita*. The control group showed normal behaviour but the treated group showed many abnormalities in behaviour patterns such as swimming patterns, loss of balance, surfacing activities, convulsion, rapid swimming, irregular opercula activities, etc. The mucus debris accumulated in the gill region of the fish, to clean it, the fish increased ventilation rate by rapid opening and closing of mouth, and operculum covering and coughing was observed. Many authors suggested that Profenfos irregulates the activity of acetylcholinesterase concerning the nervous system, so fish showed hyperexcitability (Shahul Hameed and Vadamalai, 1989). Imbalanced swimming patterns were also noticed by Kesherwani, *et al.*(2018). Exposure of fish with a high concentration of Profenfos showed jerky spiral movement, lying laterally at the bottom with loss of balance, similar behavioural changes were also testified earlier in the *Labeo rohita* treated with Profenfos (Nagarjuna *et al.*, 2013). Nagarju *et al.*(2013) and Rathnamma *et al.*(2008) revealed that due to insecticidal stress, many behavioral variations were seen in fish which easily prey in their natural habitat and affect the stability of the population. The same clinical signs were observed by Rahman *et al.* (2002) and EI-bouhy*et al.*(2023), when *C. Idella* was exposed to Profenfos, such as rapid semi-circular swimming, trying to

jump out of the aquarium, fast opercula movement. Respiratory capacity decreases and respiratory irritation occurs due to toxicants that damage the tissue of the gill. At the end of the exposure, the movement of the fish was stopped, remained motionless, and settled down on the bottom of the aquarium. Organophosphorous pesticides inhibit the activity of Acetylcholinesterase and fish became lethargic and showed abnormal behavior. Halappa *et al.* (2009) also noted this type of behaviour in *Labeo rohita* when exposed to Chlorpyrifos. Fish behaviour response and morphological deformities are due to the inhibition of activities of acetylcholinesterase. Caudal bending in *Labeorohita* reported by Halappa(2009) also, due to muscular acetylcholine activity inhibition. The study of behaviour response gives a direct approach to toxicants in fish. Radhaiah*et al.* (1987) and Warner *et al.* (1996) has been observed that abnormal behaviour in fish, was due to their diversified biochemical and physiological.

CONCLUSION

Fish are the most valuable aquatic vertebrates with high nutritional values, and valuable for medicinal ethical, economic, and industrial purposes. Organophosphorous compounds are effect directly nervous system of fish and show abnormal behaviour of fish. It is concluded that this study based on those parameters, which are very sensitive indicators of toxicant effect, can be used as potential biomarkers of pesticide toxicology for further investigations in the field of environmental biomonitoring. Insecticide toxicity is influenced by the duration of exposure and concentration of the toxicant. Due to the effect of insecticide, the ability of fish to remember their previous behaviour gradually decreases. Fishes are unable to adapt themselves to this changed environment. Profenfos is a highly toxic agrochemical even a minute quantity of Profenfos can disturb the behavior and morphological quality of fish.

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