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Exposure of chlorpyrifos pesticide on Indian major carps (IMCs) alters behavioural, haematological and biochemical parameters

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ABSTRACT

This study explores the effects of chlorpyrifos pesticide on the behavioural, haematological and biochemical parameters of Indian major carps (IMCs). On exposure to sublethal concentration of pesticides the IMCs showed reduced feed intake, lessening their energy and impairing its growth and reproduction. They showed behavioural alterations in the form of uncoordinated movements, erratic swimming, convulsions, excess mucus secretion, decreased opercular movements, loss of balance, faster opercular activity, infrequent hyper-excitability, drowning and change in body pigmentation and muscle fasciculation as well. The results of acute toxicity show that 100% mortality occurs at 22.5 mg/l concentration of chloropyriphos exposure for 96 hrs. Bochemical analysis showed that after pesticide exposure blood sugar level has been increased due to glycogenolysis. The content of glycogen, total proteins and total lipids decreased whereas the levels of total free sugars and total free amino acids increased. Further, the IMCs exposed to the chloropyrifos showed significantly increased blood plasma concentration of ammonia, Calcium ion (Ca^{2+}) and Cholinesterase activity while reduction in triglyceride and inorganic phosphate content. The findings of the present research will help the policy makers to make people conscious about the impact of indiscriminate use of pesticides in crop fields.

Keywords: -Chlorpyrifos, Indian major carps, Acute toxicity, Acetylcholinesterase, Haematological, Biochemical.

Introduction

Pesticides are either natural or chemically synthesized compounds that are used to kill, repel, or regulate the growth of a variety of pests. It has been categorized based on their detrimental effects and includes acaricides, herbicides, insecticides, fungicides, rodenticides, nematicides etc. Chemically these pesticides include organophosphate, organocholorines, pyrethroids and carbamates (Rani et al., 2017; Pathak et al., 2022). In spite of its advantages, pesticides are intimidating the long-term survival of major ecosystems by disruption of ecological relationships between organisms and loss of biodiversity. Contamination of freshwater aquatic ecosystem by these pesticides occurs mainly due to intensive agriculture combined with surface runoff and subsurface drainage. Fishes are one of the most important members of the aquatic food chain, and through them these toxicant pesticides may reach human beings as well (Drishya et al., 2016). These pesticides at acute and sublethal concentrations cause alterations in the hormonal and enzymological responses of these fishes. Therefore, understanding the level of toxicity of pesticide is essential for the protection of our already dwindling freshwater fauna (Rohani, 2023).

Aquaculture has developed as one of the most promising and fastest growing food producing sectors in the world. India occupies first position in the global aquaculture production and produced 1.8 million tonnes of fish according to the report of the Food and Agriculture Organization (FAO, 2019). Carp accounts for half of the world inland aquaculture production. India is also regarded as 'Carp country' as carps contributes maximum to the fisheries industry of the country (FAO, 2019) and the major species which contributes to the production are Indian major

carps (IMCs) viz., Labeo rohita, Catla catla, Cirrihinus mrigala (Kanu et al., 2023). It is imperative to study the toxic effects of pesticides on IMCs since they establish a vital link in food chain and their pollution by pesticides imbalances the aquatic system (Cordeiro Bentes et al., 2022). Haematological and biochemical studies on fishes have supposed to be of superior implication because of the growing importance on pisciculture and greater cognizance of the contamination of natural freshwater resources. Pesticide-induced haematological and biochemical changes may be of some value in assessing the impact of exposure to these chemicals and may serve as tools for biological monitoring (Ghayyur et al., 2021).

The present study involves detail investigation of the deleterious effects of an organophosphate pesticide chloropyrifos on fresh water Indian major carps namely Labeo rohita (Ham.), Catla catla (Ham.) and Cirrihinus mrigala (Bloch). The three major carps have been selected as the model of experiment because of its low-cost culture and management and have been proposed for use as a test organism in toxicological assays for its suitability for toxicity testing, wide geographical distribution and availability throughout the year (Jayaprakash and Shettu, 2013). For these aquatic species, at present, there are no standard mandates for consideration of pesticide toxicity which is an area of great concern to both the public and regulatory authorities (Ceger et al., 2023). Quantitative assessment of physiological responses in the form of behavioural, haematological and biochemical parameters have been chosen for the assessment of the sublethal effects of chloropyrifos. Chlorpyrifos is an organophosphate pesticide and has been used on crops to kill numerous pests, counting insects and worms. It acts on the nervous systems of insects by inhibiting the acetylcholinesterase enzyme (Sparks et al., 2020). Based on acute toxicity information, chlorpyrifos is considered hazardous to humans causing disorder to nervous system, persistent developmental disorders, and autoimmune disorders by the World Health Organization (WHO, 2019). Its high level of solubility restricts its use in certain areas where the water table is close to the surface (Kök et al., 1999). The effects of this pesticide on IMCs have been richly documented with poor understanding of behavioural and haematological changes. Therefore, the present study endeavour to investigate the acute toxicity effects of chloropyrifos on behavioural changes, haematological parameters and biochemical parameters in fish along with enzymetic changes in the form of acetylcholinesterase.

Materials and methods

In the present investigation, the three Indian major carps (IMCs) viz., Labeo rohita (Hamilton, 1822), Catla catla (Hamilton, 1822) and Cirrihinus mrigala (Bloch, 1795) have been selected as the model of experiment because of its wide geographical distribution, availability throughout the year with low-cost culture and management, and its suitability for toxicity testing (Boucaud-Maitre et al., 2019).

Acclimatization of fish in laboratory

Live specimens of freshwater carp, Catla catla (Ham.), Labeo rohita (Ham.) and Cirrhinus mrigala (Bloch) were collected from the local pond of Patna, Bihar with the help of fisherman and carefully packaged into aerated polythene bags filled with tap water. In the laboratory, fish were disinfected by treatment of 0.05% potassium permanganate (KMnO4) to make the walls free from fungal attack. They were then transferred into large rectangular plastic tanks containing 500 liters of dechlorinated tap water for acclimatization for 15 days. The acclimatized Catla catla (Ham.) of length 7.5±1.5 cm and weight 12.0±3 g, Labeo rohita (Ham.) of length 6.5±1.5 cm and weight 10±5 g and Cirrhinus mrigala (Bloch) of length 8.0±1.5 cm and weight 10±2 g were sorted and starved for 24 hr before starting the experiment.

The chlorine free groundwater was used with aerators fixed in the entire tank and the pH of the water was adjusted and maintained to 7.4 - 7.8. During acclimatization, water of the tank was changed daily and fish were fed to satiation with dried shrimps twice a day and remaining food was removed daily. No selection or identification of sexes was made. The experiment was conducted under natural photoperiod and temperature in months of September – October, 2022. The water quality parameters were carried out by standard method of A.P.H., Association, (1915) (Ahmad et al., 2021). The physicochemical characteristics of experimental water used were as follows: pH 7.40±0.40; dissolved oxygen 8.35±0.15 mg/l; temp. 23.00±20C; free carbon dioxide 6.5±0.5 mg/l; total hardness as calcium carbonate 135±5.25 mg/l; and electrical conductivity 285.36±60.45 µmho/

cm. For feeding, commercial readymade feed were purchased from Gold Mohur Foods and Feeds Pvt. Ltd, Sanathnagar, Mumbai-79.

Preparation of stock solution of pesticides

The Chlorpyrifos is a white crystalline or irregularly flaked solid, insoluble in water. It has a very faint mercaptan-type odor. Its IUPAC name is diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-?5-phosphane with molecular formula C9H11Cl3NO3PS. (National Center for Biotechnology Information, 2023). Stock solution of chlorpyrifos (an organophosphate) from Rallis India Ltd were purchased and prepared in acetone separately. The stock solutions for 35% emulsifiable concentrate of chlorpyrifos were prepared in 95% acetone to yield a concentration of 100 mg/100 ml which were further diluted with distilled water to get a working solution. All reagents used were of analytical grade.

Behavioural changes in IMCs and determination of LC50 values

The data of behavioral changes and mortality rate of the fish were recorded at four different exposure periods 24, 48, 72 and 96 hr. The dead fish were removed immediately. The toxicity tests were conducted to choose the mortality range from 10% to 90% in static tests. The fish were exposed to several concentrations for defined time intervals, and mortality or immobility for each group was recorded. Five replicates, each containing ten fish were subjected to each of the two pesticides separately at eight different concentrations of 20.5, 21.0, 21.5, 22.0, 22.5, 23.0, 23.5, and 24.0 mg/l of chloropyriphos. Control groups, each having ten fish kept in tap water containing 0.4 ml/l acetone was run concurrently. All experiments were carried out in cylindrical glass aquaria containing 30 liters of test solution. All solutions (control and test) were replaced by freshly prepared one daily and dead fish were immediately removed. The concentration that produced 50% mortality (LC50) in test species had been noted. The LC50 values were calculated by Finney's probit analysis (1971). The data on the mortality rate of fish was recorded. The concentration that produced 50% mortality in test species was noted. The statistics involved were the estimation of the best fit dose-response line and the evaluation of the adequacy of the fitted line (Buikema et al., 1982).

Haematological profile of IMCs before and after exposure to chlorpyrifos

Before exposure to the pesticide, blood from all three classes of IMCs was collected separately. Examinations of fish were performed after 96 hr exposure period with at an exposure level of 96 hr with LC50 of 21.0 mg/l. The IMCs in the control group were monitored concurrently. The test was performed with major carps in 300 liter capacity tanks. Each tank contained the three types of fish in a set of twenty-four i.e. eight tanks treated for 96 hr with LC50 of chlorpyrifos and one control tank for each of the three IMCs respectively. Experiments for all the treated and control fish were carried out in replicates of five. Presence of the tested substance (above 80% of the nominal concentration) was ensured through a 12 hr exchange of the water bath. Twenty four experimental (8 fish from each of the IMCs and twenty-four control IMCs were selected at random and used for haematological examination at the end of the 96 hr exposure. The fish were caught gently in a small net, avoiding stress as much as possible, and immediately anaesthetized in MS-222 (Sandoz) using a concentration of 1/15000. The time taken for the fish to be anaesthetized was usually about 2-3 min, as shown by loss of equilibrium and by immobility when touched. When this occurred the fish was placed on its back in a V-section trough and blood taken by cardiac puncture using a 2 cm3 sterile plastic syringe and a No. 21 swg needle as described by Klontz and Smith (1968). Blood was sampled by vas caudalis using an in syringe and plasma was obtained by centrifuging blood samples in a cooled centrifuge (4°C, 837×g). Plasma samples were held at -800C until analysis (Lawrence et al., 2020).

Haematological parameters like haemoglobin (Hb), haematocrit (Hct) or packed cell volume (PCV), total erythrocyte count or RBC count and total leucocyte count or WBC count were measured using the standard method described by Chung et al., (2015). RBC and WBC were counted in haemocytometer (Improved Neubauer Weber Scientific Ltd). The PCV was determined by haematocrit reader after centrifuging heparinised capillary tubes filled with blood for 5 minutes at 5000 ×g and estimation of Hb in blood was done by using Sahli Haemometer (Marinefield, Germany). Mean corpuscular volume (MCV), Mean corpuscular

haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were calculated using the following formula (Delwatta et al., 2018):

$$MCV = \frac{Haematocrit \text{ or PCV (\%)}}{\text{RBC count (EC in million mm}^3\text{)}} \times 10$$

$$MCHC = \frac{Haemoglobin \text{ (g/dl)}}{\text{Haematocrit of PVC (\%)}} \times 100$$

$$MCH = \frac{Haemoglobin \text{ (g/dl)}}{\text{RBC count (EC in million mm}^3\text{)}} \times 10$$

Biochemical analysis

For biochemical analysis, blood plasma glucose level (GLU) was determined using Abnova kit, India and the amount of glucose expressed as mg/dl. Estimation of total amino acids was done by colorimetric assay kit and was expressed in µg/ml. Quantitative determination of total protein was performed by Assay-Genie kit method and was expressed in µg/ml. The quantity of glycogen was assessed by colorimetric glycogen assay kit (Cell Biolabs, Inc, Kolkata, India) and was expressed in µg/ml. Colorimetric detection of total carbohydrates (sugar) was done by kit method of Cell Biolabs, Inc, Kolkata, India (Copur et al., 2003) and was expressed in µg/ml. Total lipids was estimated by Malondialdehyde (MDA) Colorimetric Assay Kit (TBA Method) (Elabscience) and was expressed in µg/ml. The plasma triglyceride concentration (TRIG) was estimated through Triglyceride Assay Kit (Colorimetric) as per Guo et al., (2011) and the amount of triglyceride expressed as milli-mole per liter (Assay-Genie). The plasma cholinesterase (ChE) activity level was determined by the Garmavy et al., (2023) assay method using Kit (Crest Biosystem, India) and the amount is expressed as µmole/min/mL. The blood ammonia was estimated by colorimetric assay kit (Sigma-Aldrich) and was expressed in mmol/l. Plasma calcium (Ca2+) is estimated by Calcium Assay Kit of Assay-Genie. The estimation of inorganic phosphate (PHOS) in blood plasma is done through Colorimetric Phosphate Assay Kit.

Statistics and data analysis

Statistical investigations were executed by a one-way ANOVA (analysis of variance). Differences between means were determined by Duncan's multiple range test (DMRT) ($p < 0.05$). The correlation between hematological variables was analyzed by the Pearson coefficient for linear correlation at $p = 0.05$.

Results

Acute toxicity and behaviour changes of IMCs exposed to chlorpyrifos

Pesticides have the potential to enter aquatic habitats from direct application, terrestrial runoff or wind borne drift. A pesticide's capacity to harm fish and aquatic animals is largely a function of its toxicity, exposure time, dose and persistence in the environment. Exposure of fish and other aquatic animals to any pesticide depends on its bioavailability, bioconcentration, biomagnification, and persistence in the environment. The results in Table 1 shows that percentage of deaths increases as the concentration of chlorpyriphos increases from 20.5 mg/l to 24.0 mg/l. Hundred percent deaths have been recorded in the two classes of fishes i.e., Catla catla and Cirrhinus mrigala at concentrations of 22.5 mg/l and above after 96 hr of exposure while only 90% deaths have been observed in Labeo rohita under similar condition. Labeo rohita showed 100% mortality after exposure to 23.0 mg/l and above of pesticide chloropyrifos for 96 hours.

Behavioral responses are quicker with subtle toxicity parameter compared to mortality, indicating that behavioral changes of fish can be easily be monitored for unintended releases of insecticides in water bodies. From the result it is evident that the Catla catla, Labeo rohita and Cirrhinus mrigala showed behavioural alterations against chloropyrifos intoxication in the form of uncoordinated movements, erratic swimming, convulsions, excess mucus secretion, decreased opercular movements, loss of balance, faster opercular activity, infrequent hyper excitability, drowning and change in body pigmentation and muscle fasciculation as well. The symptoms of lethargy, refusal of feeding, respiratory distress became more apparent with increase in duration of exposure at all test concentration of chloropyrifos (Table 2 - Table 4). After exposure to the sublethal concentration of pesticides the selected carps showed reduced feed intake which ultimately lessens the energy intake which impaires its growth and reproduction also.

Table-1:

Percentage lethality of the three Carps after exposure to eight different concentrations of Chloropyrifos

Concentration of Chloropyrifos in mg/l	Exposure time in hrs.	Death percentage (%)		
		Catla catla	*Labeo rohita*	*Cirrhinus mrigala*
20.5	24	10	10	10
	48	20	20	20
	72	30	30	30
	96	40	50	40
21.0	24	10	10	10
	48	20	20	20
	72	40	50	30
	96	60	70	40
21.5	24	20	20	20
	48	20	50	20
	72	50	60	50
	96	80	80	80
22.0	24	20	30	20
	48	30	50	40
	72	60	70	50
	96	80	80	70
22.5	24	30	30	30
	48	50	50	40
	72	60	70	60
	96	100	90	80
23.0	24	30	50	30
	48	50	60	50
	72	70	80	80
	96	100	100	100
23.5	24	50	50	50
	48	70	80	60
	72	90	90	80
	96	100	100	100
24.0	24	80	70	60
	48	90	70	80
	72	100	100	100
	96	100	100	100

Effect of chlorpyrifos on haematological and Biochemical profiles of IMCs

Pesticides have been reported to significantly damage haematological and biochemical processes when they enter into the organs of fishes. In animals, any stress could inflict excessive energy demand, which is immediately fulfilled by blood glucose. As a consequence, the blood sugar level could increase as observed in the present experimental IMCs. Accordingly, reduction in the glycogen reserve in the blood could be subjected to glycogenolysis with the resultant inhibition of glycogenesis. The control fish, C. catla, L. rohita and C. mrigala showed 75.00 mg/dL, 70.00 mg/dL and 72.00 mg/dL of total proteins respectively in their blood. Similarly reduction in total glycogen content in Catla catla as compared to control was recorded and showed significant decline in its content (4.58 mg/dL) at 24.0 mg/l of chloropyrifos on 48 hrs of exposure (Table 5). Similarly Labeo rohita and Cirrhinus mrigala showed a significant decline in glycogen content to 4.45 and 4.55 mg/dL of blood respectively at 24.0 mg/l after exposure to chloropyrifos for 48 hrs. Reduction in total lipids in blood of Catla catla, Labeo rohita and Cirrhinus mrigala was recorded to be 179.27 mg/

dL, 175.27 mg/dL and 175.27 mg/dL respectively at 24.0 mg/l of chloropyrifos on 48 hrs of exposure as compared to control (396.65 mg/dL, Catla catla; 390.65 mg/dL, Labeo rohita and 387.65 mg/dL in Cirrhinus mrigala). In the same way, increase in total amino acids and total free sugars were recorded to be 630.00 mg/dL and 272.15 mg/dL respectively in Catla catla, 627 mg/dL and 270.15 mg/dL respectively in Labeo rohita and 625.00 mg/dL and 273.15 mg/dL respectively in Cirrhinus mrigala at 24.0 mg/l chloropyrifos on 24 hrs of exposure as compared to control . This is suggestive of degradation of proteins and glycogen with the resultant increase of total free amino acids and sugars. The decline in the total lipids in the blood of Catla catla, Labeo rohita and Cirrhinus mrigala under study indicates the utilization of lipids to meet the energy demand during the stress caused by chloropyrifos.

Table-5:

Biochemical composition of blood serum of Catla catla, Labeo rohita and Cirrhinus mrigala **in control and 48 h of exposure to eight different concentrations (Conc^n) of Chloropyrifos in µg/ml**

Chloropyrifos Conc^n	Total amino acids	Total proteins	Glycogen	Total sugars (carbohydrate)	Total lipids
Catla catla					
Control	455.00±11.63	75.00±5.54	20.75±1.45	150.00±6.11	396.65±15.41
20.5	470.00±14.26 NS	71.80±1.35*	18.60±1.45*	162.00±10.15 NS	255±22.11 NS
21.0	482.00±12.21NS	63.65±1.35*	16.45±1.35*	173.00±9.75*	343±21.24*
21.5	502.00±11.25*	54.25±1.65*	13.20±1.15*	205.00±9.62*	315±11.65*
22.0	511.00±12.65	45.00±1.65*	10.35±1.16*	225.00±10.61*	282.45±17.64*
22.5	542.00±6.58*	36.80±1.17*	9.35±1.21*	238.00±11.50*	201.55±16.64*
23.0	572.25±7.45*	32.70±1.16*	8.35±1.25*	245.00±15.12*	192.16±11.65*
23.5	602±7.46*	28.00±2.35*	6.27±1.35*	255.10±11.15*	181.14±11.50*
24.0	630.00±13.25*	23.25±2.75	4.58±1.27*	275.15±7.15*	175.27±10.90
Labeo rohita					
Control	450.00±11.63	70.00±5.54	18.75±1.45	140.00±6.11	390.65±15.41
20.5	468.72±14.15 NS	67.80±1.35*	17.6031.45*	165.00±10.15 NS	378±22.19 NS
21.0	485.00±12.21NS	62.65±1.35*	16.45±1.35*	175.24±10.21*	345±21.45*
21.5	507.12±11.00*	55.85±1.80*	12.20±1.22*	205.75±9.89*	318±11.75*
22.0	515.00±12.34	42.59±1.40*	10.25±1.12*	223.45±10.11*	281.40±17.86*
22.5	545.00±6.52*	37.81±1.35*	9.35±1.36*	235.52±11.90*	205.35±16.73*
23.0	575.25±7.49*	33.62±1.25*	8.25±1.81*	241.76±15.55*	185.65±11.34*
23.5	610±7.20*	24.08±2.15*	7.27±1.20*	265.84±11.21*	179.85±11.28*
24.0	630.00±13.10*	20.25±2.66	5.45±1.15*	275.15±7.40*	170.29±11.10
Cirrhinus mrigala					
Control	455.00±11.53	73.00±5.85	17.55±2.65	148.56±6.75	384.68±16.75
20.5	472.00±14.26 NS	65.80±1.62*	16.85±1.25*	164.78±10.15 NS	362.85±21.11 NS
21.0	484.00±12.21 NS	61.65±1.35*	15.75±1.23*	175.55±9.75*	357.54±28.65*
21.5	508.00±11.10*	54.53±1.54*	13.15±1.11*	215.48±9.66*	325.28±11.89*
22.0	512.00±12.32	48.59±1.23*	12.28±1.19*	237.35±10.72*	293.84±17.74*
22.5	535.00±6.39*	39.85±1.13*	8.87±1.01*	255.28±11.62*	242.69±16.23*
23.0	575.25±7.80*	32.68±1.19*	6.56±1.75*	259.24±15.35*	189.37±11.43*
23.5	623±7.48*	26.45±2.38*	6.54±1.35*	268.18±11.24*	180.42±11.65*
24.0	639.08±13.75*	22.28±2.78	4.78±1.25*	276.55±7.35*	173.79±10.22

***= Significant at p>0.05; NS= not significant; + indicates increase over control; indicates decrease over control**

Table-3:
Biochemical profiles of blood plasma of Catla catla, Labeo rohita and Cirrhinus mrigala affected by acute exposure to Chloropyrifos

Indices	Catla catla		Labeo rohita		Cirrhinus mrigala	
	Control	Experimental	Control	Experimental	Control	Experimental
GLU (mmol/l)	3.64±0.75a	4.17±1.84a	3.55±0.15a	4.70±21.12a	3.65±1.15a	4.66±1.16a
TRIG(mmol/l)	0.97±0.12a	0.85±0.19a	0.95±0.15a	0.85±0.14a	0.96±0.12a	0.85±0.15a
Ca2+(mmol/l)	2.53±0.18a	2.82±0.38a	2.13±0.15a	2.71±0.31a	2.25±0.16a	2.65±1.05a
ChE µmole/min/mL	2.03±1.30a	2.54±0.99a	2.00±0.95a	2.48±0.71a	2.05±0.15a	2.30±0.14a
PHOS (mmol/l)	1.46±0.22a	1.38±0.16a	1.40±0.25a	1.35±0.11a	1.41±0.17a	1.35±0.12a
NH3 (mmol/l)	555	721**	515	628**	525	637**

Note: Number of fishes (n) in each group=8; (?± SD); Groups with different alphabetic superscripts differ significantly at $p < 0.05$ (ANOVA) Significant $^{**}p < 0.01$. [GLU: plasma glucose; TRIG: triglyceride; Ca2+: Calcium ion; ChE: Cholinesterase activity; PHOS: inorganic phosphate; NH3: ammonia]

Discussion:

Pesticides are substances used to control pests, including insects and plant diseases. On a global scale approximately over five billion pounds of conventional pesticides are being in use in different areas like agricultural lands, forests, rangelands management, disease control, domestic use and many more areas annually (Akter et al., 2020). The investigations of the effects of pesticides, or any other pollutants, on fishes, aimed at delineating the pollution effects, mainly centre around two broad scientific approaches viz. ecological monitoring and laboratory investigations. Quantitative assessment of the effects of pesticides on fishes has got cardinal importance in fishery management both from the biological and ecological points of view.

In our study, it had been found that maximum number i.e., hundred percent of deaths for chloropyriphos exposure occurs in all the IMCs occured at 23.5 mg/l to 24.0 mg/l concentration for 72 hrs to 96 hrs. The present findings gain support from other works (Tiwari et al., 2019; Ray and Shaju 2023; Clasen et al., 2018; Agrawal et al., 2010). The chloropyrifos adversely alter the fish physiology and thus have potential to interfere with behaviours that may be essential for the survival of IMCs (Vagi and Petsas, 2020). One of the most important manifestations of the toxic action of chemical is the over-stimulation or depression of behavioural activity. Fish experiencing acute exposures to sublethal concentrations of pesticide exhibit significant feeding impairment, with potentially severe consequences for their ecological fitness. Chlorpyrifos insecticide has been designed to be operative either by direct contact or digestion or by inhalation (Floyd, 2008).

From our result it is evident that the IMCs showed behavioural alterations against chloropyrifos intoxication in the form of uncoordinated movements, erratic swimming, convulsions, excess mucus secretion, decreased opercular movements, loss of balance, faster opercular activity, infrequent hyper excitability, drowning and change in body pigmentation and muscle fasciculation as well. However, these symptomed were insignificant after 24 hrs of exposure. But after 72 hrs to 96 hr of exposure, the degree of intensity of aforementioned symptoms became moderate to severe. During the course of symptom development, all the carps first loose their appetite and refuse to feed. In due course, they become lethal and their conditions started deteriorating. Organophosphorous compounds are known to induce neurotoxicity. Irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and hitting to the walls of the test tank before finally sinking to the bottom just before death all reveal neurotoxicity (Ghazala et al., 2014). The behavioural and morphological changes observed in the present work, may be due to the inhibition of acetycholinesterase activity.

Blood is a pathophysiological indicator of the whole body; consequently, blood parameters are

important in diagnosing the structural and functional status of fish exposed to toxicants. Alterations in the blood biochemical profile point toward modifications in the metabolism and biochemical processes of any organism, caused due to the effects of various pollutants (Monteiro et al., 2009; Jee et al., 2005). From our study, it is evident that the glycogen content, total proteins and total lipids decreased in the blood of IMCs under the magnifying toxicity of chloropyrifos whereas the levels of total free sugars and total free amino acids increased in a dose dependent manner. In the same way, increase in total amino acids and total free sugars were recorded in IMCs exposed to chloropyrifos as compared to control. It has also been documented that analyses of blood biochemical abnormalities in the fish could act as a subtle gauge to measure the early signs of pollutants stress over the analytical techniques (Colla et al., 2014; Conde-Avila et al., 2021). Our result also showed that the glycogen content, total proteins and total lipids decreased in the blood of Catla catla, Labeo rohita and Cirrhinus mrigala under the toxicity of chloropyrifos whereas the levels of total free sugars and total free amino acids increased. As a consequence, the blood sugar level could increase as observed in the present study. The glycogen reserve in the blood could be subjected to glycogenolysis with the resultant depletion (Costa et al., 2019; Daneshmehr et al., 2016). In addition, the chloropyrifos caused reduction in total proteins in the blood of IMCs. Elevated level of blood glucose may possibly cause hyperglycemic state because of the response of the hormone triggered by stress (Sathyamoorthi et al., 2019).

The results of haematological profile showed that acute exposure to chloropyrifos at the concentration of 23 mg/l had significant effect on indices like RBC, Hb, PCV, MCV, MCH and MCHC in a dose dependent manner. Thses results are in agreement with the study of Woryi et al., (2020) and Hedayati et al., (2019). Our results also exhibited a significant decline in concentration of triglyceride, inorganic phosphate and Calcium ion (Ca^{2+}) as well as cholinesterase activity while significantly increased concentration of ammonia (NH_3) during the study.

Chloropyrifos caused an increase in plasma ammonia level perhaps due to an increase in amino acids catabolism and a failure of ammonia excretion mechanisms in blood biochemical profile of Indian major carps after acute exposure (Bujjamma and Padmavathi, 2018). Again, after the exposure of pesticides to the IMCs, serum calcium levels decreased, continuing until the end of the experiment in the pesticide treated groups similar to the findings of other workers (Gharavi-Nakhjavani et al., 2023, Liu et al., 2022). Neurotransmission occurs via acetylcholine mediation which acts as an excitatory transmitter besides aiding as a preganglionic transmitter in the sympathetic nervous system and a postganglionic transmitter in the parasympathetic nervous system (Fulton and Key, 2001). The elevated level of acetylcholine derails the normal functioning of the nervous system. Therefore, the activity of acetylcholinesterase (AChE) is responsible for the hydrolysis of acetylcholine to choline and acetate (Colovic et al., 2013). This alteration deactivates the neurotransmitters which regulate the concentration in the synapse (Yang et al., 2021). Our results also showed a similar trend where chlorpyrifos has been found to inhibit the cholinesterase activity in tested IMCs. Thus from our study, it is concluded that chloropyrifos is highly toxic to fish which is greatly reflected in its behavioural, haematological and biochemical alterations. This connection will help to bridge the disciplines of ecotoxicology and conservation biology in their common goal of guiding the recovery of threatened and endangered species.

Conclusion:

This study clearly demonstrated the adverse impact of chloropyriphos pesticide on behavioural, haematological and biochemical parameters of freshwater IMCs. The IMCs exposed to chloropyriphos showed adverse impact in a dose dependent manner. We concluded that the alterations in parameters may be a result of the target tissue damage and dysfunction induced by the toxicants and that these parameters can be thus used as rapid and sensitive indicators of monitoring toward the impact of toxicants on aquatic organisms and ultimately whole of the ecosystem. The findings of the present research will help the policy makers to make people conscious about the impact of indiscriminate use of pesticides in crop fields on normal physiological development of fish and other aquatic organisms.

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