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Effect of Organophosphate, Monocrotophos on Behavioural, Haematological, Histological and Biochemical Indices in the Subtropical Catfish *Heteropneustes fossilis* (Bloch, 1794)

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A study was conducted to evaluate the impact of acute sub-lethal toxicity of monocrotophos on behavioural, biochemical, haematological and histopathological changes in *Heteropneustes fossilis* (49.53±0.71 g). Probit analysis showed the 96 h LC_{50} of monocrotophos for *H. fossilis* was 20 ppm. Followed by the LC_{50} value, sub-lethal concentrations for acute exposure of monocrotophos were 3 ppm in T_1, 6 ppm in T_2 and 8 ppm in T_3 for 72 h of the experimental period. The behavioural responses observed in treated fish were: erratic movement, imbalance in swimming, surfacing, and hyperactivities. A gradual reduction in total RBC count, haemoglobin, monocyte, and basophil contents were observed with an increased concentration of monocrotophos. On other hand, the total WBC count, neutrophil, basophil and blood ESR showed reversed trend ($p<0.05$). Total tissue protein content of gill, liver and kidney was altered and decreased significantly ($p<0.05$) in monocrotophos-treated fish. Tissue lipid peroxidation (LPO) and catalase activities in gill, liver and kidney were also altered after 24 h, 72 h and followed an increasing trend in exposed fish which differed significantly ($p<0.05$) from the control group. Compared to the control, significant changes were observed in the histopathological architecture of blood cells and gill tissue. The overall result showed that exposure to monocrotophos severely affects fish behaviour and physiology. Therefore the misuse of the chemical may be avoided to reduce the negative impact on aquatic animals.

(***Key words:*** *Catfish, Haematology, Histology, Monocrotophos, Oxidative stress, Toxicity*)

The aquatic ecosystem has been polluted by continuous discharge of wastewater/runoff from agricultural fields. The use of pesticides during agricultural practices has increased significantly during the last few decades for pest and weed control (Ortiz-Ordoñez *et al*., 2011; Kaur and Kaur, 2018; Jacquin *et al*., 2019;). In the Indian subcontinent, many agricultural fields are located adjacent to water bodies and therefore, there is always a high risk of water contamination by the pesticide residues through run off which could adversely impact non-target aquatic biota (Hedayati and Niazie, 2015; Doherty *et al*., 2016). Fishes being conventionally dominant fauna serving as bioindicators of water quality in aquatic ecosystems get exposed to the contaminants through the skin, gills or oral routes which are likely to cause damage to their vital organs and adversely impact metabolism and enhance oxidative stress (Blahova *et al*., 2014; Jacquin *et al*., 2019). The cellular and enzymatic alterations in these animals could be useful markers to evaluate the level of pesticide residue contamination in water bodies (Agrahari *et al*., 2006; Ortiz-Ordoñez *et al*., 2011; Lakshmaiah, 2016; Özaslan *et al*., 2018).

The order Siluriformes (catfish) comprises 4,100 species, which constitute 6.3% of all vertebrate species and 12% of all fish species. *Heteropneustes fossilis* commonly found in freshwaters of the Indian subcontinent is rich in protein and has high nutritional and therapeutic values and is therefore consumed by a large population in India, Pakistan, Srilanka, Bangladesh and Myanmar (Rahman *et al*., 2017). It inhabits mainly the slow and shallow water bodies - ponds, ditches, swamps, marshlands, and sometimes muddy rivers. As

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it provides large concentrations of protein, iron (226 mg 100 g^{-1}) and calcium, the fish is dubbed an essential food fish (Saha and Guha, 1939) and fetch high prices in the market (Alok *et al*., 1993). Now-a-days organophosphate pesticides are used mainly in the agriculture industry for their biodegradability with short environmental residence time. There are several pesticides and insecticides used in agriculture such as sumithion, lorsbon, aluminium chloride, endosulphan, monocrotophos, chlorpyrifos, dichlorvos, profenofos, dimethoate, etc. Among them, monocrotophos is a cost-effective organophosphate and widely used pesticide in crop fields in many countries. Several studies have reported that aquatic animals were affected by monocrotophos (Velmurugan *et al*., 2007). It also affects the internal organs and alters the histoarchitecture of tissues, metabolic activities which ultimately affect the human beings through the food chain and bioaccumulation process. Although the toxicity of this compound on a few freshwater fishes has been reported earlier (Tamizhazhagan & Pugazhendy, 2016), the impact on *H. fossilis* has not been explored yet. Hence, the present study aimed at filling the research gap by studying the effects of this pesticide on the blood cells, vital tissue organs and stress enzyme activities of the fish.

MATERIALS AND METHODS

Collection and acclimatization of experimental animal

The fish used for our study were healthy *H. fossilis* juveniles. The fish in the length and weight range of (13.9±1.18 cm, 49.53±0.71 g) were collected from freshwater ponds located near organically managed paddy fields in Odisha, India with no risk of prior pesticide contamination. All the test animals were given bath treatment with 10 ppm $KMnO_4$ solution for 5 min to minimize the risk of infection and then kept in a plastic tub of 1000 L capacity having dechlorinated water (pH 7.4±0.38, temperature 28.43±0.84°C) and acclimatized in laboratory conditions for a period of 15 days, prior to the experiment. During the acclimatization period, fishes were fed with chicken liver and egg white on a daily basis. The water was changed after 30 min of feeding and the same amount of bore well water was filled to maintain the desired water level and optimum water quality.

Test chemical

Monocrotophos is an alkenyl phosphate that is the dimethyl (E)-1-methyl-2-(methylcarbomoyl) vinyl phosphate. Monocrotophos is classified as an extremely hazardous pesticide in India (ITRC, 1989). Monocrotophos (Monokem, 36% SL) was obtained from Sumitomo Chemical Pvt. Ltd., Chennai, India. Proper measurement was done to prepare the desired monocrotophos concentration for the present experiment.

Experimental design

The 96 h LC_{50} value, calculated by probit analysis as per Finney (1971) was found to be 20 ppm by following EPA guidelines (EPA, 2002). Three monocrotophos concentrations (3 ppm in T_1, 6 ppm in T_2 and 8 ppm in T_3) in triplicate along with control (without chemical) were taken for the experiment. Plastic tubs of 500 L were filled with 400 L seasoned bore well water and desired quantity of pesticide was added to the treatment groups except for the control. In each tank, 23 fish were stocked (49.53±0.71 g, 13.9±1.18 cm) and observed for 72 h of experimental duration. Fish were kept starved throughout the experimental period. Water quality parameters were tested twice throughout the experimental period using the methods of APHA (2005). Values for temperature, dissolved oxygen (DO), pH, free carbon dioxide, total alkalinity were 28±2°C, 7±0.5 ppm, 7.3±0.38, 0.85±0.2 ppm and 143±3 ppm, respectively. Clear and close observation was maintained after 24 and 72 h respectively. The cumulative mortality percentage and behavioural changes in fish were recorded.

Sampling procedure, collection and processing of samples

Three fish were randomly taken for blood collection from both control and treated groups after 24 h and 72 h. Clove oil (Apollo Pharmacy, India, 50 µL L^{-1}) was used to anaesthetize the fish before blood collection (Haque *et al*., 2021). Blood was drawn from the caudal vein using a disposable syringe (23G) rinsed with 2.7% EDTA solution. Blood was collected in EDTA coated vials and mixed properly to prevent clotting. At every 24 and 72 h intervals of the test period, gills, liver and kidney tissues of the fish from different treatments including control were sampled carefully. To prepare 5% tissue homogenate, collected tissues were homogenized using a mechanical teflon coated tissue homogenizer

(MICCRA D9, Italy) in 0.25 M chilled sucrose solution. The entire process was done in ice-cold conditions to avoid enzymatic degradation. Tissue homogenate was centrifuged at 10000 rpm for 15 min at 4°C by using a cooling centrifuge. After centrifugation, supernatant samples were collected and transferred to another vial and stored at -20°C till further analysis.

Histopathological study

Both control and treated fish were dissected to collect gill, liver and kidney tissue after 24 h and 72 h of the experimental period. Tissues were washed in saturated saline solution (0.9% saline) for 5 min to make them free from blood and debris. Small pieces of tissue were taken for processing and fixed in Bouin's fluid for 24 h and 48 h (Reddy and Rao, 2008). After that, all processes were carried out to prepare proper histological slides by following the method of Karmakar *et al*. (2021). Histological changes in the structures of these tissues were documented using a phase-contrast light microscope (Olympus, Japan FSX, BSW). Histopathological examinations were carried out as described by Roberts (2012).

Haematological study

Blood from both control and treated fish was collected by puncturing the lateral line through a disposable syringe and transferred to individual sterilized glass vials containing 100 µl 2.7% EDTA anticoagulant (Haque *et al*., 2021). The blood samples were used to estimate the haematological parameters. Haemoglobin (Hb) was estimated by the acid haematin method taking N/10 HCL in a hemometer. RBC numbers were counted taking blood and Hayem's fluid (1:200) using an improved Neubauer haemocytometer. White blood cells (WBC) were counted using the haemocytometer by taking Turk's fluid diluted blood in 1:20 ratio. Both RBC and WBC were observed under a binocular microscope (Wintrobe, 1967). The Wintrobe tube method described by (Sinton, 1948) was adopted to calculate erythrocyte sedimentation rate (ESR). Differential counts of WBC subtypes (Basophils, eosinophils, neutrophil, lymphocytes, monocytes) and the morphological studies of RBC were made by preparing blood smear using Leishman's stain (Lance and Elsey, 1999).

Tissue protein determination

Protein estimation of each tissue sample was done using Folin-Ciocalteau indicator (Lowry *et al*., 1951) and absorbance was measured in a spectrophotometer at 700 nm taking bovine serum albumin as standard. The tissue protein contents were expressed as mg g^{-1} tissue and the values were used for calculating for tissue biochemical assay.

Biochemical analysis

For the biochemical study, tissues from various organs were obtained quickly, weighed and homogenized with phosphate buffer (0.05 M, pH 7.4) to prevent osmotic damage. The homogenized mixture was then centrifuged in a cooling centrifuge at 4°C with 10,000 rpm for 10 min and the supernatant was collected and stored at -20°C in a deep freezer (Cellfrost) for further analysis. Lipid peroxidation (LPO) level was measured with absorbance spectra of malondialdehyde (MDA) at 532 nm (Ohkawa *et al*., 1979) and expressed in nmol mg^{-1} protein. The catalase activity was assessed in a UV-VIS Spectrophotometer (Systronics) at 340 nm taking phosphate buffer (pH 7.4) and hydrogen peroxide (Cohen *et al*., 1970).

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using the statistical software SPSS 22.0 version to calculate the mean values of different parameters, and a post hoc comparison of mean values was performed by Duncan's multiple range test (DMRT) to observe the significant difference among means at 5% probability level ($p < 0.05$).

RESULTS AND DISCUSSION

Alterations in fish behaviour and 96 h LC50

In our present acute toxicity study, monocrotophos exposed fish showed varying degrees of clinical signs of toxicity. Visual observation showed that in comparison to control, monocrotophos exposed fish showed the following behavioural changes *viz*., fast opercular movement, loss of equilibrium, frequent surfacing, increase secretion of mucus, irregular movement, erratic swimming, etc. (Table 1). The behavioural abnormalities in treated fish were intense with increasing concentrations of monocrotophos. The 96 h LC_{50} of monocrotophos for *Heteropneustes fossilis* was determined as 24 ppm (mg L^{-1}), and the cumulative mortality data for LC_{50} test is presented in Fig. 1.

Table 1. *Behavioural changes in H. fossilis exposed to sub-lethal concentration of monocrotophos for 24 h and 72 h*

Behaviour (24 h & 72 h)	Experimental groups			
	Control	T_1	T_2	T_3
Water surfacing	-	+	++	+++
Lethargic	-	+	+	++
Mucus secretion	-	+	+	++
Irregular movement	-	+	++	++
Hypoxic condition	-	+	++	+++
Loss of equilibrium	-	+	++	+++

None (-), Moderate (+), High (++), Very high (+++)
All data are recorded on the basis of clear observation on a frequent basis

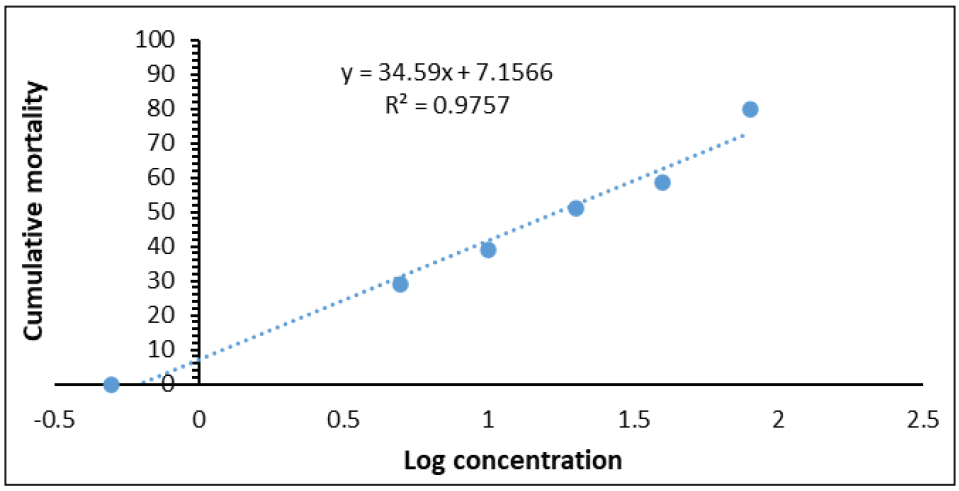

Fig. 1. *Probit line for 96 h LC50 of monocrotophos for Heteropneustes fossilis*

Microscopic observation of blood cells

In the present study, mature erythrocytes in the blood of *H. fossilis* were elliptical with a centrally placed nucleus (Fig. 2. A1, A2). Even at low concentration, erythrocytes were found to be swollen and spherical and changes such as hemolysis, clumping of RBC were observed after 24 h whereas breakage in RBC membrane was noticed after 72 h (Fig. 2. B1, B2). Fish exposed to medium concentration indicated nuclear hypertrophy, irregular nucleus at 24 h and triangular-shaped cells after 72 h (Fig. 2. C1, C2). After an exposure period of 24 h at a high concentration of monocrotophos, erythrocytes exhibited dislocation of the nucleus forming vacuoles. The severe attack of this pesticide at high concentration led to echinocytosis and nuclear shrinkage after 72 h of exposure (Fig. 2. D1, D2).

Histopathological observations

The primary and secondary lamellae were systematically arranged along with epithelial cells in control (Fig. 3. A1, A2). Histopathological changes in gills, such as curling and fusion of secondary lamellae and mild hyperplasia of epithelial cells were observed in fish exposed to low concentrations of monocrotophos (Fig. 3. B1, B2). At medium concentration, gill indicated sloughing of epithelium layer from secondary lamellae after 24 h and necrosis of epithelial cells, severe hyperplasia, and oedema after 72 h of exposure (Fig. 3. C1, C2). Complete losses of gill architecture, rupture of lamellae, severe necrosis and hyperplasia, desquamation and distortion of primary and secondary lamellae were noticed at high concentration of pesticide (Fig. 3. D1, D2).

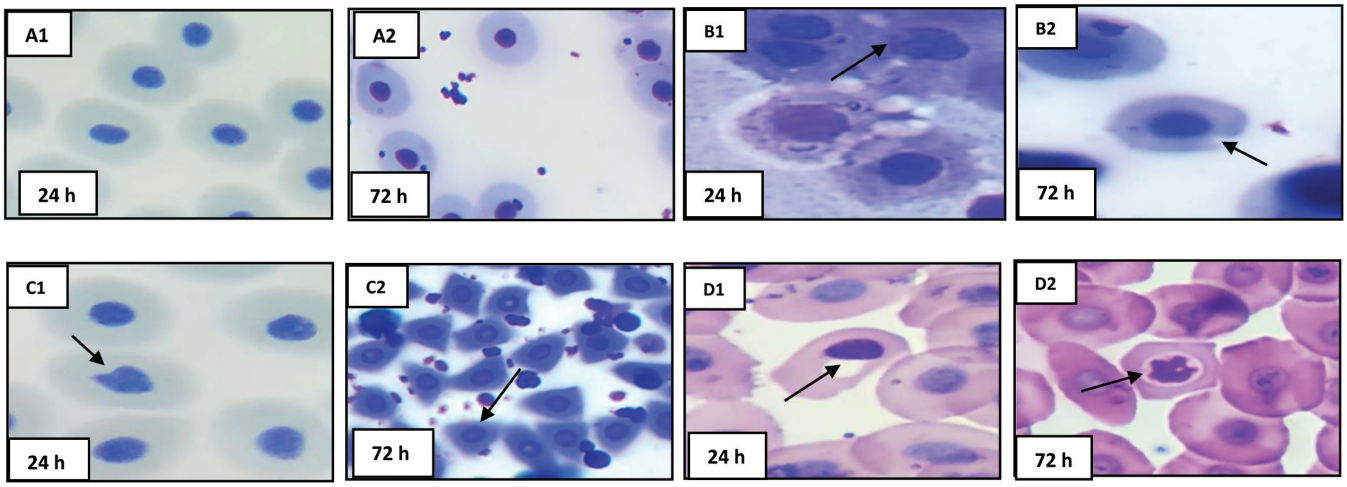

Fig. 2. *Histopathological alteration of erythrocytes blood cells of Heteropneustes fossilis exposed to various concentrations of monocrotophos. A: Erythrocytes of fishes from control tank, [Erythrocytes are elliptical in shape with centrally placed nucleus after 24 h and 72 h (A1, A2)]. B: Erythrocytes of fishes from T_1 tank, [hemolysis and clumping of RBC after 24 h (B1) and breakage in RBC membrane after 72 h (B2)]. C: Erythrocytes of fishes from T_2 tank, [nuclear hypertrophy, irregular nucleus after 24 h (C1) and triangular shaped cells after 72 h (C2)]. D: Erythrocytes of fishes from T_3 tank, [dislocation of nucleus forming vacuoles after 24 h (D1) and echinocytosis, nuclear shrinkage after 72 h (D2)]*

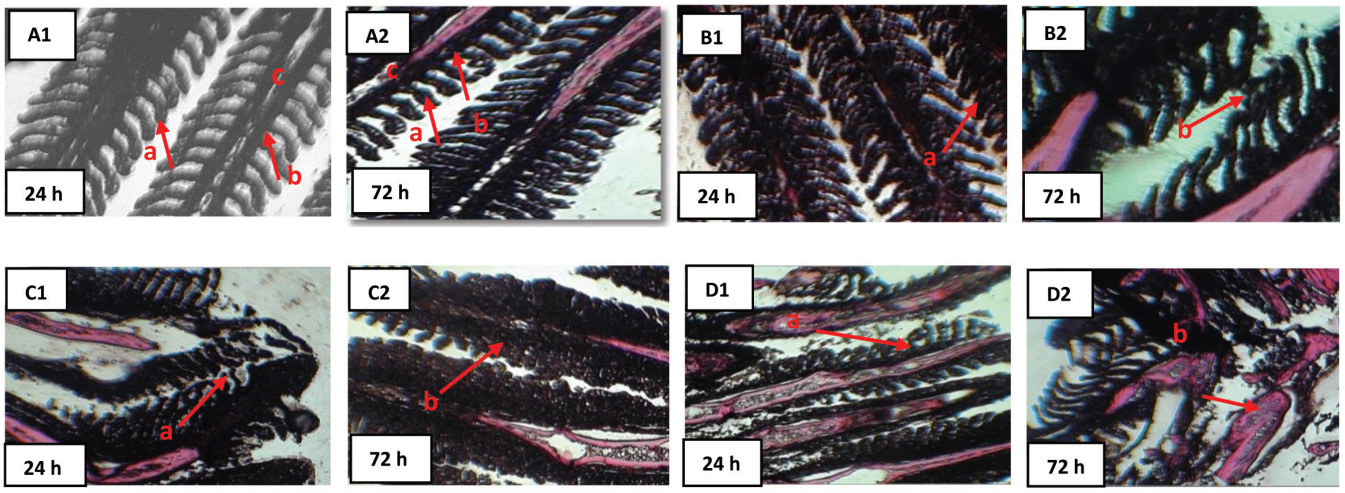

Fig. 3. *Histopathological alteration of gill tissue of Heteropneustes fossilis exposed to various concentrations of monocrotophos. A1 & A2: Gill tissue of fishes from control tank [(a) secondary lamella (SGL) (b) primary lamella (PGL) (c) cartilaginous core after 24h and 72 h]. B1 & B2: Gill tissue from T_1 tank, [(a) swelling of SGL after 24 h (b) Curling of SGL after 72 h]. C1 & C2: Gill tissue from T_2 tank, [(a) Curling of SGL and epithelial lifting after 24 h (b) severe hyperplasia after 72 h]. D1 & D2: Gill tissue from T_3 tank, [(a) disruptor of SGL after 24 h (b) disruptor of SGL and PGL, necrosis after 72 h]*

Biochemical analysis

Variations in protein contents in all tissues of treated animals were found to be significant ($p<0.05$). Higher protein content was observed in the control and it gradually decreased with increasing monocrotophos concentration in T_1, T_2 and T_3 (Table 2). The protein content of liver tissue significantly decreased along with the other treatment groups at 24 h and 72 h exposure to monocrotophos (Table 2). A fall in protein content was also observed in the kidney of *H. fossilis* exposed to sub-lethal concentrations of pesticide whereas it was higher in control at 24 h and 72 h, respectively (Table 2).

On exposure to monocrotophos, LPO activity increased by 29.22%, 62%, and 83.64% in T_1, T_2 and

T_3 respectively in gill tissue (Table 3). A similar trend was noticed in liver tissue, which increased by 19.76%, 36.29%, 56.39% in T_1, T_2 and T_3, respectively after 24 h exposure. Further, the activities were increased to 30, 55%, and 61.25% in T_1, T_2 and T_3 respectively after 72 h of exposure (Table 3). Variations in LPO activities of all tissues were statistically significant ($p<0.05$). LPO contents in kidney increased by 11.2%, 43.7% and 47.2% after 24 h and 38.6%, 51.4%, 53.4% after 72 h in response to low, medium and high concentrations (Table 3).

Compared to control catalase activity increased by 120%, 338.1%, 590.4% after 24 h and 198.41%, 347.28%, 614% after 72 h exposure in T_1, T_2 and T_3 respectively (Table 4). Under control conditions, the catalase activity was found less in liver tissue. But catalase activities were increased by 104%, 200%, and 249% after 24 h and 164.06%, 244.53% and 280.47% after 72 h in T_1, T_2 and T_3 respectively (Table 4). A similar trend was observed in kidney tissue also where catalase activity increased by 47%, 120.1%, 272.16% after 24 h of exposure and by 60.39%, 230.4%, and 373.20% after 72 h in T_1, T_2 and T_3 respectively (Table 4). Significant variations in the activity of all tissues were observed ($p<0.05$).

Haematological parameters

The mean along with the standard deviation of all the haematological parameters of the fish are presented in Table 5. The RBC count significantly decreased by 1.69%. 2.83%, 4.5% after 24 h and 2.2%, 3.5%, 5.4% after 72 h at T_1, T_2 and T_3 respectively ($p<0.05$). The number of WBC indicated a significant reverse trend and the value increased by 2.4%, 16.8%, 28.2% at 24 h and 8.8%, 22%, 31.4% at 72 h exposed to T_1, T_2 and T_3 respectively ($p<0.05$). The Hb value decreased by 2.6%, 7.6%, 12.9% after 24 h of exposure and 2.7%, 9.6%, 14.2% after 72 h of exposure. The variation was found to be significant at 24 h and 72 h ($p<0.05$). Pesticide stimulated the ESR by 11.6%, 37.2%, 55.8% in 24 h and 18.6%, 44.1%, 65.1% in 72 h at low, medium and

Table 2. *Tissue protein content changes in H. fossilis exposed to sub-lethal concentration of monocrotophos for 24 h and 72 h*

Parameters		Experimental groups				*p value*
		Control	T_1	T_2	T_3	
Protein (mg g^{-1} tissue)	Gill 24 h	103.141±0.382^d	94.876±0.344^c	91.460±0.292^b	86.501±0.335^a	<0.001
	Gill 72 h	102.021±0.21^d	91.736±0.477^c	88.485±0.397^b	83.306±0.382^a	0.000
	Liver 24 h	59.614±0.291^d	56.474±0.307^c	52.507±0.240^b	48.209±0.146^a	<0.002
	Liver 72 h	59.003±0.087^d	53.113±0.146^c	49.256±0.191^b	45.179±0.480^a	<0.001
	Kidney 24 h	92.066±0.344^d	87.603±0.252^c	81.157±0.668^b	77.190±0.165^a	0.000
	Kidney 72 h	91.823±02134^d	85.454±0.252^c	76.529±0.286^b	71.791±0.386^a	<0.001

Values are expressed in mean ± SE (n = 3)
Mean values in the same row with different superscripts differ significantly ($p<0.05$)

Table 3. *Tissue lipid peroxidation (LPO) changes in H. fossilis exposed to sub-lethal concentration of monocrotophos for 24 h and 72 h*

Parameters		Experimental groups				*p value*
		Control	T_1	T_2	T_3	
LPO (nmol mg^{-1} protein)	Gill 24 h	1.764±0.023^a	2.058±0.031^b	2.365±0.073^c	2.636±0.019^d	0.001
	Gill 72 h	1.612±0.098^a	2.280±0.020^b	2.862±0.035^c	3.258±0.031^d	0.000
	Liver 24 h	2.609±0.016^a	3.124±0.031^b	3.556±0.016^c	4.080±0.008^d	0.002
	Liver 72 h	2.539±0.097^a	3.395±0.032^b	4.044±0.023^c	4.218±0.012^d	0.001
	Kidney 24 h	4.236±0.019^a	4.711±0.023^b	6.089±0.027^c	6.235±0.012^d	0.000
	Kidney 72 h	4.112±0.099^a	5.871±0.012^b	6.413±0.008^c	6.498±0.016^d	0.002

Values are expressed in mean ± SE (n = 3)
Mean values in the same row with different superscripts differ significantly ($p<0.05$)

Table 4. *Catalase activity (CAT) changes in H. fossilis exposed to sub-lethal concentration of monocrotophos for 24 h and 72 h*

Parameters		Experimental groups				*p value*
		Control	T_1	T_2	T_3	
Catalase (U mg^{-1} protein)	Gill 24 h	0.420±0.012[a]	0.927±0.013[b]	1.840±0.012[c]	2.900±0.023[d]	0.002
	Gill 72 h	0.409±0.033[a]	1.253±0.127[b]	2.420±0.042[c]	3.000±0.020[d]	0.001
	Liver 24 h	0.853±0.024[a]	1.747±0.053[b]	2.560±0.060[c]	2.980±0.042[d]	0.000
	Liver 72 h	0.832±0.011[a]	2.253±0.024[b]	2.940±0.012[c]	3.247±0.018[d]	0.002
	Kidney 24 h	0.647±0.007[a]	0.953±0.018[b]	1.423±0.034[c]	2.407±0.035[d]	0.001
	Kidney 72 h	0.621±0.012[a]	1.107±0.035[b]	2.280±0.040[c]	3.060±0.050[d]	0.000

Values are expressed in mean ± SE (n = 3)
Mean values in the same row with different superscripts differ significantly ($p < 0.05$)

Table 5. *Haematological parameters in H. fossilis exposed to sub-lethal concentration of monocrotophos for 24 h and 72 h*

Parameters		Experimental groups				*p value*
		Control	T_1	T_2	T_3	
RBC (10^6 mm^{-3})	24 h	2.94±0.01[a]	2.91±0.01[b]	2.89±0.01[c]	2.86±0.01[d]	0.002
	72 h	2.98±0.04[a]	2.93±0.01[b]	2.88±0.01[c]	2.84±0.01[d]	0.001
WBC (10^4 mm^{-3})	24 h	7.95± 0.01[a]	8.10±0.10[a]	9.23±0.15[c]	10.13±0.15[d]	0.000
	72 h	7.9± 0.01[a]	8.60±0.10[b]	9.73±0.15[c]	10.47±0.15[d]	0.002
Hb (g 100 mL^{-1})	24 h	8.77±0.06[a]	8.63±0.06[a]	8.53±0.06[b]	8.10±0.10[c]	0.001
	72 h	8.67±0.06[a]	8.63±0.06[b]	8.40±0.10[c]	7.80±0.10[d]	0.000
ESR (mm hr^{-1})	24 h	1.43±0.06[a]	1.60±0.10[b]	1.97±0.06[c]	2.23±0.06[d]	0.002
	72 h	1.33±0.08[a]	1.70±0.10[b]	2.07±0.06[c]	2.37±0.06[d]	0.001
Lymphocyte (%)	24 h	39.00±1.01[a]	39.33±1.15[a]	40.33±1.15[a]	41.02±1.02[a]	0.000
	72 h	39.8±1.1[a]	39.33±1.15[ab]	40.33±1.15[a]	41.66±1.52b[d]	0.001
Monocyte (%)	24 h	6.66±1.15[a]	5.66±2.08[a]	4.66±1.52[a]	4.03±1.04[a]	0.000
	72 h	6.56±1.11[a]	6.02±1.73[a]	4.66±1.52[a]	3.33±2.51[a]	0.003
Neutrophil (%)	24 h	48.01±1.73[a]	48.33±2.08[a]	49.11±3.04[a]	49.66±2.51[a]	0.001
	72 h	46.21±1.71[a]	48.66±2.51[a]	49.02±3.09[a]	50.33±3.05[a]	0.000
Eosinophil (%)	24 h	5.12±1.03[a]	5.33±0.67[a]	5.13±1.04[a]	5.66±0.88[a]	0.002
	72 h	5.51±1.21[a]	4.66±1.52[a]	5.34±1.23[a]	4.86±2.08[a]	0.001
Basophill (%)	24 h	1.33±0.57[a]	1.33±0.57[a]	1.22 ± 1.01[a]	1.21±0.57[a]	0.000
	72 h	1.31±0.51[a]	1.33±0.57[a]	1.22 ± 1.01[a]	0.66±0.57[a]	0.000

high concentrations, respectively, and the variation was found to be significant ($p<0.05$). The maximum increase in lymphocytes and neutrophils was reported by 5.1%, 3.4% after 24 h and 6.8 %, 4.8% after 72 h on exposure to high concentration. However, other subtypes of leucocytes such as basophil, eosinophil and monocytes did not show significant alterations in response to pesticide stress (Table 5).

Behavioural response due to toxicity

Any toxicant causes stress on the organisms; the behavioural changes are the immediate responses to the toxicant and are indicators of possible stress (Robinson, 2009). In the present study, fishes from the control tank have shown natural behaviour *i.e.*, they were very active in movement through the experimental tank. In

the case of monocrotophos-treated fishes, they exhibited irregular, erratic and darting swimming activities due to loss of equilibrium and excess mucus secretion. Similar observations were made by (Reddy and Rao, 2008) in fish with organophosphorus pesticides, profenofos at 3.55 mg cm^{-2}. After monocrotophos exposure, we observed an increase in surfacing and gulping of surface water by fish to avoid breathing in the experiment tank (poisoned water). Moreover, hypoxic condition arises primarily due to damage to the gills of pesticides exposed fish which altered oxygen uptake and increased surfacing (Velmurugan *et al*., 2007).

Changes in structure of blood cells

In the present study, mature erythrocytes in the blood of *H. fossilis* were elliptical with a centrally placed nucleus (Fig. 1). This might be due to the chemical toxicants present in monocrotophos which affected blood cells causing haemorrhage in fish. In agreement with our study, El-Naggar *et al*. (2009) also reported similar effects in *Oreochromis niloticus* exposed to heavy metals, necrosis and haemorrhage in *Labeo rohita* exposed to zinc (Loganathan *et al*., 2006) and *H. fossilis* exposed to thiodan.

Histopathological alterations

The results from our present study are in agreement with the histopathological work carried out by (Katuli *et al*., 2014) in *Rutilus rutilus*, (Rosety-Rodríguez *et al*., 2002) in *Scophthalmus maximus*, (Maharajan and Parurukmani, 2012) in *Catla catla* and (Ghasemzadeh *et al*., 2015) in *Scatophagus argus*. In their study, it was reported that epithelial lifting, necrotic lamellae in gill tissues, edema, lamellar fusion and deformities in secondary gill lamellae on exposure to pesticides. Other studies have also reported histopathological changes in gills on exposure to chlorpyrifos in *Oreochromis niloticus* (Zahran *et al*., 2018) and in *Channa punctatus* (Devi and Mishra, 2013). The results from our present study confirm the above findings *i.e*., mucous accumulation inside secondary lamellae and decreased inter-lamellar cell mass which has also been reported by (Karmakar *et al*., 2016) in *Labeo rohita* on exposure to malathion.

Biochemical observations

Several workers have also observed a declining trend in tissue protein under toxic stress of various chemicals (Tilak and Yakobu, 2002; Nagaraju and Rathnamma, 2013; Rohankar *et al*., 2012). Jaffer *et al*. (2017) observed the reduced protein contents in *Cyprinus carpio* as the marker of chlorpyrifos water contamination. In the present study, the results clearly indicated that there was a decreased amount of protein and glycogen content in tissues to prevent the effect of pesticides. The decline in total tissue protein might be due to reduced or inhibition of protein synthesis due to tissue necrosis (Jyothirmayee *et al*., 2006).

Organophosphate pesticides, including monocrotophos induce oxidative tissue damage resulting from the release of reactive oxygen species (ROS), (Hincal *et al*., 1995). As high reactivity of ROS, most components of the cellular structure and function may become potential targets of oxidative damage. ROS may induce DNA damage (Kappus & Mahmutoglu, 1987), protein damage (Bainy *et al*., 1996) and methemoglobin formation (Andersson *et al*., 1987). ROS-induced damage involves the peroxidation of unsaturated fatty acids (Kappus and Mahmutoglu, 1987), in comparison to other reports, our experiment has also shown a significant ($p<0.05$) increase in LPO values in gill, kidney, and liver of monocrotophos exposed groups after 24 and 72 h. The protein damage and increased LPO levels in pesticide-treated *C. carpio* can result from antioxidant enzyme impairments caused by ROS attack (Clasen *et al*., 2018).

ROS formed during biotransformation of xenobiotics damage cell structures via oxidation. To reduce the ROS effects, the cell produces enzymes, such as catalase involved in the antioxidant process (van der Oost *et al*., 2003; Jana *et al*., 2021). Catalase is an important enzyme which plays a role to neutralize H_2O_2 by decomposing it into H_2O and molecular O_2. Pesticide induced inhibition of catalase activity has also been reported in *Channa punctatus* by (Sarma *et al*., 2012) on exposure to endosulfan (Sayeed *et al*., 2003), delta methrin (Tripathi and Singh, 2013) and alphamethrin in *Danio rerio* (Ansari and Ansari, 2014). However, the results of the present study showed a significant increment of catalase activity in gills, liver and kidney tissues of monocrotophos exposed fish in comparison to control group fish. In the present study, *H. fossilis* activated catalase enzyme as the cellular defence mechanism against monocrotophos induced lipid peroxidation and oxidative stress and corroborate the result of previous works. In agreement with our result, some studies were

reported, such as in *Leporinus obtusidens* (Moraes *et al*., 2007) and *Rutilus rutilus* (Keramati *et al*., 2010). The increase in catalase activity observed in the present study indicated the excess production of H_2O_2 due to the metabolism of monocrotophos as a defence of the organism against oxidative damage as catalase acts on H_2O_2. Stimulation of catalase activity was observed in the liver tissue of *Goodea atripinnis* treated with Yerbimat (Ortiz-Ordoñez *et al*., 2011) and in *Carassius auratus* exposed to herbicide aminotriazole (Vasylkiv *et al*., 2011). Various authors reported that oxidative damage in fish induced by pesticides leads to enhancement of LPO and catalase and reduction in protein in different tissues (Pereira *et al*., 2013; Doherty *et al*., 2016; Ullah *et al*., 2018).

Haematological analysis

The change in haematological parameters as indicators of stress provides important information concerning the pathologic state of fish (van der Oost *et al*., 2003, Kumar *et al*., 2008, Kumar *et al*., 2014; Karmakar *et al*., 2021). Our present study has shown that there was a significant ($p<0.05$) decreasing trend in RBC and Hb content in monocrotophos-treated fish blood which clearly indicated the anaemia as a result of inhibition of erythropoiesis, haemosynthesis and increase in the rate of RBC destruction in haemopoietic organs. These values signify that high doses of pesticides produced anaerobic conditions and limit the oxygen carrying capacity and thereby decreasing mobility. Similar reduction in RBC was also mentioned for cypermethrin-treated *L. rohita* (Das and Mukherjee, 2003), common carp (*C. carpio*) treated with diazinone (Svoboda *et al*., 2001) and African catfish (*C. gariepinus*) treated with diazinone (Adedeji *et al*., 2009). It was reported to have a similar reduction in RBC of fishes (Deoi *et al*., 2004; Jaffer *et al*., 2017; Maiti *et al*., 2021) after toxicity exposure. In support to our findings, the Hb contents and RBC of *Prochilodus lineatus* showed significantly lower values at different concentrations of herbicide clomazone. Agrochemical pollutants can cause ROS-induced anaemia in fish via the oxidation of haemoglobin or other cellular components (Pereira *et al*., 2013). Hedayati and Niazie (2015) and Blahova *et al*. (2014) reported the changes in the haematological parameter in carps after acute exposure to pesticides. Alterations in leukocytes can be connected with the response of fish organisms to stress reactions (Evans *et al*., 2008; Vani *et al*., 2011; Blahova *et al*., 2014). Agrahari *et al*. (2006) reported an increase in ESR and changes in differential counting of leucocytes in *Chana punctatus* exposed to monocrotophos at different duration periods. The presence of anisocytosis and poikilocytosis in *Channa punctatus* indicated toxic effects agrochemical on the red cell morphology due to pesticide impact (Mishra *et al*., 2017). Morphological deformations of RBC such as irregular margins, echinocytosis, lobopodial projections in response to toxicants were reported by various authors (Massar *et al*., 2012; Kaur and Kaur, 2015; Sadiqul *et al*., 2016; Mohapatra *et al*., 2021) and are in agreement with the findings of our present study.

Contamination of freshwater habitats by monocrotophos could seriously impair the physiological alterations in *H. fossilis*. The results of the present study clearly indicated the deleterious impact of monocrotophos on blood components, tissue and biochemical parameters of gill, kidney and liver in fish which could be used as biomarkers for diagnosis of aquatic contamination due to the specific pesticides. Accumulation of this pesticide in the tissue of this edible fish could pose a serious environmental risk to the human population, which will be our future area of research. Therefore preventive measures are to be adopted to minimize the contamination of water bodies by this potentially harmful pesticide.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICAL STATEMENT

This experiment was conducted with the approval of the Research Ethics Committee of Odisha University of Agriculture & Technology, Odisha, India. The experimental animals were reared as per recommended guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division, Ministry of Environment and Forests, Govt. of India for the use of animals in scientific research experiments.

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