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Effect of organophosphate profenofos exposure on the hematological parameters of common carp (*Labeo rohita*)

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ABSTRACT

The organophosphate insecticide profenofos (PFF) is one of the major contaminants in freshwater ecosystems and causes adverse health effects in aquatic organisms, especially fish. Blood parameters are essential biomarkers to evaluate the physiological status of stressed fish. The current study was designed to evaluate the sublethal impacts of PFF on hematological indices in the common carp, Labeo rohita, which included red blood cell (RBC) and white blood cell (WBC) count, packed cell volume (PCV), hemoglobin (Hb) content, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Fish were exposed to PFF at 0.2, 0.6, 0.8, and 1.0 µg/L for 24, 48, 72, and 96 h, respectively. Exposure caused significant reductions in Hb, RBC, Hct, and MCHC, while WBC, MCV, and MCH values were increased compared with the control group. These results indicate that both lethal and sublethal concentrations of PFF disturb the normal hematological parameters, thus showing a toxic impact, affecting the metabolic and physiological performance of *L. rohita*. Such changes in fish health may cause disturbances in the ecological balance by cascading the altered species interactions and food web dynamics to higher trophic levels.

Introduction

Organophosphorus (OP) insecticides, widely used in agriculture and households, are of significant environmental concern because of their persistence and toxicity in ecosystems. These insecticides, including parathion and malathion, were introduced in the 1970s and are chemically stable due to their hydrophobicity and lipophilic affinity (Prabhavathy Das *et al*., 2006; Ahmad *et al*., 2024). Although hydrolysis primarily degrades these compounds, pH, light, and microbial populations influence their persistence in soil and water (Ali *et al*., 2009). OPs are more stable under an acidic environment (pH 3-6) than under neutral or basic conditions; thus, they are resistant contaminants in some waters (Ali *et al*., 2009). Profenofos (PFF), a unique OP with an S-alkyl substituent, is applied extensively to control insect pests in major crops, including cotton and maize (Reddy *et al*., 2008; Kavitha *et al*., 2009). Although OPs inhibit acetylcholinesterase (AChE), leading to the disruption of the nervous system, the specific effects of PFF on non-target organisms, especially in freshwater ecosystems, are poorly understood (Ahmad *et al*., 2024; Shaw *et al*., 1995). This knowledge gap is particularly important given the widespread application and environmental persistence of PFF (Tejada *et al*., 2001; Farrag *et al*., 2007).

The environmental persistence and toxicity of PFF, especially in freshwater ecosystems, pose ecological threats through runoff, spray drift, and leaching, which affect aquatic organisms such as fish (Cosgrove *et al*., 2019). On being ingested or absorbed, these pesticides tend to accumulate in fish tissues, leading to health impacts and even mortality (Tahir *et al*., 2021). OP pesticides, mainly PFF, cause significant disruptions in the biological processes of organisms by inhibiting AChE, causing muscle spasms, neurological stress, and even death in the exposed organisms (Ahmad *et al*., 2024; Aiwan *et al*., 2009). Having been noted as very good indicators of water quality, fish are sensitive to toxicants, and hematological assessments could help in revealing stress responses to pollutants like PFF (Kubra *et al*., 2022). However, a limited number of studies on PFF's specific hematological effects in fish hinders the usual extensive risk assessment and ecological impact evaluation; this opens another frontier that necessitates explicit research into the sublethal toxicity of this pesticide within aquatic systems.

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Labeo rohita (Rohu) is an economically and ecologically important freshwater fish native to South Asia and is widely used in aquaculture because of high market demand and a fast growth rate (Ligina *et al*., 2022). A key contributor to local diets and economies, *L. rohita* is often cultured in riverine and pond systems, exposing it to environmental pollutants, such as pesticides. With its specific physiological features and habitat preferences, this species is used as a sensitive bioindicator for assessing aquatic pollution. Because of its high commercial value, studying the effects of PFF on *L. rohita* is important in understanding the health of aquaculture and ecosystems. Hematological alterations in L. rohita, changes in the counts of red blood cells (RBC) and white blood cells (WBC), may indicate immune responses to pollutants, reflecting broader ecological effects of contamination (Cai *et al*., 2019). Despite extensive research on organophosphorus pesticides, studies on the sublethal hematological effects of PFF in *L. rohita* remain limited. Existing literature primarily focuses on acetylcholinesterase inhibition and acute toxicity, with insufficient data on hematological responses, immune alterations, and long-term ecological impacts in freshwater ecosystems.

Considering the aforementioned, we hypothesize in this study that PFF exposure induces hematological changes in *L. rohita*, affecting key blood parameters. Therefore, the objective of the present study is to evaluate PFF's sublethal toxic effects on the hematology of *L. rohita.*

Materials and methods

Experimental design

L. rohita specimens were collected from the fish pond at IFTM University. All experiments were performed at the laboratory situated at the Department of Zoology, School of Sciences, IFTM University, Moradabad, India. Healthy, adult fish averaging 14 cm in length and 20 g in body weight (BW) were selected and acclimated in laboratory aquaria (28°C ± 1°C) for 21 days. The fish were fed an organic, oil -free cake made from groundnut, provided for 3 hours each day, and fresh groundwater was supplied to the aquaria daily. Commercial-grade PFF (Curacron; Syngenta India Ltd.) was used for waterborne exposure without additional solvents or vehicles. A total of 50 *L. rohita* were divided into four experimental exposure groups and one control group, with 10 fish per aquarium. The experimental groups were exposed to PFF concentrations of 0.2 µg/L, 0.6 µg/L, 0.8 µg/L, and 1.0 µg/L for durations of 24, 48, 72, and 96 hours, respectively. The control group was kept under the same conditions without PFF exposure. Water in the aquaria was fully exchanged daily to maintain consistent PFF exposure levels. Fish mortality was monitored at logarithmic time intervals from 24 to 96 hours. Fish were deemed dead when opercular respiratory movements ceased and there was no response to tactile stimuli. All experiments were carried out in triplicate. Fish were maintained in laboratory aquaria with daily replenishment of fresh groundwater to ensure stable water quality conditions. Water samples were collected at regular intervals to monitor key physicochemical parameters. Sampling was conducted at the start of the experiment and every 24 hours for the duration of the exposure period (96 hours). While seasonal variations in groundwater quality were not specifically analyzed in this study, water parameters such as temperature, pH, and dissolved oxygen were consistently monitored to minimize potential environmental fluctuations.

Determination of LC50

Standard safety protocols were strictly followed throughout the experiment, as per established guidelines (El-Bouhy *et al*., 2023). The median lethal concentration (LC50), or the concentration at which 50% mortality occurred, was determined using semi-logarithmic graphing (Rathnamma and Nagaraju, 2013). Mortality percentages across the various PFF concentrations are displayed in Figure 1, providing visual confirmation of the LC50 value.

Water parameters

During the experiment, water quality parameters—temperature, pH, and dissolved oxygen (DO)—were monitored (Cooke and Schreer, 2001). Temperature was measured using a thermometer on a 1-liter water sample, and readings were recorded once stable. pH was assessed using the electrometric method. Results showed a slight increase in pH as PFF concentrations rose. Dissolved oxygen, a key indicator of the physical, chemical, and biological activity in water, was measured using Winkler's method and expressed in mg/L. DO levels are primarily influenced by oxygen diffusion from the air and photosynthetic activity, with factors such as water movement and temperature also affecting oxygen solubility. Interestingly, DO levels showed a slight increase with higher PFF concentrations, although differences between treatment groups were not statistically significant ($P > 0.05$). Higher concentrations of pollutants can induce stress in fish, potentially leading to a decrease in their metabolic rate and oxygen consumption. This could result in higher residual DO levels in the water.

Blood sampling

Blood from the fish was collected by puncturing the caudal peduncle with the help of a 24-gauge needle and stored in 0.1% EDTA-treated vials. Total red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), hematocrit (Ht)/PCV, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined by adopting the method of Jenkins *et al*. (2003). All experiments were conducted with the permission of the ethical committee of the IFTM University, Moradabad, In-

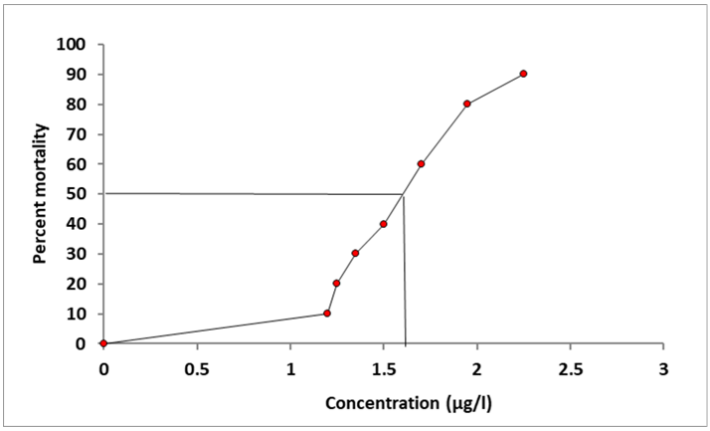

Figure 1: Effects of different concentrations of profenofos on percent mortality of *L. rohita*

dia (approval number: 52525/S181703).

Estimation of hemoglobin

Hemoglobin estimation was performed using Sahli's hemoglobinometer by adding 0.02 mL of blood to a tube containing 0.1N HCl, mixing thoroughly, and diluting with distilled water until the color matched the standard glass. The final hemoglobin concentration was recorded in grams and percentages (Shahzadi *et al*., 2024).

Estimation of Red Blood Cells (RBCs)

Fish blood was diluted 200× using an RBC diluting fluid and mixed by rolling the pipette between the palms. After preparing the sample, it was loaded into a Neubauer chamber, and the RBCs were counted under a microscope (Ajiboye et al., 2016). The total number of RBCs was counted by using the formula:

Estimation of white blood cells (WBCs)

Blood was drawn to scale 0.5 in a WBC diluting pipette, mixed with WBC diluting fluid up to scale 2.0, and thoroughly mixed by rolling the pipette. The prepared sample was loaded into a Neubauer chamber, and after 3 minutes, the total WBC count per mm^3 was calculated using the observed average WBC count in the 1×1 mm ruled area (Ullah et al., 2024):

Estimation of hematocrit

2 ml of fish blood was drawn from the vein and transferred into a clean, 0.1% EDTA-treated, dry test tube and shaken gently. The blood was drawn from the test tubes and transferred into a Wintrobe tube until the measurement of 100 mm. The tube was centrifuged for 30 minutes at 3000 r.p.m. The RBC was taken, and the value was recorded in mm (Thiour-Mauprivez, 2019).

Estimation of mean corpuscular volume (MCV)

Mean Corpuscular Volume (MCV) was derived from the hematocrit value of the blood sample and the RBC count using the method of Nour-Eldrin (1973). The mean volume of RBCs was calculated from the RBC and HCT using the formula of Stoskopf (Tripathi *et al*., 2002):

Estimation of mean corpuscular hemoglobin (MCH)

The MCH value expresses the average hemoglobin content of a single red cell pictogram (pg). The mean corpuscular hemoglobin concentration (MCHC) is calculated from two accurate and reproducible observations, i.e., packed cell volume and the amount of hemoglobin in 100 ml of blood. These parameters were calculated using the formula of Tripathi *et al*. (2002).

Estimation of mean corpuscular hemoglobin concentration (MCHC)

MCHC reflects the standard concentration of hemoglobin in the red blood cells in a given volume of blood. MCHC was obtained by the following formula and expressed in terms of gram percent (g%). MCHC was calculated using the following formula, according to Ramesh *et al*. (2008).

Statistical Analysis

Statistical analyses were performed using SPSS version 16.0 (IBM, Armonk, NY, USA). Data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. One-way analysis of variance (ANOVA) was conducted to assess significant differences among treatment groups, with results presented as mean ± standard error of the mean (SEM). Post-hoc comparisons were performed using Dunnett's test to determine significant differences from the control group. Statistical significance was set at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

Results and discussion

Effects on water quality parameters

In this study, *L. rohita* exposed to selected concentrations of 0.2, 0.6, 0.8, and 1.0 µg/l of PFF showed significant changes in water parameters and hematological profiles, accompanied by higher mortality rates within 24 to 96 hours (Figure 1). As the concentration of profenofos increases from 0.2 µg/L to 1.0 µg/L, the percent mortality also increases, indicating heightened toxicity with higher doses. The

trend shows a sharp rise in mortality beyond 0.6 µg/L. Herein, a significant increase in temperature, pH, and DO levels was observed with rising PFF concentrations (Table 1). Temperature shows a consistent increase with both time and higher PFF concentrations (Figure 2). The values are significantly different from the control at each concentration level (P1 to P4) after 24 hours. This trend persists throughout the experiment, with the highest temperature recorded at 96 hours for P4 (29.66 ± 0.01°C). The temperature rise suggests that PFF exposure may disrupt thermoregulation or metabolic activity in *L. rohita.* The pattern is clear, with higher concentrations of PFF correlating with increased temperatures, which may further stress the aquatic environment (Figure 3). The pH values also showed variation over time and concentration levels. While the pH fluctuates in the control group, a general upward trend is observed with increasing PFF concentrations. After 96 hours, pH values rise significantly for P4 (7.2 ± 0.07), indicating a shift towards alkalinity. This shift in pH could be attributed to metabolic changes in the fish or alterations in water chemistry due to PFF's chemical properties. However, the large fluctuations, particularly at P2, demand further investigation into potential mechanisms behind these variations. Also, DO levels increase over time across all PFF concentrations, with significant differences observed from the control, particularly after 72 hours (Figure 4). For instance, at 96 hours, DO levels in P4 reach 7.3 ± 0.04 mg/L, showing a marked increase. This rise may reflect increased respiratory stress or a compensatory mechanism in response to the toxic effects of PFF. The observed increase in water temperature and pH is consistent with reports by Hossain *et al.* (2005), who found that a temperature range between

Table 1: Hydrobiological parameters in *Labeo rohita* on exposure to PFF (P1 = 0.2 µg/L, P2 = 0.6 µg/L, P3 = 0.8 µg/L, P4 = 1.0 µg/L)

Parameters	Exposure duration (hrs)	Control	P1	P2	P3	P4
Temperature	24	25.3 ± 0.07	25.6 ± 0.07*	25.7 ± 0.07*	25.72 ± 0.08*	26.06 ± 0.09*
	48	26.26 ± 0.06	26.6 ± 0.02*	26.98 ± 0.08*	27.3 ± 0.07*	27.42 ± 0.08*
	72	27.4 ± 0.07	27.7 ± 0.03*	28.1 ± 0.07*	28.22 ± 0.05*	28.58 ± 0.10*
	96	28.7 ± 0.07	29 ± 0.01*	29.26 ± 0.05*	29.32 ± 0.05*	29.66 ± 0.01*
pH	24	6.26 ± 0.05	6.4 ± 0.07	6.36 ± 0.09	6.54 ± 0.05*	6.64 ± 0.05*
	48	6.38 ± 0.02	6.46 ± 0.05	5.56 ± 0.05	6.66 ± 0.05*	6.76 ± 0.05*
	72	6.52 ± 0.03	6.58 ± 0.05	6.67 ± 0.05	6.68 ± 0.07*	6.72 ± 0.08*
	96	6.69 ± 0.05	6.8 ± 0.07	6.9 ± 0.07	7.0 ± 0.07*	7.2 ± 0.07*
Dissolved Oxygen	24	5.36 ± 0.05	5.44 ± 0.05	5.54 ± 0.02	5.66 ± 0.05*	5.74 ± 0.05*
	48	5.56 ± 0.04	5.68 ± 0.03	5.76 ± 0.05*	5.84 ± 0.04*	5.88 ± 0.03*
	72	5.89 ± 0.05	5.96 ± 0.03	5.96 ± 0.03	6.26 ± 0.05*	6.44 ± 0.05*
	96	6.5 ± 0.05	6.72 ± 0.06	6.94 ± 0.05*	7.06 ± 0.02*	7.3 ± 0.04*

***Significantly different from the control treatment**

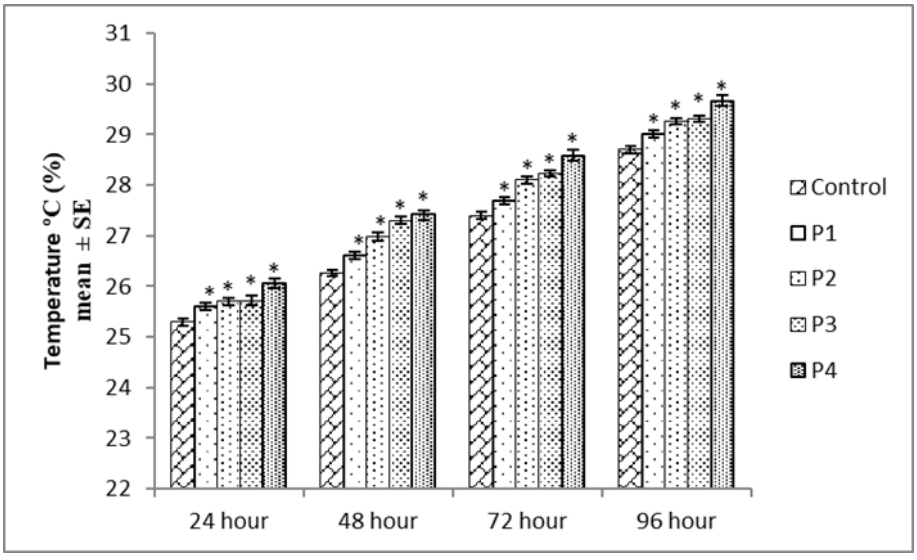

Figure 2: Effect of profenofos exposure on water temperature in the habitat of *L. rohita* at different concentrations (P1=0.2 µg/L, P2=0.6 µg/L, P3=0.8 µg/L, P4=1.0 µg/L) over 24, 48, 72, and 96 hours. *Indicates a statistically significant difference compared to the control group

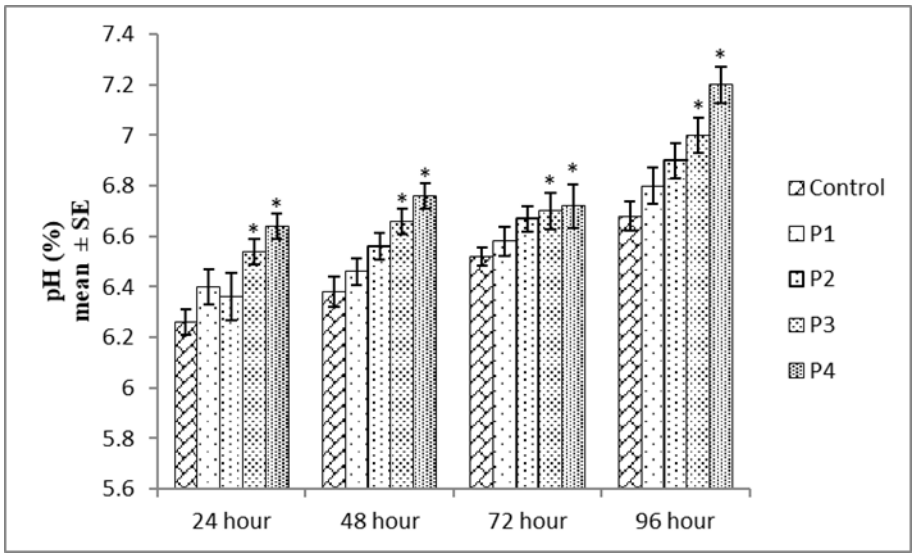

Figure 3: Effect of profenofos exposure on water pH in the habitat of *L. rohita* at different concentrations (P1=0.2 µg/L, P2=0.6 µg/L, P3=0.8 µg/L, P4=1.0 µg/L) over 24, 48, 72, and 96 hours. *Indicates a statistically significant difference compared to the control group

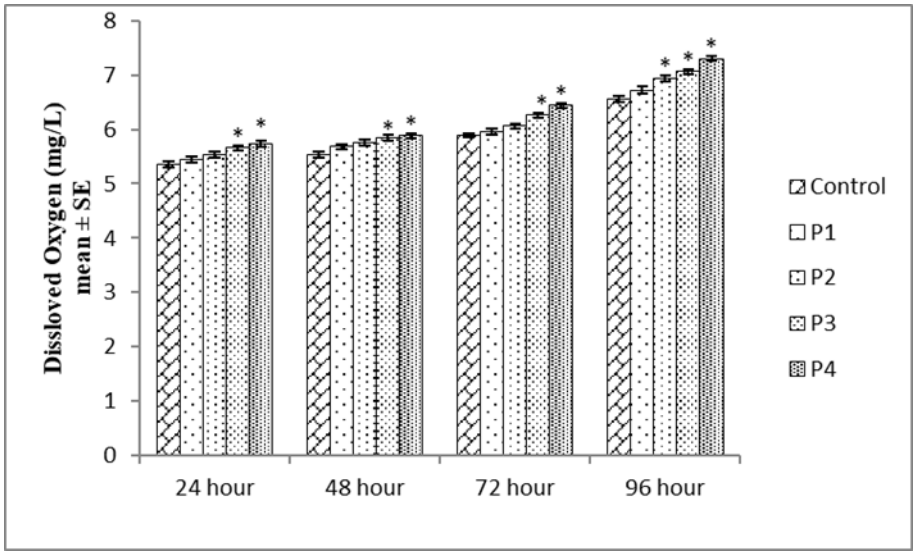

Figure 4: Effect of profenofos exposure on water DO in the habitat of *L. rohita* at different concentrations (P1=0.2 µg/L, P2=0.6 µg/L, P3=0.8 µg/L, P4=1.0 µg/L) over 24, 48, 72, and 96 hours. *Indicates a statistically significant difference compared to the control group

25ºC and 33ºC is optimal for fish. However, it is not fully certain whether the temperature rise was solely due to the effect of PFF, as temperature fluctuations were also observed in the control group. Seasonal variations may have contributed to this phenomenon. Similarly, the rise in pH across all groups suggests that factors other than PFF exposure may have influenced the pH levels, as supported by control group data. DO levels generally increased with higher PFF concentrations, yet no significant differences were noted between groups, indicating that this change may not be directly related to PFF exposure.

Effects on hemoglobin, RBC, and WBC count

The data given in Table 2 shows the effect of profenofos exposure on various hematological parameters of *L. rohita.* In this, hemoglobin levels show a progressive decline across all treatment groups, with a clear concentration-dependent reduction (Figure 5). By 96 hours, the highest exposure group (P4) exhibited the most decrease in hemoglobin levels (6.72 ± 0.02 g/dL) compared to the control (9.53 ± 0.13 g/dL), suggesting that profenofos adversely impacts oxygen transport capacity. Similarly, RBC count followed a declining trend with increasing profenofos concentrations and exposure times (Figure 6). The RBC count at 96 hours in the P4 group dropped to 1.05 ± 0.01 × 10^6/mm^3, compared to the control value of 3.30 ± 0.20 × 10^6/mm^3. This decline in RBC count could indicate hemolysis or impaired erythropoiesis induced by profenofos

Table 2: Hematological parameters of *Labeo rohita* on exposure to PFF (P1 = 0.2 µg/L, P2 = 0.6 µg/L, P3 = 0.8 µg/L, P4 = 1.0 µg/L)

Parameters	Exposure duration (hrs)	Control	P1	P2	P3	P4
Haemoglobin	24	9.56 ± 0.13	9.46 ± 0.13	8.90 ± 0.10*	8.36 ± 0.05*	7.76 ± 0.09*
	48	9.55 ± 0.13	9.33 ± 0.02	8.65 ± 0.02*	8.21 ± 0.02*	7.57 ± 0.02*
	72	9.54 ± 0.13	9.06 ± 0.03*	8.32 ± 0.04*	7.90 ± 0.04*	7.25 ± 0.03*
	96	9.53 ± 0.13	8.82 ± 0.02*	7.98 ± 0.02*	7.48 ± 0.02*	6.72 ± 0.02*
RBC Count ($\times 10^6$/mm3)	24	3.3 3 ± 0.20	3.20 ± 0.17*	2.76 ± 0.10*	2.70 ± 0.00*	2.44 ± 0.01*
	48	3.3 2 ± 0.20	3.18 ± 0.24*	2.64 ± 0.17*	2.58 ± 0.02*	2.41 ± 0.01*
	72	3.3 1 ± 0.20	3.00 ± 0.03*	2.40 ± 0.03*	2.28 ± 0.02*	2.07 ± 0.02*
	96	3.3 0 ± 0.20	2.81 ± 0.01*	2.07 ± 0.02*	1.68 ± 0.02*	1.05 ± 0.01*
Haematocrit gm (%)	24	27.24 ± 0.05	26.38 ± 0.05*	25.22 ± 0.08*	24.40 ± 0.13*	23.12 ± 0.14*
	48	27.23 ± 0.05	26.16 ± 0.02	25.08 ± 0.02*	24.10 ± 0.03*	23.12 ± 0.14*
	72	27.22 ± 0.05	25.79 ± 0.04*	24.73 ± 0.04*	23.89 ± 0.05*	23.10 ± 0.03*
	96	27.21 ± 0.05	24.68 ± 0.02*	24.18 ± 0.02*	23.34 ± 0.03*	22.10 ± 0.03*
WBC Count ($\times 10^3$/mm3)	24	4.65 ± 0.01	4.76 ± 0.01*	4.85 ± 0.01*	5.15 ± 0.01*	5.24 ± 0.01*
	48	4.66 ± 0.01	4.75 ± 0.02	4.89 ± 0.02*	5.25 ± 0.02*	5.28 ± 0.04*
	72	4.67 ± 0.01	5.08 ± 0.02*	5.26 ± 0.03*	5.93 ± 0.01*	6.13 ± 0.02*
	96	4.68 ± 0.01	5.52 ± 0.01*	5.88 ± 0.02*	6.38 ± 0.21*	7.19 ± 0.02*
MCV (%)	24	83.15 ± 0.01	88.29 ± 0.03*	91.40 ± 0.37*	97.23 ± 0.02*	100.55 ± 0.04*
	48	83.17 ± 0.01	89.35 ± 1.21	92.45 ± 0.01*	98.17 ± 0.01*	100.77 ± 0.02*
	72	83.20 ± 0.01	90.04 ± 0.04*	93.22 ± 0.04*	99.54 ± 0.03*	100.84 ± 0.03*
	96	83.21 ± 0.01	93.25 ± 0.03*	96.64 ± 0.03*	99.85 ± 0.03*	110.17 ± 0.02*
MCH (%)	24	25.29 ± 0.03	27.14 ± 0.01*	30.54 ± 0.03*	32.73 ± 0.02*	35.41 ± 0.03*
	48	25.30 ± 0.03	27.45 ± 0.01*	32.62 ± 0.00*	34.42 ± 0.00*	37.45 ± 0.01*
	72	25.32 ± 0.03	27.80 ± 0.03*	33.17 ± 0.02*	35.58 ± 0.02*	38.32 ± 0.02*
	96	25.35 ± 0.03	27.91 ± 0.03*	34.48 ± 0.02*	36.08 ± 0.02*	40.07 ± 0.02*
MCHC (%)	24	33.51 ± 0.02	32.87 ± 0.22	30.32 ± 0.03*	27.42 ± 0.02*	26.68 ± 0.03*
	48	33.50 ± 0.01	31.79 ± 0.02*	28.62± 0.02*	26.50 ± 0.03*	25.68 ± 0.05*
	72	33.49 ± 0.03	29.37 ± 0.02*	26.21 ± 0.03*	24.38 ± 0.02*	21.62 ± 0.02*
	96	33.46 ± 0.04	28.70 ± 0.03*	25.87 ± 0.02*	24.07 ± 0.02*	± 0.05*

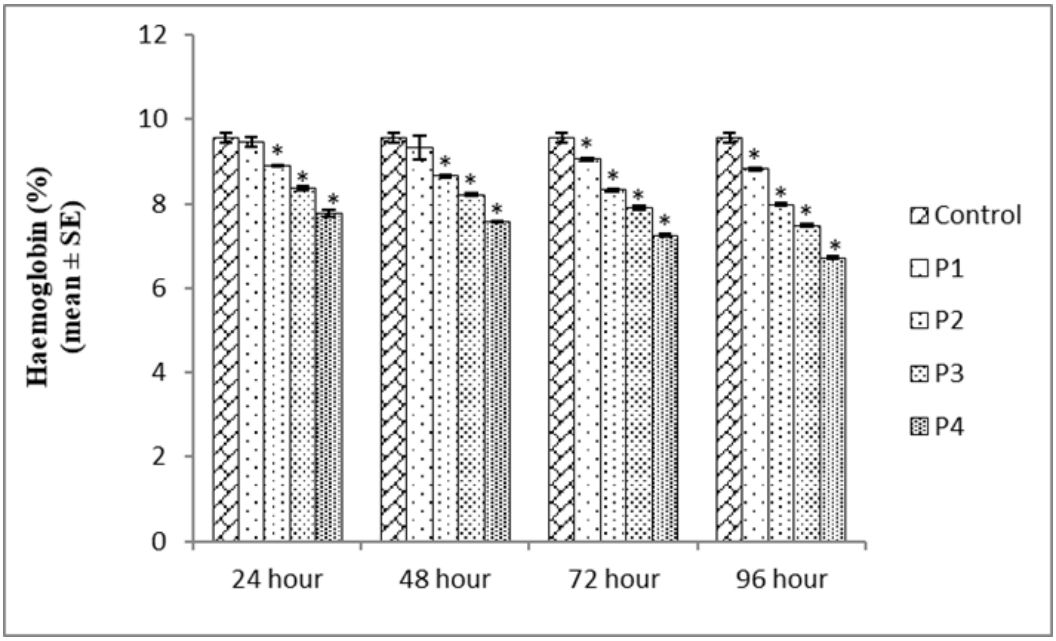

Figure 5: Effect of profenofos exposure on hemoglobin levels (%) in *L. rohita* at varying concentrations (P1=0.2 µg/L, P2=0.6 µg/L, P3=0.8 µg/L, P4=1.0 µg/L) over 24, 48, 72, and 96 hours. *Indicates a statistically significant difference compared to the control group

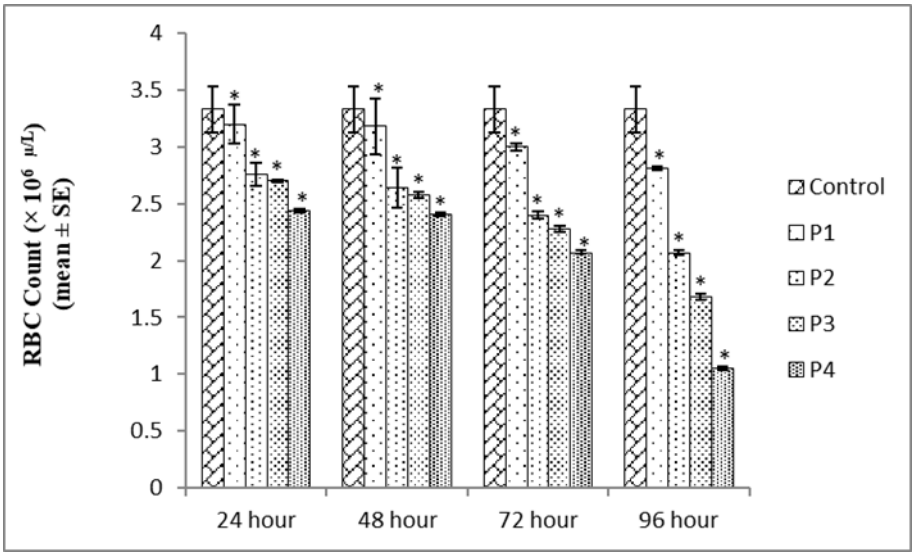

Figure 6: Effect of profenofos exposure on red blood cell (RBC) count in *L. rohita* at varying concentrations (P1 = 0.2 µg/L, P2 = 0.6 µg/L, P3 = 0.8 µg/L, P4 = 1.0 µg/L) over 24, 48, 72, and 96 hours. *Indicates a statistically significant difference compared to the control group

toxicity. Also, WBC count exhibited an increasing trend with higher profenofos concentrations and longer exposures **(Figure 7).** At 96 hours, the WBC count in the P4 group rose to 7.19 ± 0.0206 × 10^3/mm^3, compared to the control (4.68 ± 0.019 × 10^3/mm^3), which may suggest an inflammatory or immune response triggered by profenofos toxicity.

The decrease in Hb and RBC levels indicates the symptoms of anemia, which could result from hemolysis or inhibition of erythropoiesis due to PFF exposure (Shukla *et al*., 2024). Similar reductions in RBC counts have been observed in various fish species exposed to other biocides (Nithyanandam *et al*., 2007; Nataraj *et al*., 2017; Jamil *et al*., 2024). The decline in Hb levels could disrupt oxygen delivery to tissues, reducing metabolic rates and energy production, as suggested by Joshi *et al*. (2002) and Mahmood *et al*. (2023). The observed anemia may be due to the destruction of erythrocytes, as noted in earlier studies (Kumari *et al*., 2010). The increase in WBC count might be due to an immune response to PFF exposure, possibly involving leukocytosis as part of the fish's defense mechanism (Tabassum *et al*., 2020; Das *et al*., 2024). This increase may indicate an increased immunological activity or mobilization of WBC from hematopoietic organs (Ibrahim *et al*., 2024; Navruz *et al*., 2023).

Effects on HCT, MCV, MCH, and MCHC

As shown in Table 2, HCT levels also demonstrated a declining trend, starting from 27.24% in control fish to 23.12% in P4 at 24 hours. This decrease continued through 96 hours, where HCT dropped to 22.10% in the highest exposure group (Figure 8). Reduced hematocrit indicates lesser blood oxygenation, which could result from lowered RBC count and hemoglobin levels, further stressing the fish's respiratory function. In contrast, MCV showed an increasing trend, with control fish having an MCV of 83.15 fL at 24 hours (Figure 9). This value increased with PFF exposure, reaching 100.55 fL at P4. By 96 hours, MCV rose significantly, with P4 showing an MCV of 110.17 fL. The increase in MCV suggests that fewer, but larger, red blood cells are present, which is characteristic of certain types of anemia or compensatory erythropoiesis. MCH also increased, indicating higher hemoglobin content per RBC in exposed fish (Figure 10). At 24 hours, MCH in control fish was 25.29 pg, while at P4, it increased to 35.41 pg. By 96 hours, MCH further increased to 40.07 pg at P4. This increase, along with MCV, could be a compensatory mechanism for the loss of RBCs. However, MCHC decreased across the exposure groups (Figure 11). Starting at 33.51% in control fish, MCHC dropped to 26.68% at P4 after 24 hours. This declining trend continued, reaching 21.0% at P4 after 96 hours. Lower MCHC indicates a reduction in hemoglobin concentration within the red blood cells, likely due to structural damage to RBCs or impaired hemoglobin synthesis. The increase in MCV and MCH suggests a mechanism to maintain oxygen transport despite a decrease in RBC count, a response also observed by Rana *et al*. (2024). The drop in MCHC values indicates a hypochromic, microcytic anemia, consistent with previous findings in fish exposed to pesticides (Ghayyur *et al*., 2020). Reduced RBC, Hb, and PCV values point to significant physiological stress caused by PFF, in line with reports by Ramesh *et al*. (2008) and Rohani *et al*. (2023).

Behavioral and physiological responses

Fish exposed to acute PFF toxicity displayed erratic swimming, excessive mucus secretion, and puffing

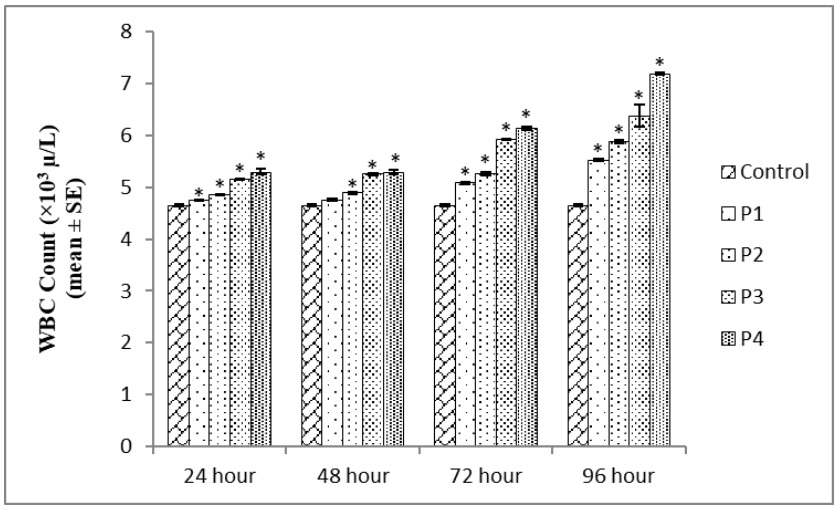

Figure 7: Effect of profenofos exposure on WBC count in *L. rohita* at varying concentrations (P1 = 0.2 µg/L, P2 = 0.6 µg/L, P3 = 0.8 µg/L, P4 = 1.0 µg/L) over 24, 48, 72, and 96 hours. *Indicates a statistically significant difference compared to the control group

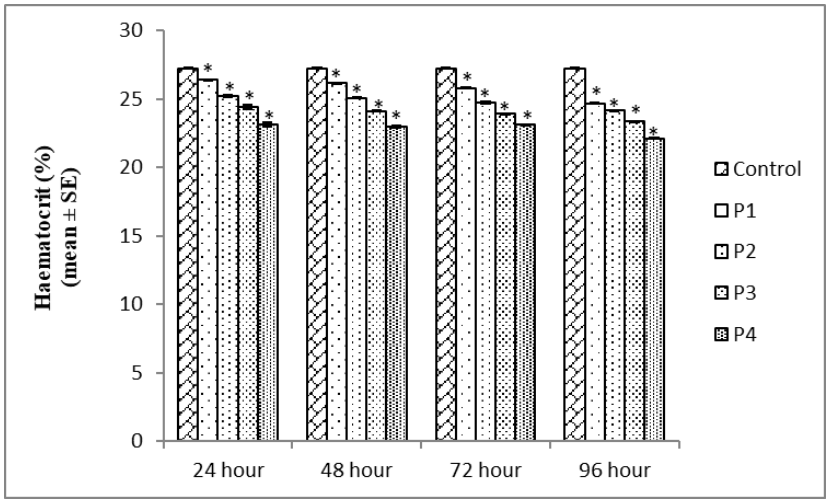

Figure 8: Effect of profenofos exposure on haematocrit (%) in *L. rohita* at varying concentrations (P1 = 0.2 µg/L, P2 = 0.6 µg/L, P3 = 0.8 µg/L, P4 = 1.0 µg/L) over 24, 48, 72, and 96 hours. *Indicates a statistically significant difference compared to the control group

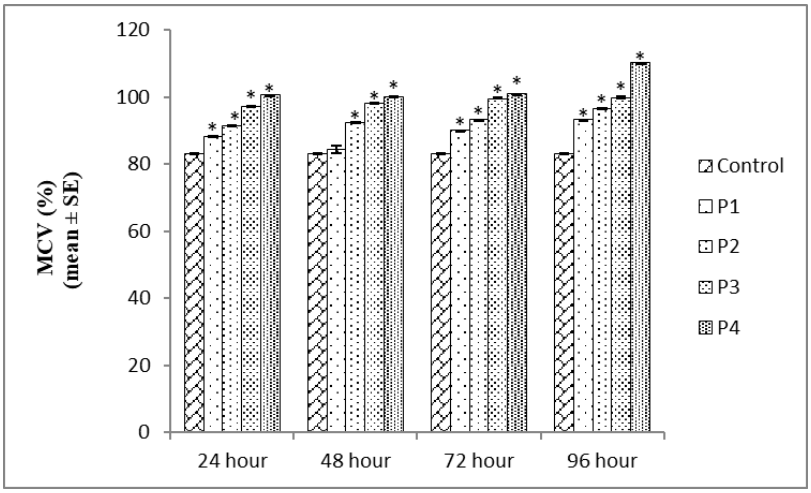

Figure 9: Effect of profenofos exposure on mean corpuscular volume (%) in *L. rohita* at varying concentrations (P1 = 0.2 µg/L, P2 = 0.6 µg/L, P3 = 0.8 µg/L, P4 = 1.0 µg/L) over 24, 48, 72, and 96 hours. *Indicates a statistically significant difference compared to the control group

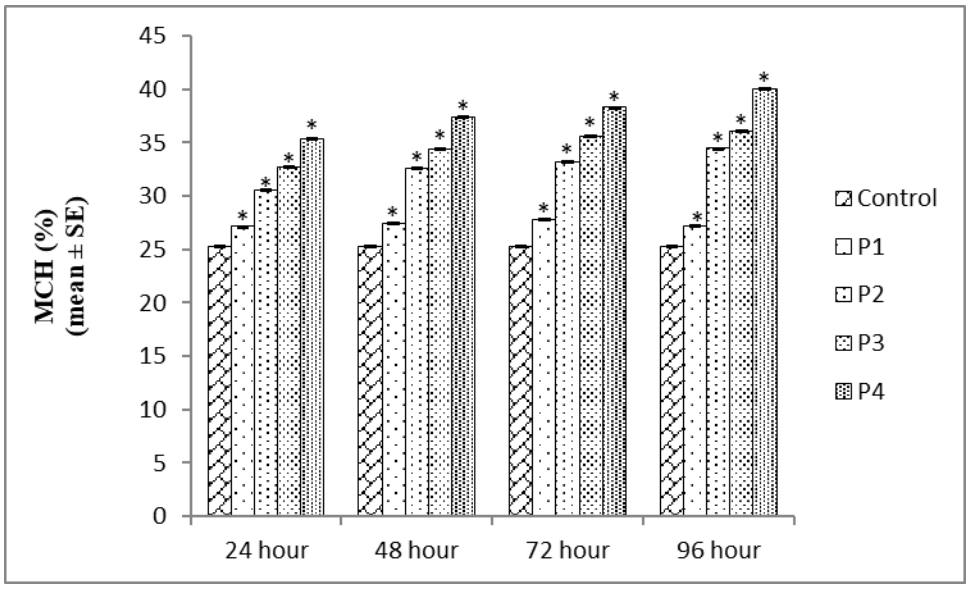

Figure 10: Effect of profenofos exposure on mean corpuscular haemoglobin (%) in *L. rohita* at varying concentrations (P1 = 0.2 µg/L, P2 = 0.6 µg/L, P3 = 0.8 µg/L, P4 = 1.0 µg/L) over 24, 48, 72, and 96 hours. *Indicates a statistically significant difference compared to the control group

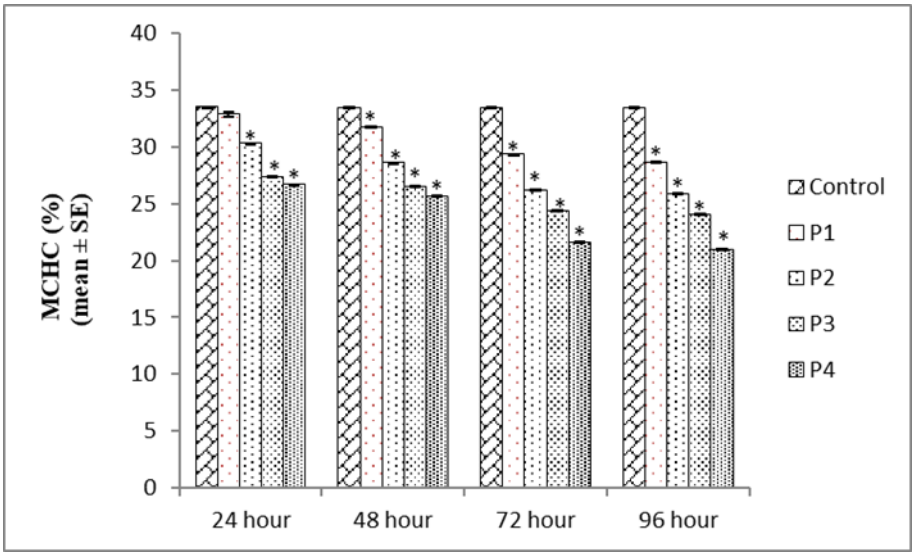

Figure 11: Effect of profenofos exposure on mean corpuscular haemoglobin concentration (%) in *L. rohita* at varying concentrations (P1 = 0.2 µg/L, P2 = 0.6 µg/L, P3 = 0.8 µg/L, P4 = 1.0 µg/L) over 24, 48, 72, and 96 hours. *Indicates a statistically significant difference compared to the control group

of gill opercula, with these behaviors becoming more pronounced during the initial stages of exposure. These behavioral responses may result from the irritating effects of PFF at acute concentrations, as previously reported by Shukla *et al*. (2024). Increased mucus secretion and gill puffing are probably protective mechanisms against the toxicant, but loss of equilibrium and erratic swimming may indicate neurodisruptions by PFF. The findings indicate exposure to PFF causes substantial alteration in water quality parameters and hematological response with elevated concentration of PFF in *L. rohita.* With increasing levels, more physiological stress and mortalities will also occur.

Conclusion

This study demonstrated that the exposure of *L. rohita* to PFF in aquatic systems resulted in significant hematological alterations, serving as a key bioindicator in ecotoxicological assessments. Changes in RBC and WBC counts, Hb concentration, PCV, MCV, MCH, and MCHC presented physiological stress and health status of fish exposed to pesticide toxicity. Although these effects are not uniform in pattern across different pesticides, they commonly lead to anemia and an increase in WBC count, indicating immune responses to toxicants. These observed hematological effects can compromise vital functions of fish, such as respiration, feeding, and

reproduction, which eventually result in reduced populations of fish and biodiversity. A decline in fish production due to pesticide contamination has risks not only to aquatic ecosystems but also to human nutrition, particularly in communities relying on fish as a protein source. Further research should be conducted to assess the cellular and molecular mechanisms by which PFF affects hematological parameters in *L. rohita* and study their impact on fish health and ecosystem sustainability. Future studies should consider long-term water quality and fish response assessments across different seasons to evaluate the broader ecological implications of pesticide exposure. This study is beneficial to human society as it contributes to food safety, environmental monitoring, and sustainable aquaculture practices.

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Ethical approval

This study was approved by the ethical committee on human and animal experiments of IFTM University, Moradabad, U.P. India (approval number: 52525/S181703).

Conflict of interest

The authors declare that they have no conflicts of interest.

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