



Effects of Sub Lethal Concentration of Cypermethrin on the Biochemical, Behavioural and Haematological Parameters of Rohu (*Labeo rohita*) Fingerlings

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study aims to examine and assess the harmful effects of the pyrethroid insecticide cypermethrin on rohu (*L. rohita*) fingerling. The acute median lethal concentration (LC₅₀) was determined by exposing fish to four different concentrations (2 ppb, 5 ppb, 10 ppb, and 15 ppb) over a 96-hour period and the LC₅₀ value was identified as 4.996 ppb. Based on the concentration obtained fish were exposed to the sub lethal concentration (0.025, 0.05 and 0.1 ppb) and one control with no chemical for a duration of 30 days for further studies to assess the impact of chronic toxicity. A sub-lethal toxicity evaluation indicates significant changes in blood parameters, characterized by an increase in total leucocytes count and glucose levels, alongside a decrease in total erythrocytes count, packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). There was also a decrease in biochemical parameters such as albumin, globulin, and total protein. Abnormal behavioural changes observed include loss of equilibrium, increased opercular movement, restlessness transitioning to lethargy, gill mucus secretion, repeated mouth movements, and twitching or convulsions. This study revealed that even minimal exposure to cypermethrin can negatively affect fish, causing notable alterations in behavioural, biochemical and haematological parameters.

Keywords: Chronic toxicity; mortality; insecticides; ecosystem.

1. INTRODUCTION

Aquaculture continues to drive growth, with global production soaring to 130.9 million tons in 2022, representing a significant 6.6 percent increase from 122.8 million tons in 2020. (FAO, 2024). With the wild fisheries resources continuing to decline, aquaculture has become a primary source of global food fish production. However, this growth has been accompanied by a rising use of synthetic chemicals such as pesticides to treat parasites, fungal infections, and eradication of unwanted or invasive organisms in and around fish culture systems to enhance productivity. However, excessive and improper use of pesticides disrupt the delicate balance of aquatic ecosystems and degrade environmental quality (Kadiru et al., 2022). This disruption threatens the long-term sustainability of aquatic resources, undermining the benefits they provide to human well-being. Consumption of pesticide-contaminated animals and their by-products can transfer toxicity to humans and wildlife, including carnivorous fish species that feed on contaminated aquatic insects, potentially propagating harmful effects up the food chain (Maurya et al., 2019).

CYP is a 4th generation halogenated type II pyrethroid (Kaviraj and Gupta, 2014) synthesized in 1974, it was first introduced to the market in 1977 as a highly potent synthetic pyrethroid insecticide. It is effective against a broad spectrum of pests, making it widely applicable in agriculture, public health, and animal husbandry

(Anonymous, 2024). Although designed to target various pest species, CYP also adversely affects beneficial insects and other non-target organisms. Notably, fish are particularly susceptible to the detrimental effects of cypermethrin (Stephenson et al., 1982), and changes in their health directly reflect the condition of their environment, particularly when exposed to pollutants such as pesticides. (Lopez-Lopez and Seden-Diaz, 2015). Insecticides can be carried to aquatic ecosystems through agricultural runoff, spray drift, atmospheric fallout, soil erosion, industrial and domestic sewage, careless disposal of empty containers, equipment washing and ground infiltration (Kadiru et al., 2022).

Haematological and biochemical parameters are very sensitive to the changes of environmental factors and its analysis is reliable in detecting stress, disease, or toxicity in fish (Witeska et al., 2023). Variations in these parameters can provide indicators of physiological disruptions caused by factors like pollution, water temperature fluctuations, oxygen depletion, and exposure to harmful chemicals. (Chhaba et al., 2024; Samajdar et al., 2015). (Bhatnagar et al., 2017; David et al., 2015; Khatun et al., 2014) reported that the fish exposed to pesticides showed considerable alteration in the level of different blood parameters, haematological parameters including red blood cells count (RBC) and haemoglobin (Hb) and white blood cells (WBC). Additionally, biochemical parameters such as total protein and glucose levels also

show considerable variation. The results indicate that the investigated parameters can be used as effective biomarkers for evaluating pesticide-induced toxicity in aquatic organisms, and enhance our understanding of the effects of pesticide exposure on fish.

In this experiment, *L. rohita* were selected for the present study due to its high economic and market value. CYP is the second most consumed insecticide in India in the year 2019-2020 (GOI 2020). Given its widespread use, assessing its impact on fish is essential. Blood parameters and behavioural responses serve as an effective tool for understanding the impact of insecticides on fish health. While many studies report such effects in various fish species, specific research on cypermethrin's impact on *L. rohita* remains limited.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted in the laboratory of the Department of Aquaculture, School of Agriculture, Sanjeev Agrawal Global Educational (S.A.G.E) University, Bhopal, Madhya Pradesh.

2.2 Fish Collection and Acclimatization

A total of 150 healthy and active rohu (*L. rohita*) fingerlings were obtained from Prayas Fish Farm situated at Chandpura, Narmadapuram, Madhya Pradesh. They were transported from farm to the laboratory in a properly aerated, oxygen-enriched plastic bag and the fish were acclimated for two weeks in a large 1000L capacity FRP (Fiber Reinforced Plastic) tank equipped with sufficient aeration. Fish were fed a commercially available formulated diet at a rate of 3% of their body weight twice per day prior to the experiment.

2.3 Chemical Collection

CYP 10 percent EC, a commercial name CYPERHIT-10, manufactured by HPM Chemicals & Fertilizers Ltd. was purchased from Shri Ram Green Garden, LG 67, Minal Mall, Bhopal, Madhya Pradesh.

2.4 Experimental Design

For the acute toxicity test (LC₅₀), a group of twenty healthy, uniform-sized fish was randomly selected and introduced into each 50 L experimental tank. The experimental setup

included four treatments with cypermethrin (CYP) and one control, each with three replicates: Control (0 ppb), T1 (2 ppb), T2 (5 ppb), T3 (10 ppb), and T4 (15 ppb). Mortality rates were assessed at regular intervals (24, 48, 72, and 96 hours), and deceased fish were promptly removed to maintain water quality. During the experiment tanks were kept under continuous aeration and without food to prevent the interference of excretory products of the fish with the test chemical (Pandey et al., 2009) and the water was renewed with a fresh concentration every 24 hours to maintain the toxicant strength. To evaluate the long-term effect, fish were exposed to 1/40th, 1/80th, and 1/160th (0.1, 0.05 and 0.025 ppb) of the LC₅₀ of CYP and one Control group (without chemical) for 90 days. During this period commercial pelleted feed obtained from the market was given twice a day at 3% of fish body weight in the morning and evening at the same timing every day.

2.5 Behavioural Observation

Any alterations in the normal behavioural patterns of the fish, including changes in swimming activity, frequency of surfacing, operculum movement rate, and responses to the chemical, were recorded.

2.6 Analysis of Haematological Parameters

Blood samples were collected using an insulin syringe from the caudal region of fish. The total erythrocyte and leucocytes count were estimated with the help of improved Standard Neubauer haemocytometer described by Dacie and Lewis (1991). The haemoglobin was estimated using the standard Sahli's method. Other parameters, including PVC, MCV, MCH, and MCHC, were estimated and calculated using the formulas described by Wintrobe et al. (1981).

2.7 Analysis of Biochemical Parameters

Total protein was estimated according to the method of Lowry et al. (1951) and Glucose was estimated by Folin-Wu method described by the Folin and Wu (1920). The methods of Kingsley (1939) were used to determine Albumin level and Globulin content was calculated by subtracting albumin values from the total serum protein.

2.8 Statistical Analysis

The data were analysed using one-way analysis of variance (ANOVA) to assess significant

differences among treatment group means, and mean comparisons were made using Duncan's Multiple Range Test (DMRT) to identify specific group differences. A p-value of less than 0.05 was considered statistically significant and all data were presented as mean \pm standard deviation. All statistical analyses were performed using SPSS software.

3. RESULTS

3.1 Acute Toxicity Test

In the control tank (C), where no pesticide was present, fish mortality was zero. In contrast, mortality rates increased with rising CYP concentrations in the other tanks results showed a dose-dependent response. After 96 hours of exposure, 100% mortality was observed in T4 (15 ppb), followed by 90% in T3 (10 ppb), 60% in

T2 (5 ppb), and 20% in T1 (2 ppb), while no mortality (0%) occurred in the control tank C (0 ppb) as shown in (Fig. 1). The median lethal level concentration (LC_{50}) was calculated to be 4.996 ppb using probit analysis software (Fig. 2).

3.2 Behavioural Changes

During the acute toxicity test, fish exposed to CYP exhibited various abnormal behaviours. These included erratic swimming, loss of equilibrium, increased opercular (gill cover) movement, restlessness followed by lethargy, gill mucous secretion, repeated opening and closing of the mouth and migration to the bottom. These behavioural changes indicate significant stress and physiological disruption in fish exposed to CYP. Behaviour of fish observed in different concentration of test chemical in respect of time is given in Table 1.

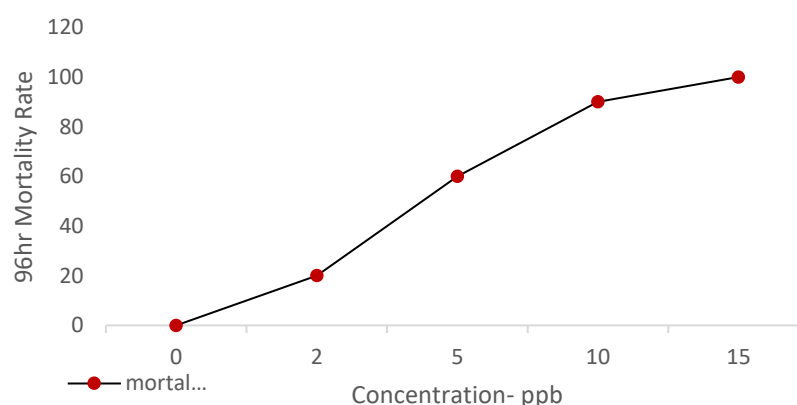


Fig. 1. Cumulative 96-hour mortality rate at different concentrations of CYP

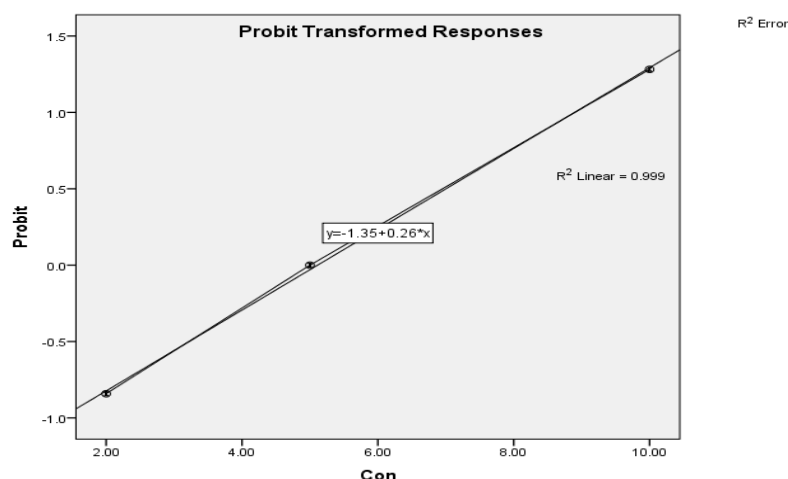


Fig. 2. Linear relationship between probit mortality and concentration of rohu (*L. rohita*) exposed to acute concentrations of CYP for 96 hours

3.3 Chronic Toxicity Test

Fish were exposed to sub-lethal concentrations of CYP at 1/40th (0.1 ppb), 1/80th (0.05) ppb, and 1/160th (0.025ppb) of the LC₅₀, along with a control group without chemical exposure.

3.3.1 Effects on haematological parameters

As the concentration of CYP increased, there was a significant ($p < 0.05$) decrease in RBC, PCV, Hb, MCV, MCH, and MCHC. Whereas, WBC count showed a significant increase with rising CYP concentrations. Changes in haematological parameters induced by CYP exposure are presented in Table 2.

3.3.2 Effect on biochemical parameters

Long-term exposure to CYP induced significant alterations in the biochemical parameters of *L. rohita*, total protein, albumin and globulin ratio levels decreased as the dose increased, while glucose levels and albumin-globulin ratio rose accordingly as shown in (Table 3).

4. DISCUSSION

In this study, the median lethal concentration 96 hrs-LC₅₀ of CYP was found to be 4.996 ppb which is the statistically derived concentration of a toxicant that expected to be lethal to 50% of a test population, based on a probabilistic model. However, it varies from species to species 27.07 µg/L on the guppy (Salako et al., 2020) and 3.14 µg/L on rainbow trout (Valisek et al., 2006) exposed to CYP. The acute toxicity test revealed that fish mortality is significantly correlated with the concentration of CYP. Fish mortality rates increased directly with CYP concentrations. Similar relation was reported by (Asadullah et al., 2010; Dawar et al., 2016; Vali et al., 2022) exposed to insecticide.

Fish are able to uptake and retain different xenobiotics dissolved in water via active or passive processes and changes in the behaviour of fish indicates the deterioration of water quality (Sharma et al., 2019). In this study, after few minutes of exposure to CYP, fish behaviour shifted from normal to abnormal, exhibiting signs of distress, including loss of equilibrium, increased opercular movement, mucous secretion, muscle spasms, and erratic swimming patterns, such as swimming upwards and gulping for air. Eventually, the fish became lethargic,

sank to the bottom, and died. Similar findings of the behavioural responses to pesticides have been reported by Velisek et al., (2006), Majumder et al., (2017) and Ghost et al., (2022). The increase in surfacing and gulping of air from surface water after toxicant exposure could be an attempt of the animal to escape from the toxicant and to avoid breathing in the contaminated water (Sharma et al., 2019). Pesticides can have devastating effects on fish populations by interfering with acetylcholine esterase (AChE) activity, a crucial enzyme responsible for regulating nerve function, leading to impaired nerve transmission, accumulation of acetylcholine in tissues, and disruption of normal behavioural patterns, this inhibition was reported in *P. reticulata* and *L. rohita* exposed to pyrethroid insecticides. (Sharbidre et al., 2011) (Marigoudar et al., 2009). Fish exposed to environmental stress may secrete mucus in large quantities to cover the body, especially the gills trying to eliminate the toxin's irritating effects (Vali et al., 2022). The abnormality observed may be due to the effect of pyrethroid insecticides known to open the sodium channels in neuronal membrane, affecting both Peripheral nervous system (PNS) and Central nervous system (CNS) in fish, causing hyperexcitability, causing tremors, incoordination, paralysis, extremely toxic to fish (Sabra et al., 2015).

Fish blood is a sensitive indicator of environmental pollution, showing distinct pathological changes in response to toxicity. Haematological parameters are crucial for detecting environmental stress and disease in fish and changes in haematological parameters can serve as indicators of stress in fish (Talas et al 2009). Upon completion of exposure to sublethal concentrations of CYP, alterations in haematological parameters were observed. There was a significant increase in WBC with each increase in concentration of CYP. The increase in WBC suggests the body's attempt to enhance its protective mechanisms against stress (Bhanagar et al., 2017) and likely linked to increased antibody production, facilitating improved survival and recovery rates in fish exposed to toxicants (Joshi et al., 2002). Consistent with findings reported for *Oreochromis niloticus*, *Clarias gariepinus*, *Channa punctatus* and *Ctenopharyngodon idella*, exposed to Cypermethrin (Majumder and Kaviraj, 2017; Amaeze et al., 2020; Ghosh et al., 2022; Ulla et al., 2022). In our present study significant decline was observed in RBC, Hb, PCV, MCV, MCH, and MCHC values with increasing

concentrations of CYP which is similar to the observations made by (Jasmin et al., 2018; Akhtar et al., 2021; Shahi et al., 2013) fish exposed to difenoconazole, cypermethrin and furadan pesticides. The reduction of total RBC, Hb, and PCV levels in fish under pesticidal stress reflect an anaemic condition, potentially caused by the inhibition of erythropoiesis or the destruction of erythrocytes (red blood cells) due to the excessive generation of reactive oxygen species (ROS) in response to pesticide toxicity (Sinha et al., 2022). The haemoglobin content decrease in fish may be caused by either destruction or impaired synthesis (Vani et al., 2012). Other haematological parameters, namely mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), were comparatively lower than those in the control group. The changes observed in these indices were directly correlated with alterations in packed cell volume (PCV), haemoglobin (Hb) concentration, and red blood cell count. In line

with the observations of other researchers (David et al., 2015; Jasmin et al., 2015).

Biochemical parameters serve as stress indicators, reflecting the physiological responses of fish to environmental stressors, including pesticides and other pollutants. Several researchers have reported significant dose-dependent alterations in biochemical blood parameters due to insecticide exposure in various fish species. These changes include increased glucose levels and decreased total protein, albumin, and globulin, as observed in *C. carpio* (Bhanu et al., 2015), *C. mrigala* (Bhatnagar et al., 2017), and *P. hypophthalmus* (Chhaba et al., 2024). These findings support the biochemical changes observed in our study. The significant increase in glucose levels may be a consequence of stress-induced gluconeogenesis, a metabolic adaptation to meet the elevated energy demands imposed by physiological stress (Ramesh et al., 2018; Quader et al., 2022). The decrease in protein

Table 1. Changes in fish behaviour over time at different test chemical concentrations

Behaviour of fish	Behavioural changes over time at different concentrations (96-hour toxicity test)				
	0 ppb	2 ppb	5 ppb	10 ppb	15 ppb
Erratic and darting movements	-	1hr	1hr	<1hr	<30min
Increased opercular movement	-	24hr	12hr	<1hr	<30min
Loss of balance	-	72hr	24hr	<1hr	<30min
Gasping	-	12hr	12hr	<1hr	<30min
Migration to the bottom	-	48hr	24hr	<1hr	<30min
Lethargy	-	48hr	12hr	<1hr	<30min

Table 2. Effect of CYP on haematological parameters of *L. rohita*

Parameters	Treatment			
	Control(0ppb)	T ₁ (0.025ppb)	T ₂ (0.05ppb)	T ₃ (0.1ppb)
Hb(g/dL)	8.2±0.11 ^d	7.6±0.11 ^c	5.1±0.11 ^b	4.6±0.11 ^a
RBC (10 ¹² /L)	2.26±0.15 ^c	2.16±0.01 ^b	1.89±0.01 ^a	1.75± 0.01 ^a
WBC (10 ⁴ /mm ⁻³)	12.94±0.01 ^a	13.48±0.01 ^b	14.68±0.01 ^c	20.24±0.01 ^d
PCV (%)	36.33±0.19 ^d	35.6±0.11 ^c	25.9±0.11 ^b	24.4±0.11 ^a
MCV (fl)	160.7±0.11 ^d	164.81±0.11 ^c	137.03±0.11 ^b	139.42±0.11 ^a
MCH (pg)	36.28±0.01 ^d	35.18±0.11 ^c	26.98±0.01 ^b	26.28±0.01 ^a
MCHC (g/dl)	22.5±0.11 ^d	21.3±0.11 ^c	19.69±0.11 ^b	18.85±0.11 ^a

Table 3. Effect of CYP on biochemical parameters of *L. rohita*

Parameters	Treatment			
	Control(0ppb)	T ₁ (0.025ppb)	T ₂ (0.05ppb)	T ₃ (0.1ppb)
Protein (µg mL ⁻¹)	2.52 ± 0.11 ^d	2.35 ± 0.01 ^c	1.98± 0.01 ^b	1.66 ± 0.01 ^a
Albumin (µg mL ⁻¹)	1.37 ± 0.01 ^d	1.28 ± 0.01 ^c	1.15 ± 0.01 ^b	0.98 ± 0.01 ^a
Globulin (µg mL ⁻¹)	1.15 ± 0.01 ^d	1.07±0.01 ^c	0.83 ± 0.01 ^b	0.68 ± 0.01 ^a
A:G (µg mL ⁻¹)	1.19 ± 0.01 ^b	1.20 ± 0.01 ^b	1.38 ± 0.01 ^a	1.44 ± 0.01 ^c
Glucose (mg 100 mL ⁻¹)	70.8 ± 0.11 ^a	94.6 ± 0.11 ^b	100.5 ± 0.11 ^c	110.5 ± 0.11 ^d

levels, albumin and globulin may be due to increased energy demands under stress, which trigger enhanced protein catabolism. This process enables fish to generate the necessary energy required for physiological adjustments, helping them cope with the adverse effects of environmental stressors (Nagaraju and Rathnamma, 2013) (Bhatnagar et al., 2017). Vani et al. (2011) and Vani et al. (2012) reported a significant reduction in albumin, globulin, and the albumin-globulin ratio in *Labeo catla* exposed to the pyrethroid insecticides deltamethrin and CYP. The decrease in albumin and globulin can be due to the decreased synthesis in the hepatocytes or of liver dysfunction (Bhanu et al., 2015).

5. CONCLUSION

The study revealed that small amounts of CYP are highly toxic to *L. rohita* fingerlings, causing significant behavioral disruptions, hematological alterations, and biochemical imbalances. The exposure showed a dose-dependent effect, leading to a decrease in red blood cell count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total protein, albumin, and globulin levels, while white blood cell (WBC) count and glucose levels increased, indicating severe physiological distress. These findings emphasize the need for strict regulations on CYP use in and around water bodies to prevent fish mortality and disruptions of aquaculture production.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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