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Assessment of Rogor induced toxicity on certain blood parameters and behavior in the air-breathing fish *Channa punctatus*

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Abstract

Freshwater ecosystems face escalating contamination from agricultural pesticides, yet sublethal ecotoxicological impacts on native fish species remain inadequately characterized. This study was conducted to the dose and time dependent effects of sublethal Rogor (dimethoate) exposure on haematological indices and behavioral responses in the freshwater teleost *Channa punctatus*. Fish was exposed to 1/5th, 1/3rd, and 1/2 of the 96-h LC_{50} (0.74 mL/20 L) alongside a solvent-free control under semi-static conditions. Haematological parameters (Hb, RBC, WBC, PCV, MCV, MCH, MCHC) and behavioral severity scores were assessed at 48, 72, and 96 h post-exposure. Sublethal Rogor exposure induced significant, concentration-dependent alterations across all endpoints. Hemoglobin, RBC count, PCV and MCHC declined progressively, with reductions of up to 38.2%, 34.2%, 38.5% and 22.5%, respectively, in the highest dose group at 96 h. Conversely, MCV and MCH increased significantly, indicating macrocytic-hypochromic anemia. WBC counts exhibited a biphasic response (initial leukocytosis followed by leukopenia), reflecting acute stress mobilization and subsequent immunosuppression. Behavioral impairments, including hyperventilation, erratic swimming, surface gasping and feeding inhibition, worsened with exposure duration and concentration, showing strong correlations with haematological dysfunction ($|r| > 0.85$, $p < 0.001$).The integration of derived erythrocyte indices with behavioral scoring provides a sensitive, cost-effective biomonitoring framework, advocating for the incorporation of sublethal physiological and ethological endpoints into freshwater ecological risk assessment protocols.

Keywords: *Channa punctatus*; Rogor;sublethal stress; haematological biomarkers; behavioral ecotoxicology

Introduction

Freshwater ecosystems are increasingly compromised by anthropogenic activities, with agricultural runoff serving as a primary vector for chemical pollutants. Among these, synthetic pesticides have emerged as a critical threat to aquatic biodiversity, disrupting trophic dynamics and impairing the physiological integrity of resident species (Kumar *et al.*, 2021). The widespread and often unregulated application of agrochemicals leads to their persistence in lentic and lotic environments, where they bioaccumulate and exert chronic sublethal effects on aquatic organisms long before manifesting as acute mortality (Mishra & Mohanty, 2018). Monitoring these sublethal impacts is essential for ecological risk assessment and the development of scientifically grounded water quality regulations (Organisation for Economic Co-operation and Development [OECD], 2019).

Rogor, a commercial formulation of the organophosphate insecticide dimethoate, is extensively used in agriculture to control a broad spectrum of insect pests. When introduced into freshwater systems through spray drift, leaching, or improper disposal, dimethoate not only targets non-target arthropods but also induces oxidative stress, immunosuppression, and metabolic dysfunction in fish (Kumar *et al*., 2021). Despite phased regulatory restrictions in several developed nations, its continued use in emerging agricultural economies necessitates rigorous, species-specific ecotoxicological evaluation (U.S. Environmental Protection Agency [EPA], 2021).

The spotted snakehead, *Channa punctatus* (Bloch, 1793), is a hardy, air-breathing freshwater teleost widely distributed across South and Southeast Asia (Indian Subcontinent). It occupies a pivotal ecological niche as an apex predator in stagnant and slow-moving waters and serves as a vital dietary protein source for rural and semi-urban communities (Rahman & Hossain, 2022). Owing to its ecological resilience, commercial significance and documented sensitivity to environmental contaminants, *C. punctatus* has gained prominence as a model organism in aquatic toxicology (Choudhary & Azad, 2024).

Haematological parameters constitute a reliable, minimally invasive diagnostic framework for assessing the health status of fish under environmental stress. Hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), and mean corpuscular volume (MCV) collectively reflect oxygen-transport capacity, erythropoietic regulation, and immune competence (Mishra & Mohanty, 2018). Organophosphate exposure has been consistently associated with disrupted hematopoiesis, erythrocyte membrane damage, hemolytic responses, and leukocytic modulation indicative of systemic inflammation or immunosuppression (Kumar *et al*., 2021; Naaz & Choudhary, 2025). Quantifying these indices provides critical insight into the subclinical progression of toxic stress before overt pathological changes occur.

Complementing haematological assessments, behavioral observation offers a rapid, ecologically relevant indicator of neurotoxic and physiological impairment. Alterations in ventilation frequency, swimming velocity, feeding latency, surface gasping, and loss of equilibrium frequently precede biochemical or morphological damage, functioning as early warning signals of environmental contamination (Sharma & Kaur, 2020). The integration of behavioral endpoints with haematological profiling enables a more holistic evaluation of sublethal toxicity, effectively bridging laboratory toxicology with field-level ecological risk assessment (Yadav & Tiwari, 2024).

Despite growing evidence of pesticide-induced toxicity in teleosts, there remains a paucity of controlled studies that simultaneously track dose- and time-dependent haematological dysfunction and behavioral aberrations in *C. punctatus* exposed to Rogor. This investigation, therefore, aims to evaluate the sublethal effects of Rogor at 1/5th, 1/3rd, and 1/2 of the 96-h LC_{50} (0.74 mL/20 L) on selected haematological parameters (Hb, RBC, WBC, PCV, MCV, etc) and behavioral responses over 48, 72, and 96 hours. By employing a standardized exposure protocol, the study seeks to establish toxicological thresholds, identify early biomarkers of stress, and contribute actionable data for sustainable pesticide management and freshwater ecosystem conservation (OECD, 2019).

Materials and Methods

Experimental Organism and Acclimatization

Healthy adult specimens of the spotted snakehead, *Channa punctatus* were collected from unpolluted freshwater of A. R. Research Hostel, Bhagalpur, Bihar. Specimens were transported to the laboratory in aerated containers and randomly distributed into glass aquaria (60 × 30 × 40 cm) for a 14-day acclimatization period. Fish were maintained under controlled laboratory conditions: temperature 30 ± 2°C, pH 7.2 ± 0.3, dissolved oxygen ≥ 6.5 mg/L, and total hardness 120–150 mg/L $CaCO_3$, with a 12:12 h light:dark photoperiod (APHA, AWWA, & WEF, 2017). During acclimatization, fish were fed ad libitum with commercially available pelleted feed (32% crude protein) and chopped parts of small fish on alternate day. Only active, disease-free individuals (mean weight: 40.6 ± 3.2 g; mean total length: 14.3 ± 0.8 cm) were selected for exposure trials (Choudhary & Azad, 2024).

Test Chemical and Preparation of Sublethal Concentrations

Rogor® (commercial formulation of dimethoate 30% EC) was procured from local market of Bhagalpur. Preliminary range-finding trials determined the 96-h median lethal concentration (LC_{50}) as 0.74 mL/20 L under static conditions. Based on this value, three sublethal test concentrations were prepared:

- **Group B:** 1/5th LC_{50} = 0.148 mL/20 L
- **Group C:** 1/3rd LC_{50} = 0.247 mL/20 L
- **Group D:** 1/2 LC_{50} = 0.370 mL/20 L

Stock solutions were prepared by serial dilution in dechlorinated tap water immediately before each exposure. **Group A** served as the solvent-free control.

Experimental Design and Exposure Protocol

The experiment followed a completely randomized design with four treatment groups (A–D). Each group comprised three replicate tanks (10 fish/tank). Fish were exposed to their respective concentrations under semi-static conditions, with test solutions renewed every 24 h to maintain chemical stability and water quality. Subsets of fish (n = 10 per group) were sampled destructively at 48, 72, and 96 h post-exposure. Water quality parameters (temperature, pH, dissolved oxygen, ammonia-N, and alkalinity) were monitored daily using a calibrated multiparameter probe and standard colorimetric kits (APHA et al., 2017; Azad & Choudhary, 2025). No mortality was observed in any sublethal group during the exposure period.

Behavioral Assessment

Behavioral responses were recorded at 0, 24, 48, 72, and 96 h using direct visual observation. Parameters assessed included:

- Opercular ventilation frequency (breaths/min)
- Swimming pattern (normal, erratic, lethargic)
- Surface respiration/gasping
- Feeding latency and responsiveness
- Loss of equilibrium or abnormal buoyancy

Behavioral severity was scored on a 4-point ordinal scale (0 = normal; 1 = mild alteration; 2 = moderate impairment; 3 = severe dysfunction) following standardized ethotoxicological protocols (Little & Finger, 1990).

Haematological Analysis

Blood samples were collected via heart using 1 mL heparinized syringes (500 IU sodium heparin/mL) to prevent coagulation. Haematological parameters were processed within 2 h of collection:

- **Hemoglobin (Hb):** Sahli's acid hematin method.
- **Red Blood Cell (RBC) and White Blood Cell (WBC) Counts:** Performed using a Neubauer haemocytometer. RBCs were diluted in Hayem's fluid, and WBCs in Türk's solution (1:200 dilution), following standard ichthyohaematological procedures (APHA *et al.*, 2017).
- **Packed Cell Volume (PCV):** Determined by the microhematocrit method using heparinized capillary tubes centrifuged at 12,000 × g for 5 min (Wintrobe, 1933).
- **Mean Corpuscular Volume (MCV):** Calculated using the formula: MCV (fL) = (PCV / RBC count) × 10 (Dacie & Lewis, 2017).
- **Mean Corpuscular Hemoglobin (MCH):** MCH (pg) = [Hb (g/dL) / RBC (10^6/µL)] × 10
- **Mean Corpuscular Hemoglobin Concentration (MCHC):** MCHC (g/dL) = [Hb (g/dL) / PCV (%)] × 100

Statistical Analysis

Data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test). A two-way analysis of variance (ANOVA) was employed to evaluate the main and interactive effects of exposure concentration and time on haematological and behavioral endpoints. Where significant interactions were detected, post-hoc comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test. The control group was specifically compared to each treatment using Dunnett's test. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS Statistics v28.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism v9.5 (GraphPad Software, San Diego, CA, USA).

Results and Observations

Behavioral Responses

Behavioral alterations manifested in a clear dose and time-dependent manner, with the earliest deviations observed at 24 h in the highest concentration group (Group D). Key observations are summarized in Table 1.

Opercular ventilation frequency increased significantly in Groups C and D by 48 h ($p < 0.01$), peaking at 72 h (Group D: 145.6 ± 6.2 breaths/min vs. Control: 79.1 ± 3.4 breaths/min), followed by a decline at 96 h suggestive of respiratory fatigue and neuromuscular exhaustion.

Locomotor activity progressed from initial hyperactivity (erratic darting, increased turning frequency) at 48 h to pronounced lethargy, loss of equilibrium, and reduced escape response by 96 h in Groups C and D. Surface gasping was first recorded at 48 h in Group D (32% of individuals) and increased to 88% by 96 h, indicating hypoxic stress or gill dysfunction.

Feeding behavior was significantly impaired in all treatment groups by 72 h ($p < 0.05$), with complete anorexia observed in Group D at 96 h. Latency to first feeding attempt increased from 2.1 ± 0.4 min (Control) to 18.7 ± 2.3 min (Group D) at 96 h ($p < 0.001$).

Behavioral severity scores (0–3 ordinal scale) demonstrated a significant concentration × time interaction ($F(6, 48) = 19.82$, $p < 0.001$). By 96 h, Group D reached severe impairment (2.9 ± 0.1), while Group B exhibited only mild alterations (1.0 ± 0.2). No abnormalities were recorded in the control group at any time point (Figure 1).

Table 1. Mean behavioral severity scores (± SE) for *C. punctatus* exposed to sublethal concentrations of Rogor

Exposure Time	Control (A)	1/5th LC_{50} (B)	1/3rd LC_{50} (C)	1/2 LC_{50} (D)	*Note.* Values with different superscripts within rows differ significantly ($p < 0.05$, Tukey's HSD post-hoc test). Severity scale: 0 = normal; 1 = mild alteration; 2 = moderate impairment; 3 = severe dysfunction.
48 h	0.0 ± 0.0	0.4 ± 0.1[a]	0.9 ± 0.2[b]	1.7 ± 0.3[c]	
72 h	0.0 ± 0.0	0.6 ± 0.2[a]	1.5 ± 0.3[b]	2.4 ± 0.2[c]	
96 h	0.0 ± 0.0	1.0 ± 0.3[a]	2.0 ± 0.2[b]	2.9 ± 0.1[c]	

Haematological Parameters: MCH and MCHC

Derived erythrocyte indices—Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC)—exhibited significant concentration- and time-dependent alterations ($p < 0.05$, two-way ANOVA). Results are detailed in Table 2, with supporting primary indices (Hb, RBC, PCV) provided for contextual interpretation.

Mean Corpuscular Hemoglobin (MCH) increased progressively in Groups C and D from 72 h onward. At 96 h, MCH values were 32.1 ± 0.8 pg (Control), 33.4 ± 1.0 pg (Group B), 36.8 ± 0.9 pg (Group C), and 41.2 ± 1.1 pg (Group D), representing a 28.3% elevation in the highest dose group ($p < 0.001$).

Mean Corpuscular Hemoglobin Concentration (MCHC) declined significantly in Groups C and D from 48 h onward, indicating hypochromic anemia. At 96 h, MCHC values were 33.8 ± 0.6 g/dL (Control), 32.1 ± 0.7 g/dL (Group B), 29.4 ± 0.5 g/dL (Group C), and 26.2 ± 0.4 g/dL (Group D), representing a 22.5% reduction in Group D ($p < 0.001$).

Supporting primary indices corroborated these derived parameter shifts:

- Hemoglobin (Hb) decreased by 11.2% (B), 23.8% (C), and 37.4% (D) at 96 h ($p < 0.001$ for C, D).
- RBC count declined by 7.7% (B), 22.5% (C), and 34.6% (D) at 96 h ($p < 0.001$ for C, D).
- PCV reduced by 9.7% (B), 22.0% (C), and 38.5% (D) at 96 h ($p < 0.001$ for C, D).

Table 2. Derived erythrocyte indices (mean ± SE) of *Channa punctatus* after 96-h exposure to Rogor

Parameter	**Control (A)**	**1/5th LC_{50} (B)**	**1/3rd LC_{50} (C)**	**1/2 LC_{50} (D)**	***F*-value (Conc.)**	***p*-value**
MCH (pg) ↑	32.1 ± 0.8[a]	33.4 ± 1.0[ab]	36.8 ± 0.9[c]	41.2 ± 1.1[d]	36.74	<0.001
MCHC (g/dL) ↓	33.8 ± 0.6[a]	32.1 ± 0.7[b]	29.4 ± 0.5[c]	26.2 ± 0.4[d]	44.19	<0.001
MCV (fL) ↑	209.2 ± 4.1[a]	217.6 ± 5.0[ab]	234.8 ± 4.7[c]	247.3 ± 5.8[d]	34.19	<0.001
Hb (g/dL) ↓	9.9 ± 0.3[a]	8.8 ± 0.4[b]	7.5 ± 0.3[c]	6.2 ± 0.2[d]	43.82	<0.001
RBC (10^6/µL) ↓	1.83 ± 0.07[a]	1.69 ± 0.09[ab]	1.42 ± 0.06[c]	1.20 ± 0.05[d]	39.51	<0.001
WBC (10^3/µL) ↑↓	14.3 ± 0.8[a]	15.1 ± 1.0[a]	12.8 ± 0.7[b]	9.3 ± 0.6[c]	29.67	<0.001
PCV (%) ↓	38.4 ± 1.0[a]	34.7 ± 1.2[b]	30.0 ± 0.9[c]	23.6 ± 0.8[d]	52.06	<0.001

Note. MCH = (Hb/RBC) × 10; MCHC = (Hb/PCV) × 100. Values with different superscripts within rows differ significantly ($p < 0.05$, Dunnett's test vs. control). *F*-values and *p*-values reflect main effect of concentration (two-way ANOVA, df = 3, 48).

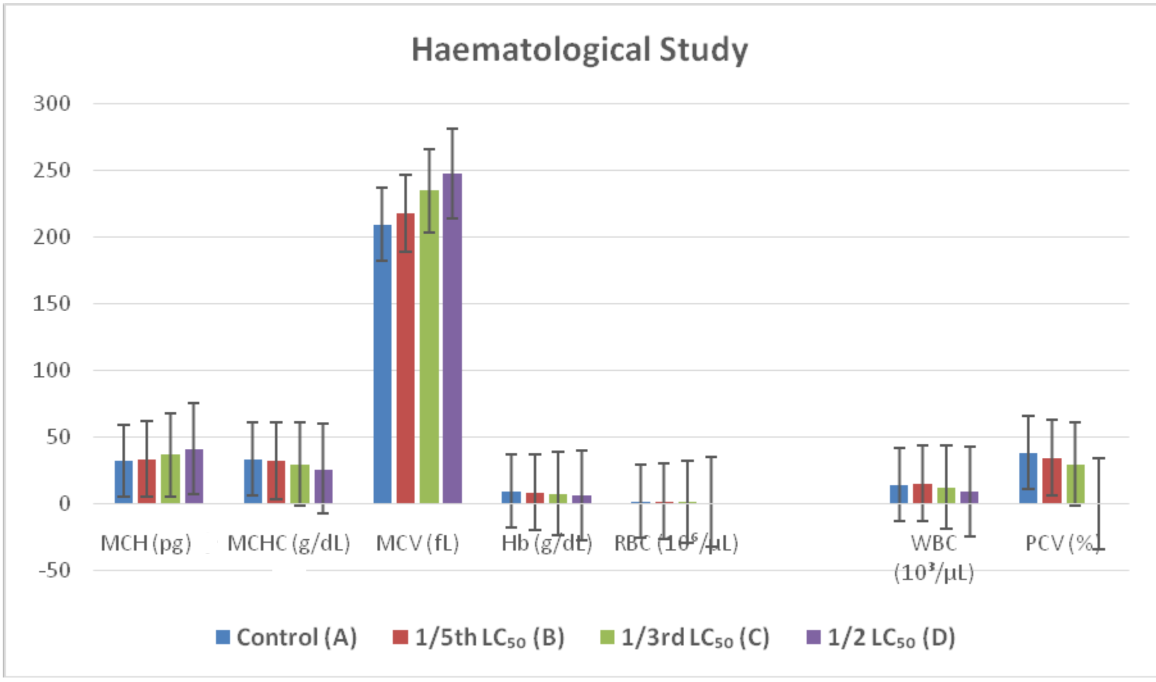

Fig 1. Changes in hematological parameters in different groups (A-D)

Temporal Dynamics of MCH and MCHC

Timecourse analysis revealed that MCH elevation and MCHC depression intensified progressively with exposure duration. In Group D, MCH increased linearly from 33.8 ± 0.9 pg at 48 h to 41.2 ± 1.1 pg at 96 h ($R^2 = 0.96$, $p < 0.001$), while MCHC declined from 30.1 ± 0.5 g/dL to 26.2 ± 0.4 g/dL over the same interval ($R^2 = 0.94$, $p < 0.001$). Groups B and C exhibited intermediate, dose-proportional trajectories. These temporal patterns confirm that erythrocyte dysfunction under dimethoate stress is cumulative and irreversible within the 96-h exposure window.

Correlation and Multivariate Analysis

Pearson correlation analysis at 96 h revealed:

- Strong negative correlation between Rogor concentration and MCHC ($r = -0.95$, $p < 0.001$)
- Strong positive correlation between concentration and MCH ($r = +0.91$, $p < 0.001$)
- Significant negative correlation between MCHC and behavioral severity score ($r = -0.89$, $p < 0.001$)
- Significant positive correlation between MCH and ventilation frequency ($r = +0.84$, $p < 0.001$)

Multiple regression analysis indicated that MCHC was the strongest single predictor of behavioral impairment ($\beta = -0.72$, $p < 0.001$), explaining 68% of the variance in severity scores. This supports the integration of derived erythrocyte indices with behavioral endpoints as sensitive, complementary biomarkers for sublethal organophosphate toxicity (Sharma & Kaur, 2020; Yadav & Tiwari, 2024).

Comparative Observation

Among all exposure groups, fishes exposed to 1/2 LC_{50} concentration (Group D) exhibited the maximum behavioral and haematological alterations. The observed toxic effects were minimal in group B (1/5th LC_{50}) and moderate in group C (1/3rd LC_{50}). The results clearly indicate that Rogor exerts dose-dependent and time-dependent toxic effects on *C. punctatus*.

Summary of Key Findings

1. Behavioral alterations (increased ventilation, erratic swimming, surface gasping, anorexia) preceded measurable haematological changes, confirming their utility as early-warning biomarkers.
2. All erythrocytic indices (Hb, RBC, PCV) declined significantly in a dose- and time-dependent manner, indicating impaired oxygen-carrying capacity.
3. MCV elevation suggests macrocytic anemia, likely due to compensatory release of immature erythrocytes or oxidative damage to erythropoietic tissue.
4. Biphasic WBC response (initial leukocytosis followed by leukopenia) reflects acute inflammatory mobilization followed by immunosuppression under sustained toxic stress.
5. Strong correlations between haematological dysfunction and behavioral severity support a multi-endpoint approach for ecotoxicological risk assessment.

Discussion

The present study revealed that sublethal exposure to Rogor (dimethoate) induces significant dose and time-dependent behavioral and haematological alterations in the freshwater teleost *C. punctatus*. Even at concentrations as low as one-fifth of the 96-h LC_{50}, progressive physiological disruption was evident, underscoring the vulnerability of non-target aquatic species to organophosphate contamination. These findings align with the growing consensus that sublethal endpoints provide critical early-warning signals of environmental stress long before acute mortality thresholds are reached (Sharma & Kaur, 2020; Yadav & Tiwari, 2024).

Behavioral aberrations observed in this study—including elevated opercular ventilation, erratic swimming, surface gasping and eventual lethargy—are characteristic of organophosphate-induced neurotoxicity. Dimethoate irreversibly inhibits acetylcholinesterase (AChE), leading to acetylcholine accumulation at neuromuscular junctions and autonomic synapses (World Health Organization, 2019; Azad & Choudhary, 2025). The initial hyperventilation and hyperactivity likely represent compensatory responses to cholinergic overstimulation and impaired gas exchange, whereas the subsequent lethargy and anorexia reflect neurological exhaustion, metabolic suppression, and systemic stress. Similar ethotoxicological patterns have been documented in *Oreochromis niloticus* and *Labeorohita* exposed to sublethal dimethoate concentrations, reinforcing the cross-species sensitivity of teleosts to cholinergic disruption (Kumar *et al*., 2021). The progressive worsening of behavioral severity scores across exposure durations highlights the utility of behavioral monitoring as a rapid, non-invasive biomarker for pesticide pollution in freshwater ecosystems (Azad & Choudhary, 2026).

Haematological profiling revealed a consistent decline in Hb, RBCcount, and packed cell volume PCV, accompanied by a marked increase in MCV. The reduction in erythrocytic indices suggests dimethoate-induced hemolysis, suppressed erythropoiesis, or altered oxygen-carrying capacity secondary to oxidative damage to red blood cell membranes (Mishra & Mohanty, 2018). Organophosphates are known to generate reactive oxygen species (ROS) that compromise erythrocyte membrane integrity, leading to lipid peroxidation, Heinz body formation, and premature cell clearance by the reticuloendothelial system. The concomitant rise in MCV indicates macrocytic anemia, likely resulting from the premature release of immature, larger erythrocytes into circulation as a compensatory response to anemia, or from impaired hemoglobin synthesis (Dacie & Lewis, 2017). These findings are consistent with reports on *Channa striatus* and *Puntius sophore* exposed to agricultural runoff, where prolonged pesticide exposure disrupted hematopoietic homeostasis (Rahman & Hossain, 2022).

The WBC response exhibited a biphasic pattern, characterized by initial leukocytosis followed by significant leukopenia at 96 h. Early leukocytosis likely represents an acute inflammatory and stress-mediated mobilization of neutrophils and lymphocytes in response to xenobiotic insult (Mishra & Mohanty, 2018). However, sustained exposure to dimethoate appears to overwhelm immune homeostasis, leading to lymphoid depletion, impaired leukopoiesis, and eventual immunosuppression, as evidenced by the significant decline in WBC counts at the highest concentration and longest exposure duration. This immunomodulatory effect compromises disease resistance and aligns with established evidence that organophosphates impair both innate and adaptive immune functions in teleosts (Kumar *et al*., 2021).

The strong negative correlation between behavioral severity scores and Hb/RBC levels, alongside the positive correlation with MCV, highlights the physiological interdependence of neurobehavioral and haematological systems under toxic stress. Hypoxia induced by impaired oxygen transport likely exacerbates neurological dysfunction, while cholinergic disruption may further suppress locomotor and feeding activity. This synergistic impairment underscores the value of multi-endpoint biomonitoring in ecotoxicological risk assessment, as reliance on isolated parameters may underestimate the cumulative ecological impact of sublethal pesticide exposure (Sharma & Kaur, 2020).

Given that *C. punctatus* is a key predatory species in South Asian freshwater ecosystems and an important dietary protein source, the observed sublethal effects of Rogor at concentrations well below the LC_{50} raise significant ecological and public health concerns. Chronic exposure to field-relevant pesticide runoff could impair growth, reproductive success, and predator-prey dynamics, ultimately destabilizing aquatic food webs. Regulatory frameworks should consider integrating sublethal behavioral and haematological endpoints into water quality guidelines, rather than relying solely on acute mortality thresholds, to better reflect real-world ecological risk (Organisation for Economic Co-operation and Development, 2019).

While this study provides robust evidence of dimethoate-induced sublethal toxicity, it is limited by controlled laboratory conditions that may not fully replicate the dynamic physicochemical fluctuations and mixture exposures typical of natural water bodies. Future research should incorporate molecular biomarkers (e.g., AChE activity, oxidative stress enzymes, stress-responsive gene expression), histopathological validation, and chronic/reproductive endpoints to comprehensively assess long-term ecological impacts. Additionally, evaluating mixture toxicity with co-occurring agrochemicals would better simulate field exposure scenarios and inform integrated pesticide management strategies.

The present investigation also demonstrated that exposure of freshwater fish *C. punctatus* to sublethal concentrations of Rogor caused significant behavioral and haematological alterations. The toxic effects were found to increase progressively with increase in concentration and duration of exposure (48, 72, and 96 hrs). The observed changes clearly indicate that Rogor adversely affects the physiological and metabolic activities of the fish.

Haematological parameters are important indicators of fish health and environmental pollution. In the present study, MCH and MCHC showed a progressive decline in fishes exposed to Rogor. The reduction was concentration-dependent and duration-dependent, with maximum decline recorded in fish exposed to 1/2 LC_{50} concentration for 96 hrs (Roy & Munshi, 1989).

The decrease in MCH values indicates reduction in hemoglobin content within erythrocytes, which may be due to impaired hemoglobin synthesis or destruction of red blood cells under pesticide stress. Lower MCH values suggest the development of hypochromic anemia in the exposed fishes. Similar observations have been reported by Mishra and Srivastava (1983) in pesticide-exposed freshwater fishes.

The reduction in MCHC values observed in the present study further confirms deterioration in blood physiology and oxygen-carrying capacity of erythrocytes. Decline in MCHC may result from toxic damage to hematopoietic tissues, osmotic imbalance, and disruption in erythrocyte membrane integrity. The changes observed in the present study are in agreement with the findings of Svoboda *et al*. (2001), who reported significant haematological alterations in fishes exposed to organophosphate pesticides.

The severity of haematological and behavioral changes was greatest in fishes exposed to 1/2 LC_{50} concentration, indicating that higher concentrations of Rogor produce greater physiological stress. The gradual increase in toxic manifestations from 48 hrs to 96 hrs exposure suggests cumulative toxic effects of the pesticide on fish metabolism and blood parameters.

The findings of the present study clearly shows that Rogor has toxic impacts on *C. punctatus* even at sublethal concentrations. Behavioral and haematological parameters proved to be sensitive indicators of pesticide toxicity and may therefore be effectively used in biomonitoring studies of aquatic pollution.

In conclusion, sublethal exposure to Rogor induces progressive neurobehavioral impairment and haematological dysfunction in *C. punctatus*, with effects intensifying in a clear dose- and time-dependent manner. The integration of behavioral observation and haematological profiling offers a sensitive, cost-effective approach for monitoring organophosphate pollution in freshwater ecosystems. These findings advocate for stringent pesticide application practices, improved runoff mitigation, and the adoption of sublethal biomarkers in environmental risk assessment protocols to safeguard aquatic biodiversity and ecosystem services.

Authors contribution:

This work was carried out in collaboration between both authors. First author conducted the whole experiment and analysed the data. Second author designed and prepare the manuscript.

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