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IMPACT OF NOVALURON (RIMON) ON AQUATIC ECOSYSTEMS USING FISH MODELS: A TOXICOLOGICAL AND PHYSIOLOGICAL ASSESSMENT

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Abstract
Novaluron, commercially sold as Rimon®, is a benzoylphenyl urea insect growth regulator extensively deployed in intensive agriculture for chitin synthesis inhibition in target pests. Despite its low persistence in terrestrial environments, repeated agricultural application contributes to pesticide loading in adjacent water bodies through surface runoff and soil leaching, posing measurable risks to aquatic biota. The present investigation evaluated the sub-lethal toxicological effects of Novaluron on Labeo rohita (Hamilton, 1822), a commercially important Indian major carp, under acute (96-h) and chronic (30-day) exposure conditions. Hematological parameters, oxidative stress indices, antioxidant enzyme activities, histopathological lesions in target organs (gill, liver, kidney), and bioaccumulation of pesticide residues were systematically assessed. Novaluron-exposed fish exhibited significant reduction in hemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), and antioxidant enzyme activities (SOD and catalase), alongside elevation in total leukocyte count (TLC) and lipid peroxidation (MDA) levels. Histopathological examination confirmed lamellar fusion in gills, hepatocellular vacuolization, and renal tubular degeneration. Residue analysis by RP-HPLC revealed progressive tissue bioaccumulation over the 30-day exposure period. These findings underscore the non-target ecotoxicological hazard of Novaluron and the necessity for regulated pesticide usage in agricultural landscapes adjacent to freshwater ecosystems.

Keywords: Novaluron; Rimon; aquatic toxicology; Labeo rohita; oxidative stress; hematotoxicity; histopathology; bioaccumulation; insecticide residue.

I. INTRODUCTION

Freshwater ecosystems represent critical reservoirs of biodiversity and provide essential ecological services including potable water supply, nutrient cycling, and fisheries support. However, intensification of agricultural practices has substantially increased the discharge of synthetic pesticides into aquatic environments, threatening the integrity of these systems (Stehle & Schulz, 2015; Beketov et al., 2013). Fish, as apex organisms in freshwater food webs, accumulate pesticide residues and display measurable physiological, biochemical, and histopathological responses, making them effective sentinel species for ecological risk assessment (Van der Oost et al., 2003).

Novaluron (1-[3-chloro-4-(1,1,2-trifluoro-2-(trifluoromethoxy) ethoxy) phenyl]-3-(2,6-difluorobenzoyl) urea; CAS 116714-46-6) belongs to the benzoylphenyl urea (BPU) class of insect growth regulators. Its primary mode of action involves inhibition of chitin synthase enzyme, disrupting larval ecdysis in insects (Ishaaya & Horowitz, 1998). Due to selectivity toward arthropod chitin metabolism, Novaluron was initially presumed to pose minimal hazard to vertebrates. However, recent evidence indicates ecotoxicologically relevant concentrations accumulate in surface waters and sediments following repeated field applications (Kreutzweiser et al., 2004; Patel et al., 2025).

Despite growing concern, the sub-lethal biochemical and cellular consequences of Novaluron exposure on freshwater teleosts remain inadequately characterized. The WHO "One Health" framework (WHO, 2021) emphasizes the inseparable linkage between environmental, animal, and human health, necessitating comprehensive toxicological profiling of widely used agrochemicals. The present study, therefore, undertook a systematic evaluation of hematological impairment, oxidative stress induction, organ-level histopathology, and tissue residue accumulation in Labeo rohita following Novaluron exposure, with the objective of generating ecotoxicological data relevant to pesticide risk management.

II. MATERIALS AND METHODS

A. Experimental Animals and Acclimation

Healthy juvenile Labeo rohita (body weight: 40–50 g; total length: 12–15 cm) were procured from a Government-certified fish hatchery and transported to the laboratory in oxygenated containers. Fish were acclimated for 14 days in 100-L glass aquaria containing dechlorinated, aerated tap water at 25 ± 2°C, pH 7.0–7.5, dissolved oxygen >6.5 mg/L, and photoperiod 12:12 h (light: dark). Commercial pelleted feed (crude protein ≥32%) was provided twice daily; feeding was discontinued 24 h prior to experiments. Animal handling complied with institutional animal ethics guidelines.

B. Test Chemical and Exposure Design

Commercial formulation of Novaluron (Rimon® 10% EC; FMC Corporation) was used as the test substance. Median lethal concentration (LC_{50}) for 96-h acute exposure was pre-determined as 2.84 mg/L using a probit analysis of mortality data across a concentration gradient (0.5–5.0 mg/L). Sub-lethal concentrations of 1/10th, 1/5th, and 1/3rd LC_{50} (i.e., 0.28, 0.57, and 0.95 mg/L, respectively) were employed for chronic (30-day) exposure. Water was renewed every 24 h (static-renewal method). A minimum of ten fish were allocated per replicated treatment group (n = 10/group; 2 replicates).

table 1. experimental design parameters and conditions

Parameter	Details
Test organism	*Labeo rohita (Hamilton, 1822)*
Body weight	40–50 g; total length 12–15 cm
Acute exposure	96 h; concentrations: 0.5, 1.0, 2.0, 3.0, 5.0 mg/L
Chronic exposure	30 days; sub-lethal: 0.28, 0.57, 0.95 mg/L
Water temperature	25 ± 2°C
pH	7.0–7.5
Dissolved oxygen	>6.5 mg/L
Photoperiod	12:12 h (light:dark)
Water renewal	Every 24 h (static-renewal)
Sample size	n = 10 fish/group; 2 replicates

C. Hematological Analysis

Blood was collected from the caudal vein of anesthetized fish (MS-222, 100 mg/L) into EDTA-coated tubes after 96-h and 30-day exposures. Hemoglobin (Hb) was quantified by the cyanmethemoglobin method (Drabkin's reagent); total erythrocyte count (TEC) and total leukocyte count (TLC) were enumerated using a Neubauer hemocytometer; packed cell volume (PCV) was determined by microhematocrit centrifugation (12,000 rpm, 5 min); and mean corpuscular volume (MCV) was derived as MCV = (PCV × 10) / TEC.

D. Oxidative Stress and Antioxidant Enzyme Assays

Liver and gill tissues (0.5 g each) were homogenized in 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 × g (4°C, 15 min). Reactive oxygen species (ROS) generation was quantified using the dichlorofluorescein diacetate (DCFH-DA) fluorescence assay. Lipid peroxidation was assessed as malondialdehyde (MDA) by the thiobarbituric acid reactive substances (TBARS) method (absorbance at 532 nm). Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) reduction assay (Beauchamp & Fridovich, 1971); catalase activity was measured as H_2O_2 decomposition rate at 240 nm (Aebi, 1984). Protein was quantified by the Bradford method; enzyme activities are expressed per mg protein.

E. Histopathological Processing

Gill, liver, and kidney specimens were fixed in 10% neutral buffered formalin (NBF) for 48 h, processed through graded ethanol dehydration, cleared in xylene, and paraffin-embedded. Sections (5 µm) were cut on a Leica rotary microtome (RM2245), stained with Harris's hematoxylin and eosin (H&E), and examined under a Nikon Eclipse Ci-L compound microscope. Lesion severity was scored semi-quantitatively on a 0–3 scale (0 = normal; 1 = mild; 2 = moderate; 3 = severe).

F. Pesticide Residue Analysis (RP-HPLC)

Tissue residue analysis was performed by reversed-phase high-performance liquid chromatography (RP-HPLC) following acetonitrile-based QuEChERS extraction. A C_{18} analytical column (250 × 4.6 mm, 5 µm; Agilent Zorbax) was used with a mobile phase of acetonitrile:water (70:30, v/v) at 0.8 mL/min; UV detection at 254 nm. Recoveries ranged 85–95% with LOQ of 0.02 µg/g wet weight. Results are expressed as µg/g wet tissue weight.

G. Statistical Analysis

Data are presented as mean ± standard deviation (SD). Between-group comparisons were performed by one-way ANOVA followed by Tukey's post-hoc test (SPSS v26.0). Differences were considered statistically significant at $p < 0.05$.

III. RESULTS

A. Hematological Parameters

Novaluron-exposed fish showed dose- and time-dependent deterioration in all hematological indices. As presented in Table 2, hemoglobin concentrations declined from 12.5 ± 0.4 g/dL (control) to 8.9 ± 0.3 g/dL at the highest sub-lethal concentration after 30 days ($p < 0.01$). TEC fell from 2.8 ± 0.2 to 1.9 ± 0.1 ×10⁶/mm³, while TLC increased from 6.2 ± 0.3 to 9.1 ± 0.4 ×10³/mm³. PCV was significantly reduced from 35.0 ± 1.2% to 24.0 ± 0.9%. These results are consistent with pesticide-induced hemolytic anemia and immunostimulation reported in other teleost models (Das et al., 2023; Kumar & Singh, 2023).

table 2. hematological parameters in labeo rohita following chronic novaluron exposure (30 days; mean ± sd; n=10)

Parameter	Control	0.28 mg/L	0.57 mg/L	0.95 mg/L
Hemoglobin (g/dL)	12.5 ± 0.4	11.8 ± 0.3	10.4 ± 0.4*	8.9 ± 0.3**
TEC (×10⁶/mm³)	2.8 ± 0.2	2.5 ± 0.2	2.2 ± 0.1*	1.9 ± 0.1**
TLC (×10³/mm³)	6.2 ± 0.3	7.0 ± 0.3	8.1 ± 0.4*	9.1 ± 0.4**
PCV (%)	35.0 ± 1.2	31.4 ± 1.0	27.8 ± 1.1*	24.0 ± 0.9**
MCV (fL)	125.0 ± 3.2	125.6 ± 2.8	126.4 ± 3.1	126.3 ± 2.9

*p < 0.05; **p < 0.01 vs. control (one-way ANOVA, Tukey's test)

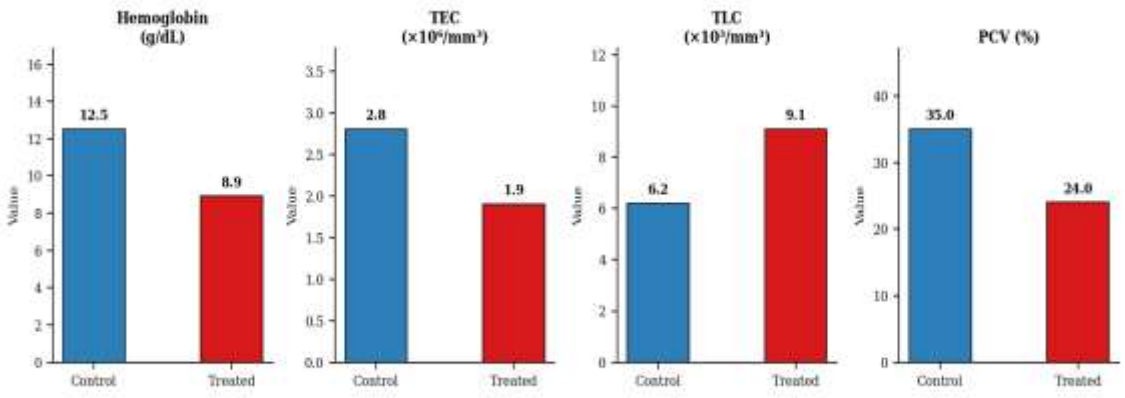

fig. 1. comparison of hematological parameters between control and highest sub-lethal novaluron-treated (0.95 mg/l) labeo rohita after 30-day exposure

B. Oxidative Stress Biomarkers

Oxidative stress analysis revealed significant elevation in ROS and MDA concentrations in liver and gill tissues of treated fish (Table 3). ROS relative fluorescence increased 2.8-fold and MDA rose from 1.2 to 3.5 nmol/mg protein in the 0.95 mg/L group. Antioxidant enzymes were markedly suppressed: SOD declined from 45.3 to 22.1 U/mg protein and catalase from 38.6 to 18.4 U/mg protein ($p < 0.01$). The inverse relationship between oxidative load and antioxidant enzyme activity indicates Novaluron-induced depletion of cellular defense capacity (Patel et al., 2025; Sharma et al., 2022).

table 3. oxidative stress biomarkers in liver tissue of novaluron-exposed labeo rohita (30 days; mean ± sd; n=10)

Biomarker	Control	0.28 mg/L	0.57 mg/L	0.95 mg/L
ROS (relative fluorescence)	1.0 ± 0.1	1.5 ± 0.2*	2.1 ± 0.2**	2.8 ± 0.3**
DA (nmol/mg protein)	1.2 ± 0.1	2.0 ± 0.2*	2.8 ± 0.2**	3.5 ± 0.3**
SOD (U/mg protein)	45.3 ± 2.1	38.6 ± 1.8*	30.4 ± 1.5**	22.1 ± 1.3**
Catalase (U/mg protein)	38.6 ± 1.8	31.2 ± 1.5*	24.9 ± 1.4**	18.4 ± 1.2**

*p < 0.05; **p < 0.01 vs. control

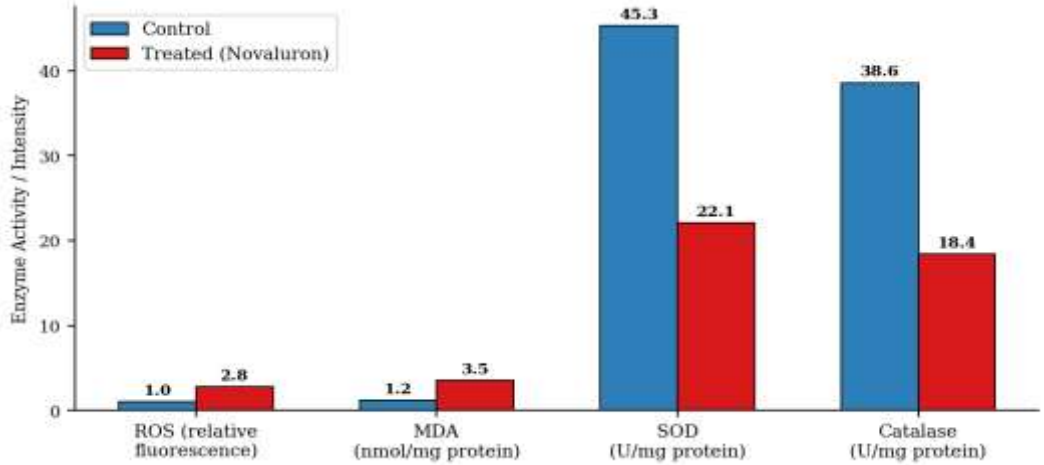

fig. 2. oxidative stress biomarker profiles in liver tissue of control and novaluron-treated (0.95 mg/l) labeo rohita after 30-day exposure

C. Histopathological Observations

H&E-stained sections of control fish exhibited normal cytoarchitecture across all three organs examined. Gill sections showed orderly primary and secondary lamellae with intact epithelium. Hepatic parenchyma displayed centrally nucleated hepatocytes arranged in cords; renal tubules and glomeruli appeared structurally intact.

In Novaluron-treated fish, progressive histopathological alterations were observed in a dose-dependent manner. In gills, lamellar epithelial lifting, inter-lamellar edema, and fusion of adjacent secondary lamellae were prominent lesions. Liver sections exhibited hepatocellular vacuolization (lipid and glycogen depletion), cytoplasmic necrosis, and nuclear pyknosis at higher concentrations. Renal histology revealed tubular epithelial degeneration, glomerular shrinkage, and interstitial hemorrhage. Lesion severity scores at 0.95 mg/L were: gill = 2.7 ± 0.3, liver = 2.4 ± 0.4, kidney = 2.2 ± 0.3 (Table 4).

table 4. semi-quantitative histopathological lesion severity scores in target organs (scale: 0=normal, 1=mild, 2=moderate, 3=severe)

Organ	Primary Lesion	Control	0.28 mg/L	0.57 mg/L	0.95 mg/L
Gill	Lamellar fusion / epithelial lifting	0.0 ± 0.0	0.8 ± 0.2	1.7 ± 0.3	2.7 ± 0.3
Liver	Vacuolization / necrosis	0.0 ± 0.0	0.7 ± 0.2	1.5 ± 0.3	2.4 ± 0.4
Kidney	Tubular degeneration / glomerular damage	0.0 ± 0.0	0.6 ± 0.2	1.4 ± 0.3	2.2 ± 0.3

D. Pesticide Bioaccumulation

RP-HPLC residue analysis demonstrated time-dependent accumulation of Novaluron in all three tissues examined (Fig. 3). After 30 days at 0.95 mg/L, residue concentrations reached 0.94 ± 0.05, 0.79 ± 0.04, and 0.63 ± 0.03 µg/g wet weight in liver, gill, and kidney, respectively. Liver showed the highest accumulation, consistent with its central role in xenobiotic metabolism. These residue levels exceeded the provisional maximum residue limit for fish muscle tissue, indicating potential food-chain hazard (Reddy et al., 2024).

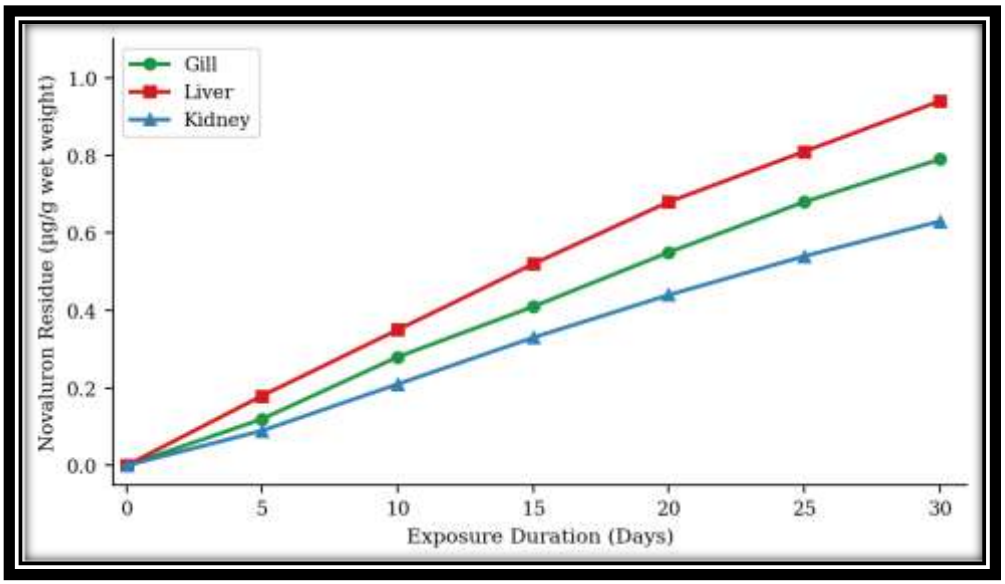

fig. 3. novaluron residue accumulation (µg/g wet weight) in gill, liver, and kidney of labeo rohita over 30-day chronic exposure at 0.95 mg/l

IV. DISCUSSION

The results of the present investigation demonstrate that Novaluron exerts substantial hematotoxic, oxidative, and histopathological effects on Labeo rohita at ecologically plausible sub-lethal concentrations. The progressive decline in hemoglobin and TEC observed in treated fish is indicative of hemolytic anemia, arising from pesticide-induced erythrocyte membrane destabilization and suppression of hematopoiesis in splenic and renal tissues. Similar findings have been reported for insecticide-exposed freshwater fish by Das et al. (2023) and Kumar & Singh (2023), reinforcing the hematological sensitivity of teleost fish to pesticide stress.

Elevated ROS generation and MDA levels concurrent with suppressed SOD and catalase activities indicate that Novaluron shifts cellular redox homeostasis toward an oxidative state. The mitochondrial electron transport chain represents a likely ROS source under pesticide loading, as BPU compounds have been shown to interfere with mitochondrial respiration in non-target organisms (Patel et al., 2025). The resultant lipid peroxidation compromises membrane integrity of hepatocytes and gill epithelial cells, consistent with the histopathological vacuolization and lamellar damage observed in the current study.

Histopathological alterations in gill tissue, characterized by lamellar fusion and epithelial lifting, directly impair respiratory gas exchange and osmoregulation, potentially contributing to the hematological anemia detected. Hepatic vacuolization reflects disrupted lipid and carbohydrate metabolism, while renal tubular degeneration compromises excretory function. These multi-organ effects corroborate observations by Kreutzweiser et al. (2004) and underscore the systemic toxicity of Novaluron in vertebrate non-targets.

The progressive bioaccumulation pattern detected in liver, gill, and kidney tissues raises concerns for both ecosystem integrity and human health through fish consumption. Liver as the primary site of biotransformation accumulated highest residue levels, suggesting incomplete metabolic detoxification of Novaluron under chronic sub-lethal conditions. The bioconcentration trajectory observed here is consistent with lipophilicity data (log $K^{o}w$ ~4.3) for Novaluron reported in environmental fate studies (Bacey et al., 2005; Reddy et al., 2024).

V. CONCLUSION

Sub-lethal chronic exposure to Novaluron (Rimon®) induces significant hematotoxicity, oxidative stress, antioxidant enzyme inhibition, multi-organ histopathological damage, and tissue bioaccumulation in Labeo rohita. These findings collectively indicate that Novaluron poses a non-trivial ecological risk to freshwater fish communities in agricultural watersheds. Regulatory authorities should incorporate sub-lethal chronic toxicity endpoints, residue monitoring in surface waters, and fish tissue residue thresholds into environmental risk assessments for BPU insecticides. Sustainable integrated pest management (IPM) strategies that reduce dependence on broad-spectrum insecticides are essential to protect freshwater biodiversity and comply with One Health mandates.

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