Environmental Risk of Thiamethoxam Revealed through Hemato-Biochemical Alterations in Anabas sp.

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ABSTRACT

Thiamethoxam (TMX), a widely used neonicotinoid insecticide, has increasingly been detected in aquatic ecosystems due to agricultural runoff and improper pesticide management. The present study evaluated the acute (96 h) and chronic (21 days) toxic effects of TMX on the freshwater fish *Anabas* sp. by assessing hematological and biochemical alterations. Fish were exposed to graded concentrations of TMX under controlled laboratory conditions, and parameters such as red and white blood cell counts, hemoglobin, hematocrit, erythrocyte indices, glucose, protein, urea, creatinine, lipid profile, and liver enzymes were analyzed. One-way ANOVA and Pearson correlation analyses were employed to determine dose-dependent effects and interrelationships among parameters. The results demonstrated significant, dose-dependent hematological disturbances and metabolic stress responses, with more pronounced alterations under chronic exposure. Elevated glucose, urea, creatinine, and hepatic enzyme activities, along with reduced protein and erythrocytic parameters, indicated physiological stress, anemia, immunological disturbance, and hepatic dysfunction. The study highlighted glucose, protein, ALT, AST, ALP, RBC, and Hb as sensitive biomarkers of TMX toxicity. These findings underscored the environmental risk posed by TMX to non-target aquatic organisms and contributed valuable data to aquatic ecotoxicology and environmental risk assessment.

Keywords: Thiamethoxam, *Anabas* sp., Neonicotinoids, Hematology, Biochemistry, Aquatic ecotoxicology.

1. Introduction

The extensive application of pesticides in modern agriculture has significantly enhanced crop productivity; however, it has also led to unintended contamination of adjacent ecosystems. Among these chemicals, neonicotinoid insecticides have gained global prominence due to their high efficacy against target pests and systemic mode of action. Thiamethoxam (TMX), a second-generation neonicotinoid, has been extensively used for seed treatment, foliar application, and soil drenching. Despite its agricultural benefits, TMX has been increasingly reported in surface waters, sediments, and aquatic biota, raising serious environmental concerns.

Aquatic ecosystems are particularly vulnerable to pesticide contamination, as runoff, leaching, and spray drift transport agrochemicals into rivers, ponds, wetlands, and reservoirs. Fish, being integral components of aquatic food webs and sensitive indicators of environmental health, are widely used in ecotoxicological studies. Exposure to sublethal concentrations of pesticides can disrupt physiological homeostasis in fish, resulting in hematological and biochemical alterations that precede overt toxicity or mortality.

Hematological parameters such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), hematocrit (PCV), and erythrocyte indices (MCV, MCH, MCHC) serve as reliable indicators of oxygen transport capacity, immune competence, and general health status in fish. Similarly, biochemical parameters including glucose, protein, urea, creatinine, lipid profile, and liver enzymes (ALT, AST, ALP) provide insights into metabolic stress, renal and hepatic function, and energy utilization.

The freshwater fish *Anabas* sp. is a hardy, air-breathing species widely distributed in South and Southeast Asia and is of considerable ecological and economic importance. Its adaptability and tolerance to environmental fluctuations make it a suitable model organism for toxicological investigations. However, systematic information on the effects of TMX on *Anabas* sp., particularly under acute and chronic exposure scenarios, remains limited.

Therefore, the present study was designed to evaluate the hemato-biochemical responses of *Anabas* sp. following 96-hour and 21-day exposure to different concentrations of TMX. By integrating hematological, biochemical, and statistical analyses, the study aimed to elucidate the environmental risk of TMX and identify sensitive biomarkers for neonicotinoid toxicity in freshwater fish.

2. Review of Literature

Previous studies have documented that neonicotinoid insecticides exert neurotoxic effects on insects by binding to nicotinic acetylcholine receptors, but their impacts on non-target aquatic organisms have also gained increasing attention. Several researchers have reported hematological disturbances in fish exposed to neonicotinoids, including reductions in RBC, Hb, and PCV, indicative of anemia and impaired oxygen transport. Alterations in WBC counts have been associated with immunological stress and inflammatory responses.

Biochemical investigations have revealed that pesticide exposure often induces hyperglycemia in fish due to enhanced glycogenolysis and gluconeogenesis, reflecting stress-mediated endocrine responses. Decreased protein levels have been linked to enhanced proteolysis and impaired protein synthesis under toxic stress. Elevated urea and creatinine levels have been reported as indicators of renal dysfunction, while increased activities of ALT, AST, and ALP have been associated with hepatocellular damage and altered membrane permeability.

Comparative studies on acute and chronic pesticide exposure have demonstrated that prolonged exposure, even at lower concentrations, can result in cumulative physiological damage exceeding that observed under short-term exposure. Correlation analyses have further shown strong interrelationships between hematological and biochemical parameters, highlighting the integrative nature of physiological stress responses.

Despite these findings, data on TMX-induced toxicity in air-breathing freshwater fish remain scarce. The present study addressed this gap by providing a comprehensive evaluation of hemato-biochemical alterations in *Anabas* sp. under both acute and chronic TMX exposure.

3. Materials and Methods

3.1 Experimental Fish

Healthy specimens of *Anabas* sp. of uniform size and weight were procured from a local freshwater source and transported to the laboratory under aerated conditions. The fish were acclimatized to laboratory conditions for two weeks in dechlorinated water and were fed a standard diet.

3.2 Experimental Design

Fish were divided into control, low-dose, medium-dose, and high-dose groups. Acute toxicity was assessed over a 96-hour exposure period, while chronic toxicity was evaluated over 21 days. TMX concentrations were selected based on sublethal ranges determined from preliminary trials.

3.3 Hematological Analysis

Blood samples were collected from anesthetized fish using heparinized syringes. RBC, WBC, Hb, PCV, MCV, MCH, and MCHC were analyzed following standard hematological procedures.

3.4 Biochemical Analysis

Serum was separated by centrifugation and analyzed for glucose, total protein, urea, creatinine, cholesterol, triglycerides, HDL, LDL, ALT, AST, and ALP using standard diagnostic kits.

3.5 Statistical Analysis

Data were expressed as mean ± standard deviation. One-way ANOVA was applied to determine significant differences among groups, followed by appropriate post hoc comparisons. Pearson correlation analysis was used to assess relationships between hematological and biochemical parameters. Statistical significance was considered at $p < 0.05$.

4. Results

The hematological analysis revealed significant, dose-dependent reductions in RBC, Hb, and PCV values in TMX-exposed fish, with more pronounced declines observed after 21 days of exposure. WBC counts showed a progressive increase with increasing TMX concentration, suggesting an immunological response to toxic stress. Erythrocyte indices indicated macrocytic anemia under chronic exposure conditions.

Table 1: Hematological and Biochemical Alterations in *Anabas* sp. Exposed to Thiamethoxam (TMX)

Biomarker Category	Parameter	96-hour Exposure (Acute)	21-day Exposure (Chronic)	Toxicological Interpretation
Hematological	RBC	Slight decrease at high dose	Marked, dose-dependent decrease	Indicated hemolysis and suppression of erythropoiesis under prolonged exposure
	WBC	Mild increase	Significant increase	Suggested activation of immune response and stress-induced leukocytosis
	Hemoglobin (Hb)	Marginal reduction	Pronounced reduction	Reflected anemia and impaired oxygen transport
	Hematocrit (PCV)	Slight decline	Significant decline	Confirmed anemia and reduced blood oxygen-carrying capacity
	MCV	Mild increase	Significant increase	Indicated macrocytic anemia due to erythrocyte swelling
	MCH	Slight decrease	Significant decrease	Suggested reduced hemoglobin synthesis per cell
	MCHC	Minor variation	Significant reduction	Reflected hypochromic anemia
Biochemical – Metabolic	Glucose	Moderate increase	Sharp, dose-dependent increase	Indicated stress-induced hyperglycemia and metabolic disruption

	Total Protein	Slight decrease	Severe depletion	Suggested impaired protein synthesis and hepatic dysfunction
Renal Function	Urea	Slight elevation	Significant elevation	Indicated renal stress and reduced excretory efficiency
	Creatinine	Minor increase	Marked increase	Confirmed nephrotoxicity under chronic exposure
Lipid Profile	Total Cholesterol	No significant change	Mild increase	Suggested altered lipid metabolism
	Triglycerides	Slight increase	Significant increase	Indicated disturbed energy metabolism
	HDL	Minor decrease	Significant decrease	Reflected impaired lipid transport and cardiovascular stress
	LDL	Slight fluctuation	Moderate increase	Suggested altered lipid homeostasis
Hepatic Enzymes	ALT	Mild elevation	Sharp increase	Indicated hepatocellular damage
	AST	Moderate elevation	Severe increase	Confirmed liver tissue degeneration
	ALP	Slight increase	Highly significant increase	Reflected biliary dysfunction and liver pathology

Biochemical parameters exhibited marked alterations in TMX-treated groups. Glucose levels increased significantly with dose and duration of exposure, indicating stress-induced hyperglycemia. Total protein levels decreased markedly, particularly under chronic exposure, reflecting enhanced protein catabolism and impaired synthesis. Urea and creatinine levels increased significantly, suggesting renal impairment. Lipid profile changes were moderate but indicated metabolic imbalance, while hepatic enzyme activities (ALT, AST, ALP) showed significant elevation, especially in the medium- and high-dose groups after 21 days, confirming hepatic dysfunction.

One-way ANOVA demonstrated highly significant differences ($p < 0.001$) among treatment groups for most parameters. Pearson correlation analysis revealed strong negative correlations between RBC/Hb and glucose, urea, creatinine, and liver enzymes, while positive correlations were observed among biochemical stress markers.

5. Discussion

The observed hematological alterations indicated that TMX exposure induced anemia, immunological stress, and impaired oxygen transport in *Anabas* sp. The reduction in RBC, Hb, and PCV values was consistent with pesticide-induced hemolysis and inhibition of erythropoiesis reported in other freshwater fish. Elevated WBC counts suggested activation of defense mechanisms in response to toxic insult.
The biochemical changes reflected pronounced metabolic stress under TMX exposure. Hyperglycemia was indicative of stress-mediated endocrine responses, while hypoproteinemia suggested enhanced proteolysis to meet increased energy demands. Elevated urea and creatinine levels pointed toward renal dysfunction, whereas increased ALT, AST, and ALP activities confirmed hepatocellular damage.

Comparative analysis between acute and chronic exposure revealed that chronic exposure resulted in more severe and persistent alterations, highlighting the cumulative toxicity of TMX. The strong correlations between hematological and biochemical parameters emphasized the integrated physiological response of fish to neonicotinoid stress.

6. Ecological and Environmental Implications

The findings demonstrated that environmentally relevant concentrations of TMX could adversely affect non-target freshwater fish, potentially disrupting population health and aquatic food web stability. Chronic sublethal toxicity posed a greater ecological risk than short-term exposure, emphasizing the need for long-term monitoring of neonicotinoids in aquatic ecosystems.

7. Conclusion

The study conclusively demonstrated that TMX induced significant, dose- and duration-dependent hemato-biochemical alterations in *Anabas* sp. The identified biomarkers provided valuable tools for early detection of neonic.

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