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Research Article

Study on the Effect of Pesticide Thiamethoxam 25% WG on Some Behavioural, Haematological, Biochemical and Histopathological Aspects of the Fish *Oreochromis niloticus*

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ABSTRACT

The widespread use of pesticides like Thiamethoxam 25% WG poses serious ecological threats to aquatic ecosystems, affecting non-target species such as *Oreochromis niloticus*. This study evaluated the acute and sublethal toxicity of Thiamethoxam, with a 24-hour LC₅₀ determined at 1 ppm. Fish exposed to a sublethal dose (0.1 ppm) exhibited behavioural changes (erratic swimming, increased opercular movement), significant decreases in haemoglobin and RBC counts, and increased WBC counts, indicating physiological stress. Biochemical parameters like carbohydrates, proteins, and lipids also declined significantly, especially after 48 hours. Histopathological analysis revealed severe gill damage, including epithelial lifting and lamellar sloughing. These findings underline the toxic effects of Thiamethoxam and highlight *O. niloticus* as a suitable bioindicator for aquatic pollution monitoring.

INTRODUCTION

Pesticides have become indispensable in modern agriculture for pest control and crop protection. However, their excessive and indiscriminate use has led to serious environmental consequences, especially in aquatic ecosystems, which act as sinks for agricultural runoff (Glashan and Hughies, 2001). Among these ecosystems, freshwater habitats are particularly vulnerable, as

pesticides entering water bodies through rainwater, irrigation runoff, or accidental spills can have long-term deleterious effects on aquatic organisms. Fish, being highly sensitive to pollutants and integral to aquatic food webs, are often used as bioindicators for environmental monitoring (Sasaki *et al.*, 1997).

Thiamethoxam, a second-generation neonicotinoid insecticide, is widely used in

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agriculture due to its systemic action and effectiveness against a broad spectrum of insect pests. Despite its agricultural benefits, recent studies have raised concerns about its ecotoxicological effects on non-target aquatic organisms. Neonicotinoids, including Thiamethoxam, act on nicotinic acetylcholine receptors (nAChRs) in the central nervous system, potentially disrupting neurophysiological processes in fish (Gibbons *et al.*, 2015). The toxic impact of Thiamethoxam on fish can manifest through changes in behaviour, blood composition, metabolic function, and tissue architecture, thereby affecting the overall health and survival of aquatic organisms (Kumari *et al.*, 2024).

Oreochromis niloticus (Nile tilapia), a widely cultured freshwater species, is frequently employed in ecotoxicological research due to its ecological and economic significance, ease of handling, and physiological sensitivity to pollutants. The present study was undertaken to investigate the acute and sublethal effects of Thiamethoxam 25% WG on *O. niloticus* by evaluating behavioural responses, haematological indices, biochemical markers, and histopathological alterations, particularly in the gills. This integrative approach provides a comprehensive understanding of pesticide toxicity and underscores the need for responsible pesticide management to protect aquatic life and ecosystem integrity (Ghaffar *et al.*, 2021; Selvi and Ilavazhahan, 2012).

MATERIALS AND METHODS

Toxicological studies play a vital role in evaluating the health risks posed by pesticides through the analysis of dose-response relationships, target organ effects, and both acute and chronic toxicities. In this study, *Oreochromis niloticus* (Nile tilapia), a hardy, fast-growing freshwater species widely used in toxicological

research, was selected due to its sensitivity to pollutants and ability to serve as a reliable bioindicator (Popma *et al.*, 1999; Sattanathan *et al.*, 2019). The fish were exposed to Thiamethoxam 25% WG, a neonicotinoid insecticide, to assess changes in behavioural, haematological, biochemical, and histopathological parameters. Acute toxicity tests determined a 24h LC₅₀ of 1.0 ppm, and sublethal exposure at 0.1 ppm was conducted for four days. Haematological assessments included haemoglobin concentration, red and white blood cell counts, while biochemical analyses involved measuring total carbohydrates, proteins, and lipids (Wintrobe, 1967; Sahil, 1962; Lowry *et al.*, 1951).

The test fish were maintained under controlled laboratory conditions and acclimated before exposure. Blood samples were collected post-exposure for physiological analysis, providing insights into the systemic effects of the pesticide. Thiamethoxam, acting on insect nicotinic receptors, is widely used in agriculture and poses ecological risks due to its persistence and systemic action (Boss *et al.*, 2011). Statistical analysis was performed using standard methods (Gupta, 1995) to identify significant deviations in haematological and biochemical markers compared to the control group. These biomarkers serve as sensitive indicators of pesticide-induced stress in fish, offering a valuable tool for environmental monitoring (Tavares-Dias and Moraes, 2007).

RESULTS

Toxicological bioassays were conducted to assess the acute and sublethal effects of Thiamethoxam 25% WG on *Oreochromis niloticus*. Pesticide contamination, especially from agricultural runoff, is a major threat to aquatic life, causing bioaccumulation and disruption of aquatic ecosystems (Glashan and Hughies, 2001; Sasaki *et al.*, 1997). The 24-hour LC₅₀ value of



Thiamethoxam was calculated as 1.0 ppm. Fish were then exposed to a sublethal concentration (0.1 ppm) for 24 and 48 hours. Behavioural abnormalities such as erratic swimming and increased opercular movement were noted, indicating acute stress responses in the exposed fish.

Haematological analysis showed a significant reduction ($p < 0.05$) in haemoglobin and RBC counts and a significant increase in WBC counts post-exposure, with more pronounced effects at 48 hours. Haemoglobin decreased by 34.26% and 39.41% at 24 and 48 hours, respectively; RBC declined by 20.75% and 21.91%; WBC increased by 6.85% and 17.91%, indicating immune response and possible anaemia (Ghaffar *et al.*, 2021; Kumari *et al.*, 2024).

Biochemical changes also reflected physiological stress: carbohydrate, protein, and lipid levels showed significant depletion with longer exposure durations, suggesting impaired energy metabolism and tissue damage.

Histopathological examinations revealed marked gill alterations including epithelial lifting, cartilage disruption, lamellar sloughing, and vacuolation—especially prominent in the 48-hour group. The gills, being the primary contact organ with waterborne toxicants, serve as sensitive indicators of aquatic pollution (Selvi and Ilavazhahan, 2012). Statistical analyses confirmed the significance of these alterations ($p < 0.05$) using Student's t-test and Pearson's correlation (SAS, 1988), reinforcing the toxic potential of Thiamethoxam even at sublethal levels.

DISCUSSION

The present study demonstrates that Thiamethoxam 25% WG, even at sublethal

concentrations, induces significant behavioural, haematological, biochemical, and histopathological alterations in *Oreochromis niloticus*. Behavioural changes such as erratic swimming and increased opercular movements observed in exposed fish are early warning indicators of stress due to pesticide exposure, consistent with findings from previous studies on organophosphates and neonicotinoids (Sasaki *et al.*, 1997). Haematological parameters such as haemoglobin, RBC, and WBC serve as crucial biomarkers of physiological disruption. The observed reduction in haemoglobin and RBC counts suggests anaemia and impaired oxygen transport, while elevated WBC counts reflect an immune response to pesticide-induced stress, aligning with earlier toxicological assessments on freshwater fish (Ghaffar *et al.*, 2021; Kumari *et al.*, 2024).

Biochemical parameters further confirmed the physiological distress, with significant reductions in total carbohydrate, protein, and lipid content in exposed fish. These reductions may be attributed to increased energy demands and the breakdown of biomolecules to cope with pesticide-induced metabolic stress (Kumari *et al.*, 2024). Histopathological analysis revealed gill tissue damage, including epithelial lifting and lamellar disruption, which are known indicators of toxicant-induced respiratory dysfunction (Selvi and Ilavazhahan, 2012). As gills are the primary contact site for waterborne toxicants, such changes underscore the ecological implications of Thiamethoxam contamination in aquatic ecosystems. The study's statistical findings reinforce the reliability of using haematological and histological markers in fish as sensitive bioindicators for pesticide toxicity (SAS Institute, 1988; Glashan and Hughies, 2001).



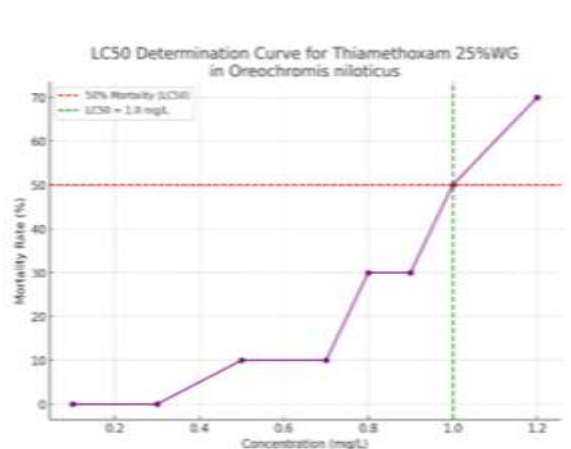


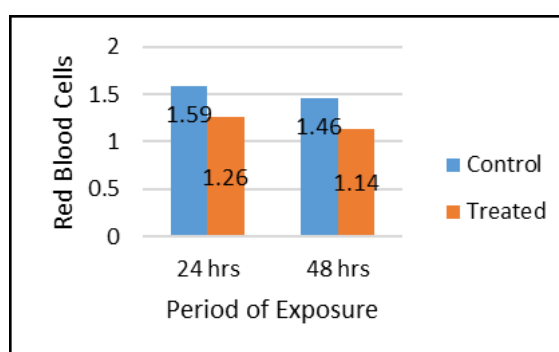
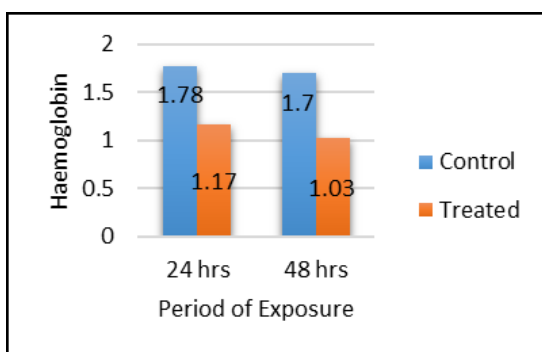
Figure.1 Showing LC₅₀ graph for the toxicant Thiamethoxam 25% WG tested on *Oreochromis niloticus*

Table.2 Changes in the Histopathological characteristics (Haemoglobin, RBC,WBC)in the blood of the fish *Oreochromis niloticus* exposed to different period of Sublethal Toxicity

Days of Exposure	Haemoglobin(Hb)		Red Blood Cells		White Blood cells	
	Control	Treated	Control	Treated	Control	Treated
24 hr	1.78±0.04	1.17±0.08	1.59±0.03	1.26±0.01	1762±4.45	1883±3.16
% Change t-test	-34.26% 66.82**		-20.75% 43.86**		6.86% -113.7**	
48 hr	1.70±0.02	1.03±0.03	1.46±0.06	1.14±0.05	1686±3.90	1988±2.03
% Change 't' value	-39.41% 74.49**		-21.91% 29.62**		17.91% -374.23**	

Table.3 Changes in the Biochemical characteristics (Carbohydrate,Protein,Lipid)in the blood of the fish *Oreochromis niloticus* exposed to different period of Sublethal Toxicity

Days of exposure	Carbohydrate		Protein		Lipid	
	Control	Treated	Control	Treated	Control	Treated
24 hr	21.45 ±0.2	17.72 ±0.08	2.58 ±0.04	2.04 ±0.007	31.32 ±0.08	29.52 ±0.08
% Change 't' value	-17.38% 20.98**		-20.93% 58.05**		-5.74% 180.0**	
48 hrs	20.02±1.3	14.88±0.03	2.45±0.06	1.96±0.05	31.0±0.07	28.03±0.12
% Change 't' value	-25.67% 253.54**		- 20% 52.03**		-9.58% 266.09**	



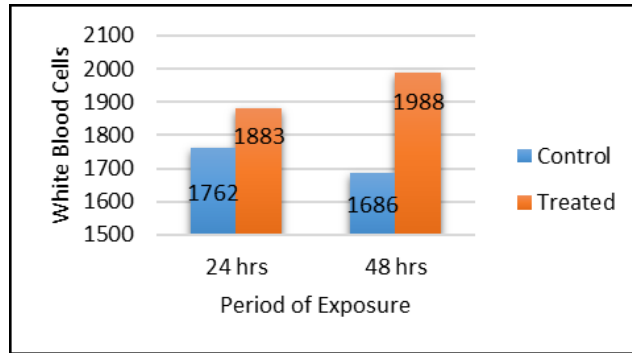


Figure.2 Showing the changes in the Haematological characteristics (Haemoglobin, RBC,WBC) in the blood of the fish *Oreochromis niloticus* exposed to different period of Sublethal Toxicity

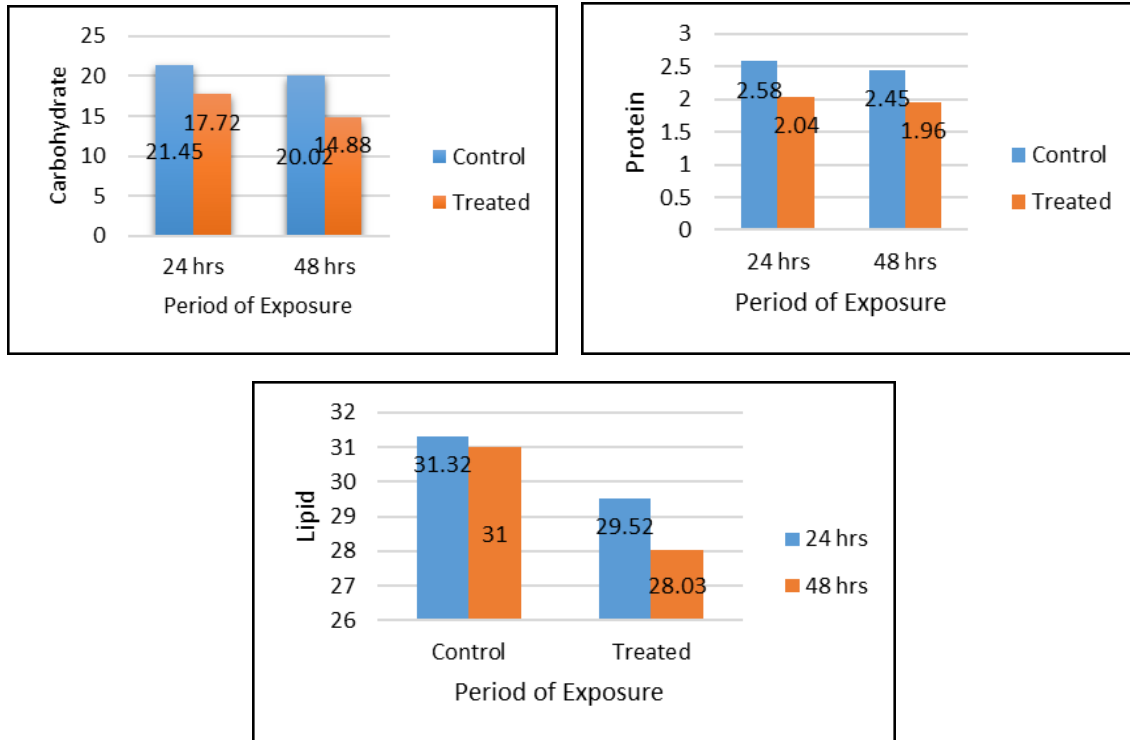
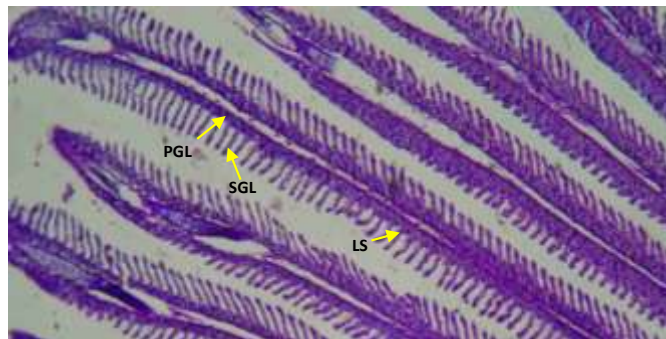
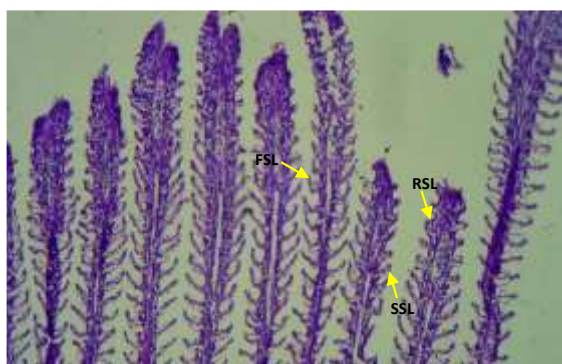


Figure.3 Showing the changes in the Biochemical characteristics (Carbohydrate, Protein, Lipid) in the blood of the fish *Oreochromis niloticus* exposed to different period of Sublethal Toxicity

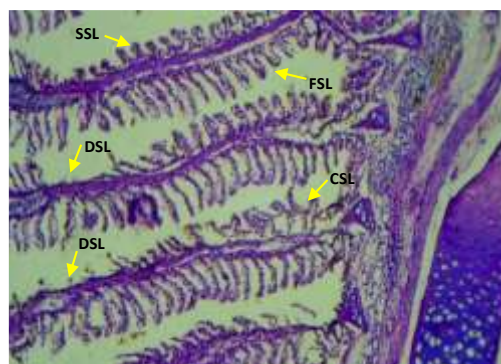
Plate.1: Effect of Pesticide Thiamethoxam on Histology of Gill in *Oreochromis niloticus* in different period of Exposure



CONTROL



24 HOURS OF EXPOSURE



48 HOURS OF EXPOSURE

- EC : Erythrocytes
- LS : Lamellar space
- PGL : Primary gill lamellae
- SGL : Secondary gill lamellae
- FSL : Fusion of secondary lamellae
- RSL : Reduction of secondary lamellae
- SSL : Shortening of secondary lamellae
- DSL : Destruction of secondary lamellae
- CSL : Curling of Secondary lamellae
- DC : Destruction of cartilage core

CONCLUSION

The present study assessed the toxicological effects of Thiamethoxam on *Oreochromis niloticus* by evaluating behavioral, haematological, biochemical, and histopathological responses. A 24-hour LC₅₀ value of 1 ppm was determined, and sublethal exposure at 0.1 ppm led to notable behavioral abnormalities such as erratic swimming and increased opercular activity, indicating stress. Haematological parameters showed significant reductions in haemoglobin and RBC counts and an increase in WBC count, reflecting immune activation. Biochemical analysis revealed marked depletion of carbohydrates, proteins, and lipids, suggesting metabolic disruption. Histopathological examination of gills showed progressive damage, including epithelial lifting, lamellar sloughing, and cartilage disruption, especially after 48 hours, indicating impaired respiratory function. These

findings highlight the physiological and structural impacts of Thiamethoxam on fish and underscore its potential ecological threat to aquatic ecosystems.

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