

Protective Role of Probiotics and *Spirulina* in Modulating Immune and Antioxidant Genes Expression Against Sumithion Toxicity in Nile Tilapia (*Oreochromis niloticus*)

Esrat Zahan Etti

Roll No: 0124/04 Registration No: 1483 Session: 2023-2024

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Fish Biology and Biotechnology

> Department of Fish Biology and Biotechnology Faculty of Fisheries Chattogram Veterinary and Animal Sciences University Chattogram-4225, Bangladesh

> > **JUNE 2025**

Authorization

I hereby declare that I am the sole author of the thesis. I also authorize the Chattogram Veterinary and Animal Sciences University (CVASU) to lend this thesis to other institutions or individuals for the purpose of scholarly research. I further authorize the CVASU to reproduce the thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

I, the undersigned, and author of this work, declare that the **electronic copy** of this thesis provided to the CVASU Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

The author June 2025 Protective Role of Probiotics and *Spirulina* in Modulating Immune and Antioxidant Genes Expression Against Sumithion Toxicity in Nile Tilapia (*Oreochromis niloticus*)

Roll No: 0124/04

Registration No: 1483

Session: 2023-2024

This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

Dr. Md. Mahiuddin Zahangir

Dr. Helena Khatoon

Supervisor

Co-Supervisor

Dr. Md. Mahiuddin Zahangir

Chairman of the Examination Committee

Department of Fish Biology and Biotechnology Faculty of Fisheries Chattogram Veterinary and Animal Sciences University Chattogram-4225, Bangladesh

JUNE 2025

ACKNOWLEDGEMENTS

All thanks belong to Allah, who has blessed me with the strength, ability, and patience to continue postgraduate education and complete the thesis for the Masters of Science (MS) degree in Fish Biology and Biotechnology.

I would really like to express my sincere gratitude, tremendous respect, and enormous indebtedness to my honorable teacher and research supervisor, **Dr. Md. Mahiuddin Zahangir**, Associate Professor and Head, Department of Fish Biology and Biotechnology, CVASU, for providing me with the opportunity to conduct research and for providing invaluable guidance and continuous support throughout this research. His passion, vision, integrity, and motivation have left an indelible impression on me. Working and studying under his direction was a wonderful honor and privilege. I owe him a huge debt of gratitude, as well as admiration for his cordial collaboration, sensitivity, and amazing sense of humor.

I also sincerely express my gratitude to my co-supervisor, **Dr. Helena Khatoon**, Associate Professor, Department of Aquaculture, Chattogram Veterinary and Animal Sciences University, Chattogram for her valuable guidance to teach me to be more confident person. I am also deeply grateful to **Dr. Md. Shahjahan**, Professor, Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University (BAU), and the members of his **Fish Ecophysiology Laboratory**, for their generous support, insightful guidance, and technical assistance, all of which significantly enriched my research experience.

I express my thanks to Md. Moudud Islam, Associate Professor and Fatema Akhter, Assistant Professor, Md. Main Uddin Mamun, Assistant Professor, Shifat Ara Noor, Lecturer, Azmaien Naziat, Lecturer, Department of Fish Biology and Biotechnology, Chattogram Veterinary and Animal Sciences University, Chattogram for their valuable suggestions during the research work. I would like to convey my heartfelt gratitude to lab members of Department of Fish Biology and Biotechnology for their tireless efforts throughout the research time. Finally, my sincere gratitude and respect to my loving parents (Md. Saiful Islam and Zulekha Begum) for their unwavering support, inspiration, blessings, forbearance, and unending love in helping me finish my study.

The Author

June 2025

SI. NO.	TITLE	PAGE NO.
	Authorization Page	ii
	Signature Page	iii
	Acknowledgements	iv
	Table of Content	V-X
	List of Figures	vii-viii
	List of Plates	ix
	List of Tables	X
	Abstract	xi
CHAPTER 1	INTRODUCTION	1–5
	Objectives	5
CHAPTER 2	REVIEW OF LITERATURE	6-17
	2.1 Sumithion	7
	2.1.1 Sumithion toxicity in fish	7-10
	2.2 Interventions in reducing toxicity in fish	10
	2.3 Spirulina platensis	10-11
	2.3.1 <i>Spirulina platensis</i> as a dietary supplement in fish	11-12
	2.4 Probiotics	12-13
	2.4.1 Application of probiotics in aquaculture	13–14
	2.5 Cytokines regulation under environmental stress	14-15
	2.6 Nile Tilapia (Oreochromis niloticus)	15-16
	2.7 Hematological profile in Nile Tilapia (Oreochromis niloticus)	16

Table of Content

	2.8 Research gap	17
CHAPTER 3	MATERIALS AND METHODS	18-26
	3.1 Experimental fish: collection and	19
	husbandry	
	3.2 Experimental design	19–20
	3.3 Sample collection	21
	3.4 Blood glucose and hemoglobin measurement	21
	3.5 Extraction of RNA	21-22
	3.6 Synthesis of complementary DNA (cDNA)	23
	3.7 Real-Time PCR analysis of <i>SOD</i> , <i>CAT</i> , <i>IFN-</i> γ , <i>IL-1</i> β , and <i>TNF-</i> α mRNAs	23-25
	3.8 Statistical analysis	25-26
CHAPTER 4	RESULTS	27-37
	4.1 Expression of antioxidant-related genes	28-29
	4.2 Expression of immune-response related genes	29-32
	4.3 Modulation in hemato-biochemical parameters	32-34
	4.4 Principal component analysis (PCA)	34-35
	4.5 Hierarchical clustering and heatmap analysis	36-37
CHAPTER 5	DISCUSSION	38-42
CHAPTER 6	CONCLUSIONS	43-44
CHAPTER 7	RECOMMENDATIONS AND FUTURE PROSPECTS	45-46

Figure No	Title	Page No
1	Design for experiment. The fish were exposed to four	20
	different treatments: control, sumithion (0.50 mg/L),	
	sumithion (0.50 mg/L) combined with probiotics (1.0	
	ml/L), and sumithion (0.50 mg/L) combined with	
	Spirulina (50 g/kg feed). Hb, hemoglobin; Glu, glucose;	
	SOD, superoxide dismutase; CAT, catalase; TNF α ,	
	tumor necrosis factor alpha; $IL-I\beta$, interleukin 1 beta;	
	and <i>IFN-γ</i> , interferon gamma.	
2	Relative expression of the anti-oxidant gene (superoxide	28
	dismutase, SOD) in Oreochromis niloticus after 42 days	
	of rearing under various treatment conditions: control;	
	sumithion (Su); sumithion with probiotics $(Su + Pr)$; and	
	sumithion with Spirulina (Su + Sp).	
3	Relative expression of the anti-oxidant gene (Catalase,	29
	CAT) in Oreochromis niloticus after 42 days of rearing	
	under various treatment conditions: control; sumithion	
	(Su); sumithion with probiotics $(Su + Pr)$; and sumithion	
	with <i>Spirulina</i> (Su + Sp).	
4	Relative expression of the immune-related gene (tumor	30
	necrosis factor-alpha, $TNF-\alpha$) in Oreochromis niloticus	
	after 42 days of rearing under various treatment	
	conditions: control; sumithion (Su); sumithion with	
	probiotics (Su + Pr); and sumithion with <i>Spirulina</i> (Su +	
	Sp).	
5	Relative expression of the immune-related gene	31
	(interleukin-1 beta, $IL-I\beta$) in Oreochromis niloticus	
	after 42 days of rearing under various treatment	
	conditions: control; sumithion (Su); sumithion with	
	probiotics (Su + Pr); and sumithion with Spirulina (Su +	
	Sp).	

List of Figures

- 6 Relative expression of the immune-related gene 32 (interferon-gamma, *IFN-γ*) in *Oreochromis niloticus* after 42 days of rearing under various treatment conditions: control; sumithion (Su); sumithion with probiotics (Su + Pr); and sumithion with *Spirulina* (Su + Sp).
 - Blood glucose (Glu) concentrations in *Oreochromis* 33
 niloticus after 42 days of rearing under various treatment
 conditions: control; sumithion (Su); sumithion with
 probiotics (Su + Pr); and sumithion with *Spirulina* (Su + Sp).
 - 8 Hemoglobin (Hb) concentrations in *Oreochromis* 34 *niloticus* after 42 days of rearing under various treatment conditions: control; sumithion (Su); sumithion with probiotics (Su + Pr); and sumithion with *Spirulina* (Su + Sp).
 - 9 Principal Component Analysis (PCA) biplot shows the 35 distribution of experimental groups (control, sumithion, sumithion + *Spirulina*, sumithion + probiotics) based on growth parameters, hematology, growth genes, antioxidant genes, and immune genes of experimental units and loading vectors.
 - 10Heatmap of Z-score normalized representation of
growth parameters, hematological, antioxidant, immune
and growth genes expression in *Oreochromis niloticus*
exposed to control, sumithion (Su), sumithion +
probiotic (Su+Pr), and sumithion + *Spirulina* (Su+Sp).

Plate No.	Title	Page No.
1	Experimental fish (Nile tilapia, Oreochromis niloticus)	19
2	Final sampling	21
3	Isolation and quantification of RNA	22
4	RT-PCR analysis	24

List of Plates

Table No.	Title	Page No.
1	Equipment and reagents used for RNA	23
	isolation	
2	List of primers used in the present study	25

List of Tables

ABSTRACT

Pesticide pollution in aquatic ecosystems is intensifying, and organophosphate like sumithion causes major threats to non-target species such as Nile tilapia (Oreochromis niloticus). This study systematically evaluates the efficiency of dietary supplementation with probiotics and Spirulina platensis to ameliorate sumithion-induced oxidative stress, immunosuppression, and hematological impairments in Nile tilapia (Oreochromis niloticus). A total of 320 O. niloticus fingerlings (12.8 ± 0.09 g) were randomly selected for four groups: control, sumithion (0.50 mg/L), sumithion supplemented with probiotics (0.50 mg/L and 1 mL/L) and sumithion supplemented with Spirulina (0.50 mg/L and 50 g/kg), each with three replicates for 6 weeks were investigated. During the experiment, blood samples were tested for hemoglobin (Hb) and glucose (Glu) concentration and at the same time liver tissues were collected for qRT-PCR analysis to evaluate the expression of anti-oxidant (superoxide dismutase, SOD and catalase, CAT) and immune (tumor necrosis factor alpha, TNF- α , interleukin beta, IL-1 β and interferon gamma, IFN- γ) genes. It was reported that exposure to sumithion caused significant (p < 0.05) physiological stress, evident in elevated oxidative (SOD and CAT) and metabolic markers, decreased hemoglobin and hyperglycemia, and downregulation of immune-related gene expression (*TNF-a*, *IL-1* β , and IFN-y). Supplementation with probiotics and Spirulina to these stressed fish improved such alterations with recovered antioxidant and immune gene expression toward homeostatic levels. Probiotics proved to exert much stronger effects on immune recovery and glucose (Glu) amelioration, while Spirulina had an imperative impact on antioxidant stabilization and hematological parameters (Hb). This research findings emphasizes to highlight therapeutic potential of functional feed additives as ecofriendly strategies to counteract pesticide-induced toxicity and demonstrate their applicability in sustainable aquaculture production systems.

Keywords: Sumithion, *Spirulina platensis*, Probiotics, Anti-oxidant genes, Immunity, Hematology, Nile Tilapia