

Probiotics Counteract Growth Impairment, Intestinal Deformities, Cellular and Nuclear Abnormalities, and Fluctuation of Immune-Related Genes Expression in Nile Tilapia (*Oreochromis niloticus*)

Atika Anjum

Roll No: 0124/03 Registration No: 1482 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 (CVASU), 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. Furthermore, I authorize CVASU for the unrestricted right to reproduce this thesis, whether in whole or in part, by means of photocopying or any other suitable method, in response to requests from academic institutions or individuals, solely 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.

Atika Anjum June 2025

Probiotics Counteract Growth Impairment, Intestinal Deformities, Cellular and Nuclear Abnormalities, and Fluctuation of Immune-Related Genes Expression in Nile Tilapia (*Oreochromis niloticus*)

Atika Anjum

Roll No: 0124/03 Registration No: 1482 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 Supervisor Dr. Subrata Kumar Ghosh 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 (CVASU) Chattogram-4225, Bangladesh

June 2025

ACKNOWLEDGMENT

All praise is due to **Almighty Allah**, the Most Gracious, the Most Merciful, for blessing me with the strength, patience, and perseverance needed to complete the thesis for the Master of Science (MS) degree in Fish Biology and Biotechnology. The boundless mercy and guidance have been my constant companions throughout this journey. Without His divine support and endless blessings, none of this would have been possible.

I am delighted to express my deepest gratitude, immense respect and profound indebtedness to my honorable and respected supervisor, **Dr. Md. Mahiuddin Zahangir**, Associate Professor and Head, Department of Fish Biology and Biotechnology, Chattogram Veterinary and Animal Sciences University (CVASU), for his invaluable guidance, unwavering support, and continuous encouragement throughout research. His insightful feedback, thoughtful suggestions, and constructive criticism have significantly enriched the quality of this thesis. His passion, vision, integrity and motivation have left an indelible impression on me. Working and studying under his supervision and direction was a privilege and honor. I owe him a huge debt of gratitude and admiration for his cordial collaboration, and scholastic guidance.

My sincere thanks also go to my **co-supervisors**, **Dr. Subrata Kumar Ghosh**, Associate Professor, Department of Fishing and Post-Harvest Technology, CVASU, whose expertise and thoughtful suggestions greatly enriched the quality of my work. His constant cooperation and mentorship have been a source of inspiration.

I would also like to extend my heartfelt appreciation to **Mrs. Shifat Ara Noor** and **Mrs. Azmaien Naziat**, Lecturer, Department of Fish Biology and Biotechnology, CVASU for their valuable suggestion, cordial support, and constructive criticism in enhancing the effectiveness of my research.

I would like to convey my sincere gratitude to my department mates, lab assistants at the Department of Fish Biology and Biotechnology, for their effort throughout the research time.

Throughout my education at Chattogram Veterinary and Animal Sciences University, I am grateful for the support, cooperation, and direction from my respected teachers of the Faculty of Fisheries. Last but not the least, I am forever indebted to my beloved parents **Mohammed Jashim Uddin** and **Shaheen Akter** and my younger brother, **Shafin Uddin** and other family member for their blessings, continuous support and encouragement and immense sacrifices that has been a source of my motivation, inspiration for being the better version of myself.

The author

June 2025

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE NO.
	AUTHORIZATION	Π
	SIGNATURE PAGE	III
	ACKNOWLEDGEMENT	IV-V
	TABLE OF CONTENTS	VI–VIII
	LIST OF PLATES	IX
	LIST OF FIGURES	Х
	LIST OF TABLES	XI
	ABSTRACT	XII
01.	INTRODUCTION	01-07
	1.1. Background	02-07
	1.2 Objectives of the study	07
02.	REVIEW OF LITERATURE	08–20
	2.1 Sumithion	09-10
	2.2 Probiotics	10-11
	2.2.1 Probiotics in Nile tilapia	10-11
	2.3 Growth hormone	11-13
	2.3.1 History of growth hormone	11
	2.3.2 Growth hormone structures	11-12
	2.3.3 Effects of sumithion on growth hormone	12-13
	2.4 Cytokines	13-14
	2.4.1 Effects of sumithion on cytokines	13
	2.4.2 Effects of probiotics on cytokines	13-14
	2.5 Antioxidant	14-15
	2.5.1 Effects of probiotics on antioxidant and stress-related	14-15
	genes	
	2.6 Effects of sumithion and probiotics on blood parameters and the gastrointestinal tract	15
	2.6.1 Effects of sumithion on blood parameters and the gastrointestinal tract	15

gastrointestinal tract2.7 Goblet cell162.8 Impact of sumithion on intestinal morphology162.9 Impact of sumithion on immunity16-172.10 Investigations on mitigating pesticide toxicity17-182.11 Nile tilapia18-192.12 Research gap19-2003.MATERIALS AND METHODS21-303.1 Experimental fish3.1 Experimental fish223.2 Experimental design22-233.3 Sampling and data collection233.4 Growth performance of Nile tilapia23-243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25-263.8 Extraction and quantification of RNA26-273.9 Preparation for cDNA283.10 Real-time PCR assays3004.RESULTS31-414.1 Growth performance of Oreochromis niloticus324.2 Changes in intestinal morphology35-374.3 Erythrocytic nuclear and cellular abnormalities34-354.4 Changes in intestinal morphology35-374.5 Expression of growth-related genes (<i>gh. igf-1, igf-2</i>) in in the liver of <i>O. niloticus</i> 404.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>)40in the liver of <i>O. niloticus</i> 40		2.6.2 Effects of probiotics on blood parameters and	15
2.8 Impact of sumithion on intestinal morphology162.9 Impact of sumithion on immunity16-172.10 Investigations on mitigating pesticide toxicity17-182.11 Nile tilapia18-192.12 Research gap19-2003. MATERIALS AND METHODS21-303.1 Experimental fish223.2 Experimental design22-233.3 Sampling and data collection233.4 Growth performance of Nile tilapia23-243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25-263.8 Extraction and quantification of RNA26-273.9 Preparation for CDNA283.10 Real-time PCR assays28-303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of Oreochromis niloticus324.2 Changes in hemato-biochemical parameters32-334.3 Erythrocytic nuclear and cellular abnormalities34-354.4 Changes in intestinal morphology35-374.5 Expression of growth-related genes (<i>gh, igf-1, igf-2</i>) in38-39the pituitary and liver of O. niloticus40		gastrointestinal tract	
2.9 Impact of sumithion on immunity16-172.10 Investigations on mitigating pesticide toxicity17-182.11 Nile tilapia18-192.12 Research gap19-2003. MATERIALS AND METHODS21-303.1 Experimental fish223.2 Experimental design22-233.3 Sampling and data collection233.4 Growth performance of Nile tilapia23-243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25-263.8 Extraction and quantification of RNA26-273.9 Preparation for cDNA283.10 Real-time PCR assays32-333.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of Oreochromis niloticus324.3 Extraction of growth-related genes (gh, igf-1, igf-2) in38-39the pituitary and liver of O. niloticus40		2.7 Goblet cell	16
2.10 Investigations on mitigating pesticide toxicity17–182.11 Nile tilapia18–192.12 Research gap19–2003. MATERIALS AND METHODS21–303.1 Experimental fish223.2 Experimental design22–233.3 Sampling and data collection233.4 Growth performance of Nile tilapia23–243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25–263.8 Extraction and quantification of RNA26–273.9 Preparation for cDNA283.10 Real-time PCR assays28–303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of Oreochromis niloticus324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (gh, igf-1, igf-2) in38–39the pituitary and liver of O. niloticus40		2.8 Impact of sumithion on intestinal morphology	16
2.11 Nile tilapia18–192.12 Research gap19–2003. MATERIALS AND METHODS21–303.1 Experimental fish223.2 Experimental design22–233.3 Sampling and data collection233.4 Growth performance of Nile tilapia23–243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25–263.8 Extraction and quantification of RNA26–273.9 Preparation for cDNA283.10 Real-time PCR assays28–303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of Oreochromis niloticus324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (SOD and CAT)40		2.9 Impact of sumithion on immunity	16-17
2.12 Research gap19–2003.MATERIALS AND METHODS21–303.1 Experimental fish223.2 Experimental design22–233.3 Sampling and data collection233.4 Growth performance of Nile tilapia23–243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25–263.8 Extraction and quantification of RNA26–273.9 Preparation for cDNA283.10 Real-time PCR assays28–303.11 Statistical analysis3004.RESULTS31-414.1 Growth performance of Oreochromis niloticus324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (SOD and CAT)40		2.10 Investigations on mitigating pesticide toxicity	17-18
03.MATERIALS AND METHODS21–303.1 Experimental fish223.2 Experimental design22–233.3 Sampling and data collection233.4 Growth performance of Nile tilapia23–243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25–263.8 Extraction and quantification of RNA26–273.9 Preparation for cDNA283.10 Real-time PCR assays28–303.11 Statistical analysis3004.RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (<i>gh, igf-1, igf-2</i>) in38–39the pituitary and liver of <i>O. niloticus</i> 40		2.11 Nile tilapia	18-19
3.1 Experimental fish223.2 Experimental design22–233.3 Sampling and data collection233.4 Growth performance of Nile tilapia23–243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25–263.8 Extraction and quantification of RNA26–273.9 Preparation for cDNA283.10 Real-time PCR assays28–303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (<i>gh, igf-1, igf-2</i>) in the pituitary and liver of <i>O. niloticus</i> 40		2.12 Research gap	19–20
3.1 Experimental design22–233.2 Experimental design233.4 Growth performance of Nile tilapia23–243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25–263.8 Extraction and quantification of RNA26–273.9 Preparation for cDNA283.10 Real-time PCR assays28–303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (gh, igf-1, igf-2) in the pituitary and liver of <i>O. niloticus</i> 40	03.	MATERIALS AND METHODS	21-30
3.3 Sampling and data collection233.4 Growth performance of Nile tilapia23-243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25-263.8 Extraction and quantification of RNA26-273.9 Preparation for cDNA283.10 Real-time PCR assays28-303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32-334.3 Erythrocytic nuclear and cellular abnormalities34-354.4 Changes in intestinal morphology35-374.5 Expression of growth-related genes (<i>gh, igf-1, igf-2</i>) in the pituitary and liver of <i>O. niloticus</i> 40		3.1 Experimental fish	22
3.4 Growth performance of Nile tilapia23–243.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25–263.8 Extraction and quantification of RNA26–273.9 Preparation for cDNA283.10 Real-time PCR assays28–303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (<i>gh, igf-1, igf-2</i>) in the pituitary and liver of <i>O. niloticus</i> 40		3.2 Experimental design	22-23
3.5 Hematobiochemical parameters analysis243.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25–263.8 Extraction and quantification of RNA26–273.9 Preparation for cDNA283.10 Real-time PCR assays28–303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (<i>gh, igf-1, igf-2</i>) in the pituitary and liver of <i>O. niloticus</i> 40		3.3 Sampling and data collection	23
3.6 Blood abnormalities analysis253.7 Histopathological observation of the intestine25–263.8 Extraction and quantification of RNA26–273.9 Preparation for cDNA283.10 Real-time PCR assays28–303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (<i>gh, igf-1, igf-2</i>) in the pituitary and liver of <i>O. niloticus</i> 40		3.4 Growth performance of Nile tilapia	23-24
3.7 Histopathological observation of the intestine25–263.8 Extraction and quantification of RNA26–273.9 Preparation for cDNA283.10 Real-time PCR assays28–303.11 Statistical analysis3004.RESULTS4.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (<i>gh, igf-1, igf-2</i>) in the pituitary and liver of <i>O. niloticus</i> 38–394.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>)40		3.5 Hematobiochemical parameters analysis	24
3.8 Extraction and quantification of RNA26-273.9 Preparation for cDNA283.10 Real-time PCR assays28-303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32-334.3 Erythrocytic nuclear and cellular abnormalities34-354.4 Changes in intestinal morphology35-374.5 Expression of growth-related genes (gh, igf-1, igf-2) in the pituitary and liver of <i>O. niloticus</i> 40		3.6 Blood abnormalities analysis	25
3.9 Preparation for cDNA283.10 Real-time PCR assays28-303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32-334.3 Erythrocytic nuclear and cellular abnormalities34-354.4 Changes in intestinal morphology35-374.5 Expression of growth-related genes (<i>gh, igf-1, igf-2</i>) in the pituitary and liver of <i>O. niloticus</i> 38-394.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>)40		3.7 Histopathological observation of the intestine	25-26
3.10 Real-time PCR assays28–303.11 Statistical analysis3004. RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (<i>gh, igf-1, igf-2</i>) in the pituitary and liver of <i>O. niloticus</i> 38–394.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>)40		3.8 Extraction and quantification of RNA	26-27
3.11 Statistical analysis3004.RESULTS31-414.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32-334.3 Erythrocytic nuclear and cellular abnormalities34-354.4 Changes in intestinal morphology35-374.5 Expression of growth-related genes (gh, igf-1, igf-2) in the pituitary and liver of <i>O. niloticus</i> 38-394.6 Expression of antioxidant-related genes (SOD and CAT)40		3.9 Preparation for cDNA	28
04.RESULTS31-414.1 Growth performance of Oreochromis niloticus324.2 Changes in hemato-biochemical parameters32-334.3 Erythrocytic nuclear and cellular abnormalities34-354.4 Changes in intestinal morphology35-374.5 Expression of growth-related genes (gh, igf-1, igf-2) in the pituitary and liver of O. niloticus38-394.6 Expression of antioxidant-related genes (SOD and CAT)40		3.10 Real-time PCR assays	28-30
4.1 Growth performance of <i>Oreochromis niloticus</i> 324.2 Changes in hemato-biochemical parameters32–334.3 Erythrocytic nuclear and cellular abnormalities34–354.4 Changes in intestinal morphology35–374.5 Expression of growth-related genes (gh, igf-1, igf-2) in the pituitary and liver of <i>O. niloticus</i> 38–394.6 Expression of antioxidant-related genes (SOD and CAT)40		3.11 Statistical analysis	30
 4.2 Changes in hemato-biochemical parameters 4.2 Changes in hemato-biochemical parameters 4.3 Erythrocytic nuclear and cellular abnormalities 4.4 Changes in intestinal morphology 4.5 Expression of growth-related genes (<i>gh</i>, <i>igf-1</i>, <i>igf-2</i>) in 4.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>) 40 	04.	RESULTS	31-41
 4.3 Erythrocytic nuclear and cellular abnormalities 34–35 4.4 Changes in intestinal morphology 35–37 4.5 Expression of growth-related genes (<i>gh</i>, <i>igf-1</i>, <i>igf-2</i>) in 38–39 the pituitary and liver of <i>O. niloticus</i> 4.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>) 40 		4.1 Growth performance of Oreochromis niloticus	32
 4.4 Changes in intestinal morphology 35–37 4.5 Expression of growth-related genes (<i>gh</i>, <i>igf-1</i>, <i>igf-2</i>) in 38–39 the pituitary and liver of <i>O. niloticus</i> 4.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>) 40 		4.2 Changes in hemato-biochemical parameters	32-33
 4.5 Expression of growth-related genes (<i>gh</i>, <i>igf-1</i>, <i>igf-2</i>) in 38–39 the pituitary and liver of <i>O</i>. <i>niloticus</i> 4.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>) 40 		4.3 Erythrocytic nuclear and cellular abnormalities	34-35
 the pituitary and liver of <i>O. niloticus</i> 4.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>) 40 		4.4 Changes in intestinal morphology	35-37
4.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>) 40		4.5 Expression of growth-related genes (gh, igf-1, igf-2) in	38-39
the Expression of unitoxiduit related genes (50D and Chir)		the pituitary and liver of O. niloticus	
in the liver of O. niloticus		4.6 Expression of antioxidant-related genes (<i>SOD</i> and <i>CAT</i>)	40
		in the liver of O. niloticus	

4.7 Expression of immune response-related genes (*TNF-a*, 41-43

IL-1\beta, IFN-\gamma) in the liver of *O. niloticus*

05.	DISCUSSION	42–48
06.	CONCLUSIONS	49-50
07.	RECOMMENDATIONS AND FUTURE PROSPECTS	51-52
	REFEREENCES	53-75
	BRIEF BIOGRAPHY OF THE AUTHOR	76

PLATE NO.	TITLE	PAGE NO.
01.	Experimental fish	22
02.	Experimental setup	23
03.	Sampling of Nile tilapia	23
04.	Measuring blood parameters	24
05.	Blood smearing for identification of cellular and nuclear	25
	abnormalities	
06.	Histology of the intestine of Nile tilapia (O. niloticus)	26
	reared with different treatments for 42 days	
07.	Extraction and quantification of RNA	27
08.	Preparation of cDNA	28
09.	Procedure for real-time PCR assay	30

LIST OF PLATES

FIGURE	TITLE	PAGE
NO.	IIILE	NO.
01.	Alteration in (A) Hemoglobin (Hb) and (B) Glucose (Glu) levels of Nile tilapia (<i>O. niloticus</i>) reared with sumithion, probiotics, and sumithion + probiotics for 42 days.	33
02.	(A) Cellular abnormalities (a-d) of erythrocytes of Nile tilapia(<i>O. niloticus</i>) reared for 42 days at different treatments.	34
	(B) Alteration in frequencies of erythrocytic cellular abnormalities, (a) Tear drop; (b) Twin; (c) Spindle; (d) Fusion, of Nile tilapia (<i>O. niloticus</i>) reared with sumithion, probiotics, and sumithion + probiotics for 42 days.	34
03.	(A) Nuclear abnormalities (a-d) of erythrocytes of Nile tilapia (<i>O. niloticus</i>) reared for 42 days at different treatments	35
	(B) Alteration in frequencies of erythrocytic nuclear abnormalities, (a) Nuclear buds; (b) Notched nuclei; (c) Nuclear bridge; (d) Karyopkinesis, of Nile tilapia (<i>O. niloticus</i>) reared with sumithion, probiotics, and sumithion+probiotics for 42 days.	35
04.	Immune response indicators in gut histology of Nile tilapia (<i>O. niloticus</i>) reared with various treatments for 42 days.	37
05.	Relative expression of (A) <i>gh</i> in the pituitary, (B) <i>igf-1</i> and (C) <i>igf-2</i> in the liver of Nile tilapia (<i>O. niloticus</i>) (n=6) exposed to different treatments (control, T1; sumithion, T2; probiotics, T3; sumithion + probiotics, T4) for 42 days.	39
06.	Relative expression of (A) <i>SOD</i> and (B) <i>CAT</i> in the liver of Nile tilapia (<i>O. niloticus</i>) (n=6) exposed to different treatments (control, T1; sumithion, T2; probiotics, T3; sumithion + probiotics, T4) for 42 days.	40
07.	Relative expression of (A) <i>TNF-</i> α and (B) <i>IL-1β</i> in the liver of Nile tilapia (<i>O. niloticus</i>) (n=6) exposed to different treatments (control, T1; sumithion, T2; probiotics, T3; sumithion + probiotics, T4) for 42 days.	41
08.	Relative expression of <i>IFN-γ</i> in the liver of Nile tilapia (O. niloticus) (n=6) exposed to different treatments (control, T1; sumithion, T2; probiotics, T3; sumithion + probiotics, T4) for 42 days.	42

LIST OF FIGURES

LIST OF TABLES

TABLE NO	TITLE	PAGE NO.
01.	Temperature cycle for cDNA preparation	28
02.	List of primers used in the real-time PCR	29
03.	Growth response of Nile tilapia (O. niloticus) reared with	32
	sumithion, probiotics, and sumithion + probiotics for 42	
	days (n=12)	
04.	Histological changes in the gut of Nile tilapia (O.	36
	niloticus) reared with various treatments for 42 days	

ABSTRACT

Probiotics are important microflora that help in improving gut health, enhancing immunity, and boosting overall well-being. This study evaluates the counteracts effects of multispecies probiotics (Bacillus subtilis, B. thuringiensis, Lactobacillus plantarum, and L. buchneri) to sumithion toxicity on growth performance, hematobiochemical parameters, intestinal morphology, and expression of growth, antioxidant, and immunerelated genes in Nile tilapia (*Oreochromis niloticus*). Juvenile $(12.84 \pm 0.09 \text{ g})$ Nile tilapia were reared with four treatment groups: T1 (control, no sumithion or probiotic), T2 (sumithion, $0.56 \mu g/L$), T3 (probiotics, 1.0 ml/L) and T4 (sumithion, 0.3 $\mu g/L$ and probiotics, 1.0 ml/L) with three replicates for each treatment for 42 days. Results showed that fish exposed to sumithion (T2) had significantly lowered (p < 0.05) weight gain (WG) and specific growth rate (SGR), while supplemented with probiotics (T4) improved the growth performance. Supplementation of probiotics increases and decreases the sumithion-induced hemoglobin (Hb) and glucose (Glu) levels, respectively. Higher frequency of erythrocytic cellular and nuclear abnormalities observed in sumithion-exposed fish compared to the control group, as abnormalities were reduced in fish treated with probiotics. Multi-species probiotics led to pronounced thickened intestinal mucosal folds, increased abundance of goblet cells, wider lamina propria, and higher number of enterocytes compared to the control group. However, exposure to sumithion resulted in a marked decline in these intestinal parameters, and fish subjected to both sumithion and probiotics treatment exhibited a relatively improved intestinal structure. Significantly higher and lower levels of mRNA for growth-related gene (gh) and insulin-like growth factor (igf-1 and igf-2) genes were found in probiotics and sumithion-exposed fish, respectively. Relative mRNA level for antioxidant genes (catalase, CAT and superoxide dismutase, SOD) was significantly decreased (p < 0.05) in fish exposed to sumithion, while the non-significant differences was observed in probiotics (T3) and sumithion and probiotics (T4) treated fish. Conversely, the expression of immune-related genes (tumor necrosis factor alpha, TNF- α , interleukin beta, *IL-1* β , and interferon gamma, *IFN-* γ), was downregulated in sumithion-treated fish, and relative mRNA levels increased following the addition of probiotics. Therefore, incorporating probiotics into the aquatic environment demonstrated beneficial effects on haemato-biochemical properties, erythrocyte structure, and immune function, ultimately enhancing growth and countering the stress induced by sumithion pesticides.

Keywords: Probiotics, Sumithion, Growth performance, Antioxidant, Immunity, Nile tilapia