Cairo University
Faculty of Veterinary Medicine
Department of Fish Diseases and Management

"Thyme and Thymol as Immunostimulants in Cultured *Oreochromis niloticus*"

Thesis presented by

Mai Saad Yaseen Farag

A thesis Submitted to Cairo University for

The Degree of M.V. Sc. of Veterinary Medical Sciences
(Fish Diseases and Management)

Under supervision of

Prof. Dr. Mohamed Moustafa Mohamed

Prof. of Fish Diseases and Management
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Mohamed Abd El-Aziz Ahmed

Prof. and head of Fish Diseases and Management Faculty of Veterinary Medicine
Cairo University

Dr. Islam Mohamed Wassif

Researcher of Microbiology Desert Research Center

2017

بسم الله الرحمن الرحيم

وأَنْ لَيْسَ لِلْإِنْسَانِ إِلَّا مَا سَعَى (٣٩)

وَأَنَّ سَعْيَهُ سَوْفَ يُرَى (٤٠) ثُمَّ يُجْزَاهُ

الْجَزَاءَ الْأَوْفَى (٤١)

صدق الله العظيم

(سورة النجم)

Supervision sheet

"THYME AND THYMOL AS IMMUNOSTIMULANTS IN CULTURED *OREOCHROMIS NILOTICUS*"

M.V. Sc. Thesis
In
Fish Diseases and Management

By

MAI SAAD YASEEN FARAG
(B.V. Sc., Cairo University 2006)

SUPERVISION COMMITTEE

PROF.DR.MOHAMED MOUSTAFA MOHAMED
Prof. of Fish Diseases and Management
Faculty of Veterinary Medicine
Cairo University

PROF. DR. MOHAMED ABD EL- AZIZ AHMED
Prof. and head of Fish Diseases and Management Department
Faculty of Veterinary Medicine
Cairo University

DR. ISLAM MOHAMED WASSIF
Researcher of microbiology
Desert reaserch center

Name Mai Saad Yaseen Farag
Nationality Egyptian
Date of birth 23/12/1983
Place of birth El Kuwait
Specification Fish Diseases and Management
Scientific degree M. V. Sc. (Fish Diseases and Management)
Thesis title: "Thyme and Thymol as immunostimulants in cultured *Oreochromis Niloticus*"
Supervision: Prof. Dr. Mohamed Moustafa Mohamed
Prof. Dr. Mohamed Abd El-Aziz Ahmed
Dr. Islam Mohamed Wassif

ABSTRACT

The present study investigated the effects of thyme and thymol as phytoadditives on growth, hematological , and non-specific immunological parameters in cultured *Oreochromis niloticus*. In this study, ninety (90) *Oreochromis niloticus* with an average body weight of (50 ± 5) were used. Fish were divided into three groups (30 fish/ group) in triplicate and fed for 8weeks with: group (1) was fed basal diet as a control group, while group (2) was fed basal diet supplemented with thyme at 10g/kg diet and and group (3) was fed basal diet supplemented with thymol at 3g/kg body weight. After 8 weeks of feeding group (2) and group (3) showed particular differences in the growth, immunological and hematological parameters between the experimental group and the control group. However, as compared to control group, a significant increase of phagocytic activity and lysozyme activity was seen in groups (2) and (3). The serum total protein, globulin levels of the treated groups were significantly higher after challenge with *Strept.*sp in comparison with the control .The results of the present study indicated that dietary supplementation of 10 g/kg of thyme and 3g/kg thymol in in commercial diets could improve growth, some non-specific immunity and biochemical parameters in *Oreochromis niloticus* and increase survival rate against experimental challenge with *Strept.*sp.

Acknowledgements

First of all, I wish to express my sincere gratitude to "ALLAH" who gives me life, Islam, and all things that make me able to finish this work.

I would like to express my sincere gratitude and deepest appreciation to ***Prof. Dr. Mohamed Moustafa*** *Prof. of Dept. of Fish Diseases and Management, Faculty of Vet Med. Cairo University, for his helpful, supervision and facilities offered throughout the course of this work.*

I would like to express my sincere gratitude and deepest appreciation to ***Prof. Dr. Mohamed abdl –Aziz*** *Prof. and head of Fish Diseases and Management, Fac. of Vet. Med. Cairo University, for his supervision, able guidance ,his kind help and suggested me such an interesting issue followed by drawing a very fine schema.*

Words cannot adequately express the feelings of gratitude I have for ***Dr. Islam Mohamed Wassif*** *researcher of microbiology desert research center, for his kind supervision, advice, and help during the progress of this work.*

Many thanks are also extended to all the staff members of the Animal health, Desert reaserch center for their kind help.
Finally I would like to thank all colleagues of the Department of Fish Diseases and Management, my friends and my family for their encouragement.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Egypt is considered as one of the fastest growing countries in the field of aquaculture, Intensification of aquaculture has become an important practice in recent years to optimize the returns. Various kinds of fresh water and marine fishes have been worldwide cultured with annual increasing in production **(Mastan 2015)**.

Oreochromis niloticus is the most widely farmed fish species in Egypt. **(Zaki *et al*., 2011)**.Tilapias are members of the Cichlidae family which are a group of warm water fish, Importance of tilapia is due to its growth and reproduction in a wide range of environmental conditions and tolerance of stress induced by handling .

Out breaks of disease have become a critical factor which has hampered the development of aquaculture in many countries. Bacterial diseases are major problems in aquaculture and account for significant losses of fish **(Mirand and Zemelman, 2002)**. The usual methods employed for the control of diseases include vaccination for prophylaxis and antimicrobial therapy for treatment **(Austin, 1985)**.

In intensive aquaculture, the use of antibiotics and chemotherapeutics for treatment and prophylaxis has been broadly criticized for its negative impact by emergence of drug resistant microorganisms and antibiotic residue, and research on interactions between growth, immunity and development of eco-friendly alternatives to antibiotics that may keep fish healthy such as plant based Immunostimulants and enhancement the immune system is the promising method for prevention fish **(Sahu *et al*., 2007).**

Medicinal plants or plant extracts are used in many countries as an alternative to synthetic drugs. Scientists are now paying attention towards herbal extracts to act as anti-microbial agent due to the presence of different phytochemical components (tannins, alkaloids, terpenoids and phenolic compounds) **(Mousavi *et al*., 2011)**

Thymus vulgaris (Thyme) and thymol is perpetual therapeutic botanical herb belongs to lamianaceae family, the remedial potential effect of *Thymus vulgaris* (thyme) and thymol is due to the presence of flavonoids, thymol, carvacrol, eugenol, phenols, luteolin and tetramethoxylated. It has numerous valuable effects including bactericides, antiseptics, antioxidants, immunstimulant, antihelmintic properties and has late been recommended as substitute as cancer prevention agent **(Monira *et al*., 2012)**.

Therefore, the objectives of this study are:

1- Monitoring the effect of thyme as whole plant and thymol on growth performance of cultured *Oreochromis niloticus*.

2- Assessment effect of thyme as whole plant and thymol on Hematological, some non-specific immunity and biochemical parameters in *Oreochromis niloticus*.

3- Monitoring the effect of thyme as whole plant and thymol on disease resistance of *Oreochromis niloticus* challenged with *Strept*.sp.

REVIEW OF LITERATURES

1. **Uses of plant as natural feed additives in aquaculture.**
2. **Thyme and thymol in aquaculture.**
3. **Effect of thyme and thymol as appetite stimulators and growth promoter.**
4. **Effect of Thyme and thymol on haematology, antioxidant activity and fish immunity.**
5. **Effect of thyme and thymol as anti-bacterial.**

1- Uses of plant as natural feed additives in aquaculture

Harada (1990) indicated that used herbs as garlic act as stimulatory effect instead of chemotherapeutics and the active compound of garlic induce fish to ingest and increase feed intake.

Levy (1997) studied that natural feed additives and herbs that contain antibiotic properties in our diet can support immune system and help to defend from certain infectious bacteria. There are many foods and herbs known to have natural antibiotic qualities .

Sakai (1999) studied that herbs are rich sources of immune-enhancing substances and herbal immunostimulants in contrast to vaccines, can modulate the innate or non-specific immune response and are currently being used to control fish and shellfish diseases especially in cases where disease outbreaks are cyclic and can be predicted.

Kamel *et al*.(2000) reported that the large variety of plant compounds used as phytogenic feed additives (PFA) are assembled according to their origin and treatment, such as herbs and spices (eg: garlic, anise, cinnamon, coriander, pepper, rosemary and thymol).

Srinivasan *et al*. (2001) Medicinal herbs are high natural source of medicinal products used in traditional medicine and chemical entities for modern drugs. Medicinal plants are largely used either directly (home remedies) or indirectly (modern medicines) by all sectors of population.

Lin and Shiau (2005) studied that the feed additives improved fish immunity and decrease mortality of fish through improvements of the differential leukocytic count (Lymphocyte, monocytes, basophils, eosinophils and neutrophils) and immunological parameters that enhanced serum bactericidal activity, phagocytic activity, antibody levels, serum complement activity and lysozyme activity with the addition of feed additives

Muniruzzaman and Chowdhry (2005) reported that herbal extracts such as garlic and clove have antibacterial activity against various fish pathogens.

Sahu *et al*. (2007) studied that Phytobiotics are plant-derived, natural compounds embedded into diets which enhanced animal health and productivity.

Venkatramalingam *et al*. (2007) reported that post larvae of Penaeus monodon had significantly higher weight gain and specific growth rate when fed with herbal appetizer, Zingiber officinalis enriched Artemia.

Goda (2008) indicated that a dietary ginseng herb (Ginsana G115) in Nile tilapia fingerlings greatly enhanced growth performance, diet utilization efficiency and hematological indices.

Windisch *et al* . (2008) classified natural feed additives according to botanical origin, processing, and composition. Phytogenic feed additives include herbs, which are non-woody

flowering plants known to have medicinal properties; spices, which are herbs with intensive smell or taste, essential oils, which are aromatic oily liquids derived from plant materials such as flowers, leaves, fruits, and roots.

Levic *et al*. (2008) studied that World Health Organization encourages using of medicinal herbs and plants to substitute or minimize the use of chemicals through the global trend to go back to the nature. Attempts to use the natural materials such as medicinal plants could be widely accepted as feed additives to enhance efficiency of feed utilization and animal productive performance.

Gabor *et al*. (2010) studied that the use of some plants as phytoadditives (garlic, onion, oregano, etc.) used in fish as immunomodulators, immunostimulants, bioproductives, antioxidants, antimicrobials, stimulants of the enzymatic equipment, and stimulants of nitrogen absorption.

Koche *et al*. (2010) reported that the antimicrobial activities of the medicinal herbs could be due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, and steroids against both Gram-positive and Gram-negative organisms.

Nayak (2010) reported that the additions of probiotic to the fish feed have been tested in fish. Overall the effects have been beneficial such as reducing stress response, increasing the activity of innate parameters and improving disease resistance.