

Cairo University

Faculty of Veterinary Medicine

Department of Fish diseases & management



Cairo University

Effect of some herbal extracts on the health status of cultured *Oreochromis niloticus*

Thesis Presented by

Sahr Badrawy Abdel Aziz

(B.V.Sc.2011, sadat University)

For the degree of

Master in veterinary Medical Science

(Fish diseases and management)

Under the supervision of

Prof. Dr.

Mohamed Sayed Mohamed Marzouk

Professor of Fish Diseases and Management

Faculty of Veterinary Medicine

Cairo University

Prof. Dr.

Dr.

Mohamed Abd ElAziz

Ahmed

**Professor and Head Department of Fish Diseases
and Management**

**Faculty of Veterinary Medicine,
Cairo University**

Waleed Salah El-Din

Assistant Professor of fish diseases Hydrobiology

**Department, National Research Centre
Dokki - Giza**

SUPERVISION SHEET

Title of Thesis:

“Effect of some herbal extracts on the health status of cultured *Oreochromis niloticus*.”

Thesis Presented by

Sahr Badrawy Abdel Aziz Mahmoud
(B.V.Sc. 2011, Sadat University)

Under the Supervision of

Prof. Dr. Mohamed Sayed Mohamed Marzouk

Professor of fish diseases and management
Faculty of Veterinary Medicine, Cairo University.

Prof. Dr. Mahmoud Abd ElAziz Ahmed

Professor and Head department of fish diseases and Management
Faculty of Veterinary Medicine, Cairo University.

Dr. Waleed Salah Eldeen

Assistant Professor of fish diseases ,Hydrobiology Department
National Research Centre, Dokki, Giza.

Cairo University
Faculty of Veterinary Medicine
Department of Fish diseases & management

Name: sahr Badrawy Abdel Aziz Mahmoud

Date of birth: 8/1/1990.

Degree: MVSc.

Nationality: Egyptian

Place of birth: Qalubeia

Title of Thesis:

“Effect of some herbal extracts on the health status of cultured *Oreochromis niloticus*”

Under the Supervision of

Prof. Dr. Mohamed Sayed Mohamed Marzouk

Professor of fish diseases and management
Faculty of Veterinary Medicine, Cairo University.

Prof. Dr. Mahmoud Abd ElAziz Ahmed

Professor and Head department of fish diseases and Management
Faculty of Veterinary Medicine, Cairo University.

Dr. Waleed Salah Eldeen

Assistant Professor of fish diseases ,Hydrobiology Department
National Research Center, Dokki, Giza.

Abstarct

The present study was conducted to determine the effect of (green onion leaves-barley seeds-rice straw) extracts on growth, some physiological parameters and immunity of tilapia fish. The experiment was carried in 21 glass tank for 60 days. Seven experimental diet were formulated at 25% crude protein representing the following levels {control (0), OE (0.5) OE (1), BE (0.5), BE (1), RE (0.5) and RE (1) g/kg diet} .210 fish (wt. 30±5g) were fed twice daily 3% of body weight. At the end of experiment there was significant increase ($P < 0.05$) in weight gain in groups fed OE and BE extracts (0.5-1g)/kg diet. After 8 weeks, serum lysozyme activity, phagocytosis showed significant increase in groups fed OE and BE extracts while there is no effect in fish fed RE extract. The analysis of AST, ALT, and ALP showed no significant changes in all treated groups. Globulin level increased in OE and BE groups. No changes in RBCs count and HB level were detected in all groups while there was significant increase in WBCs count in OE and BE groups. The blood glucose level showed significant decrease in OE groups than control one. The result of this study demonstrated that OE and BE could improve growth and fish immunity.

(OE= Onion leaves extract, BE= Barley seed extract, RE= rice straw extract).

Key words

Tilapia, growth, immunity, *Areomonis hydrophila* , herbal extracts.

TO WHOM I LOVE.....

My son Mazen

FATHER, MOTHER, AND ALL MY FAMILY

ACKNOWLEDGMENT

It is all thanking to Allah who embraced me with his kind reconciling, wise strength and support without which, such work would never been done.

*I also can't express my sincere thanks and deep gratitude to Professor Dr. **Mohamed Sayed Mohamed Marzouk**, Prof. of Fish Disease and Management, Faculty of Veterinary Medicine, Cairo University, for his planning and stimulating supervision, great help, valuable advice, moral support during the course of this thesis and with his aid this work was accomplished. No suitable words can express my deepest gratitude to him.*

*Sincere thanks and deep gratitude are particular to Prof, Dr. **Mahmed Abd El Aziz Ahmed** Prof. and Head of Fish Disease and Management, Faculty of Veterinary Medicine, Cairo University, for his valuable help and encouragement given to me during this work and his unfailing interest, stimulating supervision and willing efforts to facilitate the difficulties during this work.*

*I offer my great appreciation to Dr. **Waleed Salah El-Din**, Ass. Prof. of fish diseases in National Research Centre, Hydrobiology Department, for his supervision, guidance and help during the course of the investigation*

*I am also particularly grateful and deeply thankful to prof. Dr. **Hossam Hassan Abbas** Prof. of Fish Physiology and Head of Hydrobiology Department, National Research Centre for his supervising, stimulating discussion, sincere help and kind guidance.*

*I wish to record my gratitude to Prof. Dr. **Mona Saad Zaki** Prof. of Fish Disease Hydrobiology Department, National Research Centre for her tremendous support and encouragement and valuable support.*

*Also, I wish to express my deep appreciation to Dr. **Elham shafik Awad**, Assist. Prof. of Fish diseases, Hydrobiology Department, National Research Centre for her valuable support in this study as well as her beneficial advices.*

*I offer my great appreciation to Dr. **Nermeen mostafa** Ass. Prof. of Fish Disease and Management, Faculty of Veterinary Medicine, Cairo University, for her tremendous support, encouragement, valuable support and help in the immunity work in this study.*

Also, I wish to express my deep appreciation to Dr. Amany Mohamed Kenawy, Prof. of Fish Pathology, Hydrobiology Department, National Research Centre for her valuable support and help in the pathology work in this study as well as her beneficial advices.

Finally, my deep thanks to Academy of Scientific Research for its financial support.

CONTENTS

	Page
Introduction	1
Review of literature	4
Materials and methods	25
Results	36
Discussion	65
Conclusion	72
Summary.....	73
References.....	75
Arabic summary	89

LIST OF TABLES

Table No.	Title	Page
Table (1)	Weight (g) of different fish groups during the 8weeks of the experimental period.	36
Table (2)	Weight gain (g) of different fish groups during the 8weeks of the experimental period.	36
Table (3)	RBC _s count $\times 10^6$ and HB (g/dl) in different fish groups at the end of the experiment.	37
Table (4)	WBCs count and differential leukocytes count of different treated <i>O. niloticus</i> groups at the end of the experiment.	39
Table (5)	Total protein (g/dl) in different fish groups (<i>O. niloticus</i>).	41
Table (6)	Globulin (g/dl) in different fish groups.	42
Table (7)	A/G ratio in different fish groups.	42
Table (8)	Glucose level in different fish groups at the end of the experiment.	44
Table (9)	Creatinin (g/dl) in different fish groups at the end of the experiment.	45
Table (10)	Uric acid level (g/dl) in different fish groups at the end of the experiment.	46
Table (11)	Alkaline phosphatase (U/L) in different fish groups at the end of the experiment.	47
Table (12)	ALT and AST (U/L) levels in different fish groups at the end of the experiment.	48
Table (13)	Phagocytic activity in different fish group at 4 th and 8 th week of experiment.	50
Table (14)	Lysozyme activity (μ g/ml) of different <i>O. niloticus</i> groups at 4 th and 8 th week of experiment.	53
Table (15)	Showing mortality % of fish after challenge with <i>A. hydrophila</i> .	54

LIST OF FIGURES AND PHOTOS

No.	Title	Page
Figure (1)	Weight (g) of different fish groups during the 8weeks of the experimental period.	37
Figure (2)	Weight gain (g) of different fish groups during the 8weeks of the experimental period.	37
Figure (3)	RBCs count $\times 10^6$ in different fish groups at the end of the experiment.	38
Figure (4)	HB (g/dl) in different fish groups at the end of the experiment.	38
Figure (5)	WBC _s count $\times 10^3$ in different fish groups at the end of the experiment.	39
Figure (6)	Lymphocytes % of different treated <i>O.niloticus</i> groups at the end of the experiment.	40
Figure (7)	Heterophiles % of different treated <i>O.niloticus</i> groups at the end of the experiment.	40
Figure (8)	Monocytes % of different treated <i>O.niloticus</i> at the end of the experiment. groups	41
Figure (9)	Total protein (g/dl) in different fish group <i>O.niloticus</i> .	42
Figure (10)	Globulin (g/dl) of different <i>O.niloticus</i> groups.	43
Figure (11)	Albumin (g/dl) of different <i>O.niloticus</i> groups.	43
Figure (12)	A/G ratio of different <i>O.niloticus</i> groups.	44
Figure (13)	Glucose level (g/dl) in different fish groups at the end of the experiment.	45
Figure (14)	Creatinin (g/dl) in different fish groups at the end of the experiment.	46
Figure (15)	Uric acid level (g/dl) in different fish groups at the end of the experiment.	47
Figure (16)	Alkaline phosphatase in different fish group at the end of the experiment.	48
Figure (17)	Serum ALT (U/L) of different treated <i>O. niloticus</i> groups at the end of the experiment.	49
Figure (18)	Serum AST (U/L) of different treated <i>O. niloticus</i> groups at the end of the experiment.	49
Figure (19)	Phagocytic activity in different fish group at 4 th and 8 th week of experiment.	50
Plate (1)	phagocytic cell of <i>O.niloticus</i> groups.	51
Plate (2)	phagocytic cell of <i>O.niloticus</i> groups.	52
Figure (20):	Lysozyme activity ($\mu\text{g/ml}$) of different fish groups at 4 th and 8 th week of experiment.	53
Figure (21)	Mortality % of fish after challenge with <i>A. hydrophila</i> .	55

Photo (1)	<i>Oreochromis niloticus</i> experimentally infected with <i>Areomonas hydrophila</i> showing external hemorrhage and detached scales.	56
Photo (2)	<i>Oreochromis niloticus</i> experimentally infected with <i>Areomonas hydrophila</i> showing ulcers.	56
Photo (3)	<i>Oreochromis niloticus</i> experimentally infected with <i>Areomonas hydrophila</i> showing pale enlarged liver.	57
Photo(4)	<i>Oreochromis niloticus</i> experimentally infected with <i>Areomonas hydrophila</i> showing congested gills and skin erosions.	57
Plate (3)	Histopathology of control negative and control positive <i>Oreochromis niloticus</i> groups infected with <i>A.hydrophila</i> (hepatopancreas and spleen)	60
Plate (4)	Histopathology examination of control negative and control positive <i>Oreochromis niloticus</i> groups infected with <i>A.hydrophila</i> (intestine and kidney)	61
Plate (5)	Histopathology of <i>Oreochromis niloticus</i> groups fed on diet with onion extract challenged with <i>Areomonas hydrophila</i>	62
Plate (6)	Histopathology of <i>Oreochromis niloticus</i> groups fed on diet with barley extract challenged with <i>Areomonas hydrophila</i>	63
Plate (7)	Histopathology of <i>Oreochromis niloticus</i> groups fed on diet with rice straw extract challenged with <i>Areomonas hydrophila</i>	64

INTRODUCTION

Aquaculture accounts for more than 65% of fish available in the market. Nile tilapia is one of the most economical freshwater fishes due to its excellent biological characteristics as fast growth, popular taste and high economic value (**FAO, 2014**).

As the demands for fish increased nowadays so many fishes are being cultured at intensive levels especially those belonging to family *Cichilidae* (**Mokoro *et al.*, 2014**).

Efforts have been made to increase productivity per unit space by increasing the rearing density. This may lead to some stresses namely overcrowding, transport, handling, grading, poor water quality, sudden changes in temperature and poor nutritional status that tend to adversely affect the health of cultured fish (**Li *et al.*, 2004**). The improper conditions in aquaculture environment lead to suppression of the immune system and facilitate the spread of pathogens, producing high mortality rates (**Quesada *et al.*, 2013** and **Guo *et al.*, 2015**).

The regular administration of antimicrobials and other chemicals as feed additives lead to the spread of bacterial resistant strains (**Cabello, 2006; Rico *et al.*, 2013**). It is also cost effective and contaminates the aquatic ecosystems by leaving the harmful residues (**Romero Ormazábal *et al.*, 2012**). So the use of most chemicals is becoming more restricted in many countries (**Seyfried *et al.*, 2010**).

Vaccination is also considered to be a potential control measure against disease outbreaks in aquaculture but they are too expensive and difficult in its application on wide range (**Harikrishnan *et al.*, 2011b**).

Using of immuno stimulants in aquaculture as feed additives is being encouraged nowadays because they have many advantages over chemotherapeutics and vaccination as they are safe, easily applied to various species and more effective in controlling fish diseases. They are also having a growth promoting and an immunostimulatory effect (**Apines-Amar *et al* 2013**). In fish culture, the maintenance

of a healthy marketable product is achieved by a good nutritional status (**Bello *et al.*, 2012**).

Plants have many bioactive compounds as phenolic, polyphenolic, alkaloid, quinine, terpenoid, flavonoid and polypeptide compounds which can enhance the non-specific defense mechanisms and so increase fish resistance to diseases. (**Shukla *et al.*, 2014**). The immunostimulants from natural sources may be an alternative to the antibiotics and vaccines as the natural organic materials do not cause any threat to fish health, to the environment and to fish consumers (**Talpur *et al.*, 2013; Reverter *et al.*, 2014**).

Among these plants, onion (*Allium cepa*) contains flavonoid compounds which act as growth promoters and immunostimulants in fish (**Scalbert and Williamson, 2000**). Also, onion has a prebiotic effect due to high soluble fibers (**Binaii *et al.*, 2014**). It has also been known to have antibacterial and antioxidant effects (**Ramos *et al.*, 2006**). There are many studies indicated that onion was one of the most effective dietary additives tested that improve lysozyme activity and act as an immunostimulant as in juvenile olive flounder (*Paralichthys olivaceus*) (**Cho and Lee 2012**).

Another plant is Barley (*Hordeum vulgare*), a member of the grass family, *Poaceae*, is one of the main cereal crops grown around the world (**Madhujit *et al.*, 2006**). They contain a wide array of phytochemicals, primarily phenolic compounds including flavonols, phenolic acids and procyanidins. Some studies on the antioxidant activity and phenolic contents of barley have been reported in the literature (**Karppinen *et al.*, 2003**). The major phenolic components in barley seeds are ferulic, caffeic, and vanillic acids (**Madhujith *et al.*, 2004**).

Rice straw (*Oryza sativa*) is one of the ligno-cellulosic plants. It makes pollutions in the environment due to its disposal by burning. Therefore we try to find a new method for utilization and disposal (**Van Soest 2006**).

Therefore this study was planned to fulfill:-

1-Extraction of the most active ingredient in some plant extracts namely green onion leaves, barley seeds and rice straw.

2- Studying the effect of these plant extracts on the health status of cultured Nile tilapia (*Oreochromis niloticus*) as interpreted by growth promotion and immunostimulation.

3- Monitoring the prophylactic efficacy of such herbal extracts as assessed in *Areomonas hydrophila* experimental challenge of healthy cultured *Oreochromis niloticus*.

REVIEW OF LITERATURE

(1) Importance of herbals in fish feed and the most active ingredient in the herbal extracts

A. Importance of herbals in fish feed

B. The most active ingredient in the herbal extracts

(2) Herbal plants as growth promoters

(3) Effect of herbal extracts on hematological and physiological parameters

A. Effect of herbal extracts on hematological parameters

B. Effect of herbal extracts on physiological parameters

(4) Herbal plants as immunostimulants in aquaculture

(5) General Characteristics of Onion; barley and rice and their application in aquaculture

A. Onion (*Allium cepa*)

a) General Characteristics of *Allium cepa*

b) *Allium cepa* in aquaculture

B. Barley (*Hordeum vulgare*)

a) General Characteristics of Barley

b) Barley in aquaculture

C. Rice (*Oryza sativa*)

a) General Characteristics of Rice

b) Rice in aquaculture

(6) Challenge with *Aeromonas hydrophila* and the histopathological examination

(1) Importance of herbals in fish feed and the most active ingredient in the herbal extracts:-

A. Importance of herbals in fish feed:-

Raa *et al.* (1992) recorded that the using of immunostimulants as prophylactic control measures is considered as one of the most hopeful methods to increase the defense mechanism of fish in aquaculture disease management.

Hansen *et al.* (1993) concluded that bacterial resistance is one of the most obvious disadvantages of continuous exposure to antibiotics. As in case of *Aeromonas hydrophila* treatment the continuous exposure to oxytetracycline developed cross-resistance and negative impact on fish consumers.

Jeney and Anderson (1993) showed that the most effective method to increase the efficacy of the vaccine is the application of immunostimulants that could decrease the dose necessary for the vaccine to achieve the same effect.

Middlyng *et al.* (1996) stated that the application of vaccine may cause induction of some gross pathological changes, which affect the aesthetic appearance of the fish but the application of immunostimulants is very simple and not cause any side effect when used in appropriate dose.

Raa (1996) conducted that using immunostimulants has a good effect in controlling of fish disease and enhancing of growth.

Dalmo *et al.* (1997) showed that some plant extracts could increase the non-specific immunity of fish which is considered as the first line of defense mechanism and represents a considerable part of the immune response.