Mansoura University
Faculty of Veterinary Medicine
Department of Internal Medicine,
Infectious and Fish Diseases

Advanced Studies on Some Fungal Diseases in Fish Farms

A Thesis presented

By

Ola Mahmoud El Sayed Mohamed

B. V. Sc. Mansoura University (2009)

M.V Sc. Fish diseases and Management Mansoura University (2016)

Under supervision of

***Prof. Dr.* Viola Hassan Zaki**

Professor of Fish Diseases and Management

Head Department of Internal Medicine, Infectious and Fish Diseases

Faculty of Veterinary Medicine, Mansoura University

***Dr.* Rawia Saad Adawy**

Senior Researcher of Fish Diseases and Management

Animal Health Research Institute

Agriculture Research Institute

Submitted to

Faculty of Veterinary Medicine Mansoura University

For the degree of Ph.D. Veterinary Medical Sciences

(Fish diseases and Management)

2020

Molecular identification of pathogenic fungi isolated from freshwater fishes in Lake Manzala

Ola, M. Hashem[a], Viola , H. Zaki[b] and Rawia, S. **Adawy[a]**

Animal Health Research Institute[a] Department of Fish Diseases and Management,Faculty of Veterinary Medicine Mansoura University[b]

Abstract

The present study was carried out on 600 specimens of freshwater fishes (300 *O. niloticus and 300 C. gariepinus)* collected from Manzala fish farms in Dakahlia Province to investigate the predominant mycotic infections among freshwater fishes with special focus on incidence and its seasonal dynamics as well as the molecular characterization of the detected fungus. Naturally infected fishes showed many abnormalities in clinical and post mortem examination. Mycological examination revealed that the general incidence of fungal infection was 20.1% among the examined fishes, *C. gariepinus* recorded high incidence (22.3%) than *O. niloticus* which was (18 %). The incidence of isolated fungi from both species of fishes *C. gariepinus* and *O. niloticus* was *Saprolegnia spp. (20.8&16.6 %) , A. niger*(17.9 & 14.8 %) , *A. flavus (14.9 & 12.9%) , A. fumigatus,(8.9 & 14.8 %) ,Fusiarium spp. (5.9 & 7.4 %) , Penicillum spp.(13.4 &11.1 %) , Cladosporium spp.(5.9 & 5.5 %) , Alternaria spp*. (2.9 & 3.7 %) and *Candida albicans* respectively (8.9 & 11.1 %)

The seasonal dynamics of infection showed that the winter season recorded high rate of infection in both *C. gariepinus* (31.1 %) and *O. niloticus(23.1%)* , followed by autumn (25 &19.4 %), spring (17.2 &15.4 %) and summer (14.2&13.1%) .*Saprolegnia spp*. recorded high incidence in autumn (33.3 & 28.5 %) and in winter (32.1 & 26.3 %) in both species of fishes respectively. All species of *Aspergillus* were found in all seasons with high rate in hot seasons summer and spring. The results declared that the PCR assay was highly specific and sensitive for the detection of detected *A. flavus, A. niger, penicillim spp.* and *candida albicanis* isolated from the examined fishes (*C. gariepinus and O. niloticus*) and molecular characterization of each species was discussed.

1- Introduction

Fish is one of the more important sources of animal protein available in the tropics and has widely accepted as good source of protein and other elements for maintenance of body health. Fish proteins are noted for a high degree of digestibility and as a rich source of lysine and sulphur containing amino acids. Therefore it is suitable for complementing high carbohydrate diets especially in developed countries period **[1]**.

With more intensification to meet consumers demand, a major problem feed the progress and growth of this sector that fish often succumb to infectious diseases. Infectious fish diseases not only caused by bacteria, virus or parasite but also could be molds borne **[2]**.

Fungal diseases are the result of interaction of the pathogen, the fish and environment. Fish in intensive culture are continuously affected be environmental fluctuation and

management particles. Poor quality of water , improper hygiene , fish that are injured have other diseases and dead fish/large amounts of decomposing organic material present in the pond .All these factors should be considered for fish health control by preventing diseases rather than treatment **[3].**

Molds grow over a temp range of 10-40°C, pH range of 4-8, humidity level greater than 62% and more than 12-13% moisture, while yeasts require free water**[4]**.

Fish is mostly attacked by fungi due to change in temperature and filthy conditions of water which allow excessive zoospores to grow and the ammonia wears away the mucus which protects the skin **[5]**.

During the last 15 years or so, molecular techniques have been increasingly employed to diagnose fish diseases. These techniques include polymerase chain reaction (PCR), restriction enzyme digestion, probe hybridization, in situ hybridization, and microarray. Pathogens can be detected from asymptomatic fish by molecular diagnostic techniques so disease outbreak could be prevented. Thus Molecular techniques are potentially faster and more sensitive than culture, serology, and histology methods that are traditionally used to identify fish pathogens**[6]**.

Manzala Lake is the largest coastal lake in Egypt which is a shallow brackish lake extending between the Damietta Nile River branch and the Suez Canal with a maximum length of 50 km along the Mediterranean coast **[7].** The northern boundary of the lake is connected with some narrow inlets such as El-Gamil canal with the sea. The eastern boundary is Suez Canal that is connected with the lake via El-Qabuty canal. Economically, Manzala Lake is considered as one of the most valuable fish sources in Egypt by about 36-50% from the total annual production of the Egyptian lakes. This contributes > 4.2% of the total country fish production; ca. 1.5 million ton **[8]**.So this work was planned to throw a spotlight on the Molecular identification of the fungus isolated from freshwater fishe in Lake Manzala.

2-Material and method

(2-1) Naturally infected fishes

A total number of 600 fishes included 300 Nile tilapia *(Oreochromis niloticus)* and 300 Nile catfish *(Clarias gariepinus)* collected from Manzala fish farms in Dakahlia Governorate. The fish samples were collected during the period from April 2018 to March 2019. The fishes collected from farms were transported alive in polyethylene bag which contained about 30% of its volume fish and water and the remaining part pumped with oxygen, and sealed in a manner preventing escape of Oxygen. They were transferred immediately to the laboratory for examination.

(2-2) Clinical and postmortem examination:

Fish samples were transported alive to the laboratory and kept in a reservoir containing oxygenated dechlorinated water. .Moribund and diseased Fish were properly examined for any external clinical abnormalities and clinical alterations on the skin, scales, eyes, abdomen, peduncle, fins and abnormal behaviors. The postmortem examination was

List of Contents

List of Figure

List of Tables

List of Histogram