

## Investigation of Some Mycotic Infections with Economic Importance in Freshwater Nile Tilapia.

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### Abstract

This study was done on 200 Nile tilapia (*Oreochromis niloticus*) of different ages were collected from farms of Central Laboratory for Aquaculture Research (Abbassa, Abou Hammad, Sharkia, Egypt) during period from late November, 2014 to march, 2016. The collected fish samples were clinically diseased showed, respiratory manifestation; ulcer and hemorrhages on body surface, cotton wool like tufts on skin, fins and in eye caused opacity and detached scale. Internally the affected fish showed enlarged pale or yellow, many cases showed congested spleen and kidney, intestine free from any food particles and gall bladder enlarged and distended with bile. The Mycological examination by different method, eleven different fungal isolates were macro and microscopically identified, *Saprolegnia spp.*, *Asp. niger*, *Asp. flavus*, *Asp. ochraceus*, *Asp. terreus*, *Asp. versicolor*, *Zygomycets group*, *Penicillium spp.*, *Fusarium spp.*, *Cladosporium spp.* and *Candida albicans*. The study revealed that *Asp. niger* was the most fungus isolated and affected fish during summer season, while *Saprolegnia spp.* was the most fungus affected fish and isolated during winter season. *Saprolegnia spp.* responsible for high percentage of mortality among affected fish and also lead to decrease percentage of hatchability among affecting fish eggs. This study revealed that row garlic prevents growth of *Asp. niger*, *Asp. flavus* and *Cladosporium spp.* in vitro.

**Key words:** Aspergillus, Nile tilapia, species.

### Introduction

The aquaculture production has been increased in last decade with average 11% per year. It considers the fastest developing sector in the world food economy. The fish production increased from 13

million tons at 1990 to 37.9 million at 2001 (FAO Fishery information). The increase in the intensification aquaculture has been accompanied by out breaks of disease from an ever increasing range of pathogens (*Rajinikanth et al., 2010*). Fungi

are part of the normal microflora of fresh water and estuarine ecosystems and have worldwide distribution (*Iqbal et al., 2012*). Fungal infection occur mainly due to immune suppression which occurs due to many factors as, stressed fish by environmental conditions; fish received poor nutrition or fish suspected to physical or mechanical injury. It attack fishes of all ages and it can also prevent successful hatching when it invades fish eggs (*Chauhan, 2012*). Several species like *penicillium spp.*, *Alternaria spp.*, *Fuzarium spp.* and *Aspergillus spp.* were isolated from diseased fish (*Firoz et al., 2011*). The losses due to fungal infection may reach up to 80- 100% in incubated eggs (*Chukanhom and Hatai, 2004*). So this work is aimed to isolate and identify fungal species which affect freshwater Nile tilapia.

#### **Material and Methods:**

A total number of 200 diseased Nile tilapia (*Oreochromis niloticus*) were collected randomly from farms of Central Laboratory for Aquaculture Research during period from late November, 2014 to march, 2016, then it exposed to clinical and postmortem examination according to (*Amlacker, 1970 & Amlacker, 1970; Lucky, 1977*). Isolation of fungal species from infected fish were carried out on Three types of media including, Sabouraud's dextrose agar (SDA), Czapek's media and potato dextrose agar

(PDA) were prepared and chloramphenicol was supplemented to each predation of medium to avoid bacterial contamination (*Cruichshak et al., 1975*). Identification of purified samples were carried out according to (*Domsch et al., 1980; Kozakiewicz, 1989; and Moubasher, 1993*).

#### **In vitro trials of treatment:**

##### **Raw garlic by disc and well diffusion methods:**

Raw garlic powder was dissolved in sterile distilled water in a ratio (1: 9), and poured on standard disc then dried on oven or in the center of plates, one well was made and the solution was poured in these wells, The fungal isolates were flooded on surface of SDA by sterile cotton swab by immersion in inoculated SDB (4 days old), The prepared disc was added to the cultured medium and incubated at 24°C. The presence of inhibition zone was recorded (disc diffusion method).

#### **Result and discussion**

The main clinical picture of diseased fish showed loss of equilibrium, off food, lethargy swimming, lifted scales and ulcerated skin and unilateral eye opacity and moderate body distention as shown in plate 1: (1),(3),(4).

The main postmortem signs presence of fungal growth on the head, eyes and fins; pale eroded gills, abdominal cavity full with serous fluid; congestion in liver, kidney and spleen; gall bladder

distended with bile and intestine free from any food as shown in plate 1: (2).

Fungal infection occur during various seasons of the year and fish (*O. niloticus*) infected with various fungi (*Saprolegnia spp.*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus terreus*, *Aspergillus versicolor*, *Penicillium spp.*, *Cladosporium spp.*, *Zygomycets group* and *Candida albicans*). The highest infection rate was during winter season 66.6% of *Saprolegnia spp.* Infection, during the summer season the highest infection rate was 24.5% of *Aspergillus niger* infection. During summer season the infection rate was as following, *Asp. niger* (22.7%), *Asp. flavus* (14.9%), *Penicillium spp.* (14.4%), *Zygomycets group* (12.5%), *Asp. versicolor* (10.8%), *Asp. terreus* (7.2%), *Asp. ochraceus* (5.3%) *C. albicans* (3.6%), *Fusarium spp.* (1.7%) and *Cladosporium spp.* (2.9%). During winter season the infection rate was as following, *Saprolegnia spp.* (66.6%), *Asp. niger* (11.6%), *Asp. flavus* (4.2%), *Penicillium spp.* (5%), *Zygomycets group* (4.1%), *Asp. terreus* (2.08%), *Asp. ochraceus* (2.08%), *Asp. versicolor* (0.08%), *Cladosporium spp.* (0.8%) and *C. albicans* (0.8%). Cultural and morphological characterization of all isolates was described in plate 2,3,4:

This work supported that *Aspergillus spp.*, *Penicillium sp.* and *Rhizopus sp.* are normal

mycoflora and these species may be regarded as opportunistic pathogen (As many of them possess virulence factors which enable them to cause disease, especially under favorable predisposing conditions (**Refai et al., 2010**)). The present finding showed seven fungal genera (*Saprolegnia spp.*, *Aspergillus spp.*, *Pencillium spp.*, *Cladosporium spp.*, *Fusarium spp.*, *Rhizopus spp.* and *Candida spp.*) were isolated from Nile Tilapia (*Oreochromis niloticus*), these result is in tune with **Junaid et al. (2010)** isolated 7 fungal species from stock fish in Nigeria and these included *Asp. flavus*, *Asp. fumigatus*, *Asp. niger*, *Trichophyton verrucosum*, *Rhizopus*, *Mucor* and *Penicillium sp.*, (**Refai et al., 2010**) In another study isolated eight different genera; *Saprolegnia*, *Aspergillus*, *Fusarium*, *Mucar*, *Penicillium*, *Rhizopus*, *Scopulariopsis* and *Curvularia* were isolated from two fish species *Oreochromis sp.* and *Claris gariepinas*. In studies done by **EL-Abbassy (2007)** *Asp. niger* was considered a thermo tolerant mold which grew at temperature ranged from 8-45°C, (**Zhao et al., 2009**) reported that *Aspergillus niger* tolerate temperature degree at 40°C, all these results are in coordenance with the present study results which showed the highest incidence of *Asp. niger* infection occur with percentage of (22.7%) during summer season. In the present study, maximum infected fish were

observed during the month of early September and late April. This observation is in agreement with (De canals et al., 2001) reported that, fungal infections often start when an immune suppressions is produced by a sudden variation in temperature. Also this observation was in harmony with (Moeller, 2010), who mentioned that most of epizootic infections occur when temperatures was blew the optimal temperature range for fish species. This work revealed that raw garlic (*Allium sativum*) powder had

antifungal effect on different fungal species as *Asp.niger*, *Asp.flavus*, *Cladosporium* and *C.albicans* as shown in photo1-4, these results were in tune with (Khodavandi et al., 2010) reported that raw garlic prevent *C.albicans* growth by destruction of its cell membrane. Also agree with (Wanapat et al., 2008) reported that the main component of raw garlic is Allicin which inhibit succinate dehydrogenase in fungus. Plate (5)



Plate (1) : Lifted scales & ulcerated skin, exposing underlying musculature.  
 3 Enlarged spleen, congested internal organs,  
 2 Eye opacity, hemorrhage on head.  
 4 Degeneration of dorsal and caudal fins & hemorrhage on skin, *Nile tilapia*.

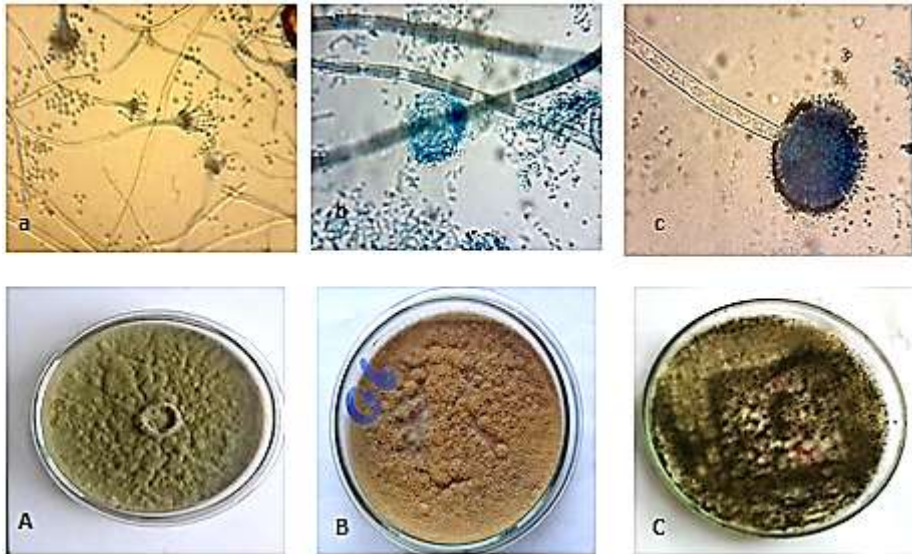


Plate (2): A, yellowish green colonies. a, very long conidiophore with radiated conidia head (*A.flavus*). B, granular, brownish yellow colonies. b, long septated hyphae and pyriform vesicle (*Aspergillus terreus*). C, granular black, c. Round vesicle with biserial phialides covering most of the vesicle (*Asp.niger*).

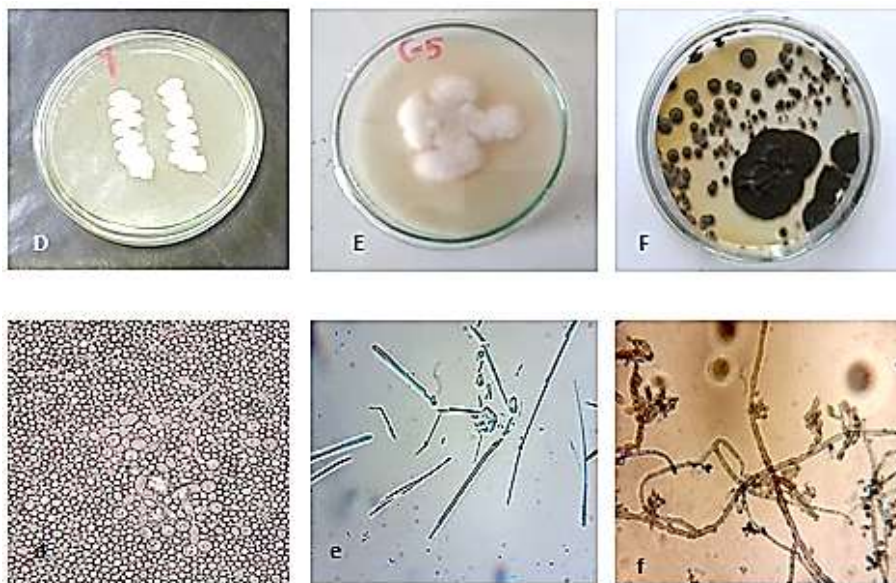


Plate (3): D, Creamy white colony d. Chlamydo-spore and pseudohyphae formation on rice agar, *Candida albicans*, E, floccose, creamy white colony, e. fusiform macroconidia, the widest diameter in the penultimate cell and have round basal cell, the apical is pointed *Fusarium spp.*, F, velvety, black colonies, f. Branched conidiophores have conidia in chains which usually one cell (*Cladosporium spp.*).

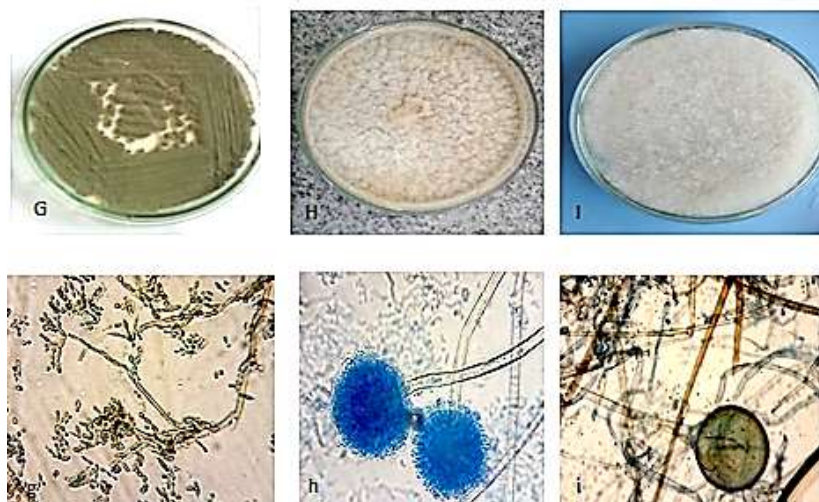


Plate (4): *G. velvety*, dark green and folded colonies, g. septated hyphae, chain of round conidia give characteristic brush appearance, *Penicillium spp.*, H. floccose white mycelium with yellow conidial head, h. globose vesicle, biserial with metulae surrounded it (*A. ochraceus*). I. an extensive and dense mycelium white in color. i. long branching hyphae with sporangia free from zoospores, *Saprolegnia spp.*.

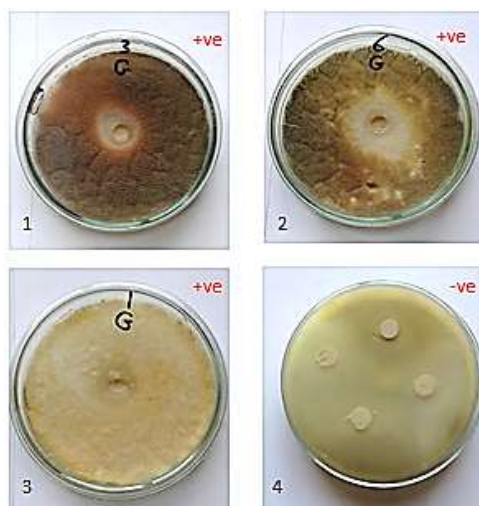


Plate (5): 1- *Asp. niger* was sensitive to raw garlic (well method). 2- *Cladosporium spp.* was sensitive to raw garlic (well method). 3- *Asp. flavus* was sensitive to raw garlic (well method). 4- *Saprolegnia spp.* not affected by raw garlic (disc method)

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## الملخص العربي

### التحقق عن بعض حالات العدوى الفطرية في الاسماك ذات الالهيه الاقتصادية مثل سمكة البلطي النيلي

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اجريت هذه الدراسة على 200 سمكة مصابه من اسماك البلطي النيلي في مراحل عمرية مختلفة تم تجميعها من المزارع التابعة للمعمل المركزى لبحوث الثروة السمكية بالعباسة خلال الفترة من اواخر شهر نوفمبر 2014 الى شهر مارس 2016 وقد خضعت هذه الاسماك للفحص الاكلينيكي والتشخيص الفطري بالطرق المختلفة لكل العزلات . اوضحت الصفات الاكلينيكية وجود تقرحات في جميع انحاء جسم السمكة والتي كانت احدى العلامات الواضحة مع وجود تهتك في الزعانف الذيلية والظهرية ونمو فطري يشبه شعيرات القطن على الزعانف وجسم السمكة ووجود بقع داكنة على الجلد وكانت بعض الاسماك تعاني من عتامة في احدى العينين واخرى كان يوجد جحوظ في عينيها. وباجراء الفحص التشريحي لوحظ تضخم في الكبد والكلى والطحال مع وجود احتقانات في جميع اعضاء التجويف البطنى والخياشيم. وقد خلصت النتائج بالاتي:

تم عزل عدد (11) نوع من الفطريات وهى السبرولجنيا, الاسبرجلس نيجر, الاسبرجلس فلافس, الاسبرجلس اوكراشياس, الاسبرجلس فيرزى كلر, الاسبرجلس تيرس, البنسليم, الكلاوسبوريم, الفيوزاريم, الزيجوميستس والكاندريا البيكانز. وقد اظهرت الدراسة ان الاسبرجلس نيجر كان الاكثر انتشارا خلال فصل الصيف والسبرولجنيا هي الاكثر انتشارا ما بين اسماك البلطي خلال فصل الشتاء وتؤدي الى نفوق نسبة عالية من الاسماك. كما اوضحت الدراسة ايضا كفاءة استخدام الثوم في علاج كل من الاسبرجلس فلافس والاسبرجلس نيجر والكلاوسبوريم في المعمل.