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Isolation and identification of pathogenic fungi on *Oreochromis aureus* (Steindachner, 1864) in the University of Basrah fish ponds

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Thirty samples of *Oreochromis aureus* were collected from University of Basrah fish ponds during the period from February until June 2014. Thirteen fish samples showed fungal infection. A sterile swab was taken from outer surface of body (head, skin, gills, abdomen, caudal fin, dorsal fin and pectoral fin). Potato dextrose agar and glucose yeast agar was used for fungal isolation. In this study six genera were identified and the most common were *Aspergillus* sp., *Alternaria* sp., *Mucor* sp., *Penicillium* sp., *Brachiomyces* sp. and *Ichthyophonus* sp. Gills and abdomen were the most affected parts of fish. Among the genera observed *Aspergillus* sp. and Mucor sp. were the most prevalent fungi infecting these fishes.

[Keywords: Fungal infection, Fish ponds, *Oreochromis aureus*]

Introduction

Fungal diseases are the result of interactions of the pathogens, the fish and the environment. Fish in intensive culture are continuously affected by environmental fluctuations and management practices. All these factors should be considered for fish health control by preventing diseases rather than treatment[1, 2]. Many fungi cause fish diseases such as *Alternaria* sp. was isolated from *Carassius auratus*, *Xiphophorus maculates* and *Poecilia reticulate*,[3] *Aspergillus*, *Mucor* and *Rhizopus* from *Cyprinus carpio* and *C. auratus*[4]; *Saprolegnia* sp. was isolated from African Catfish (*Clarias gariepinus*)[5]; *Blastomyces* sp. and *Penicillium* sp. from *Catla catla*[6], *S. diclina* was isolated from eggs of *C. carassius* in Białystok rivers, Poland[7]; *Achlya* spp. and *Saprolegnia* spp. from Indian major carps viz. *C. catla*, *Cirrhinus mrigala* and *Labeo rohita*[8]; *Ichthyophonus hoferii* from *Mugil capito*, *M. cephalus*, Bighead carp (*Hypophthalmichthys nobilis*) and *Oreochromis niloticus* were collected from 30 farms from different localities at Alexandria, Kafr El-Sheik and El-Behera in Egypt[9], *Aphanomyces invadanswas* was isolated from Nile tilapia (*O. niloticus*) eggs in Thai hatcheries[10]. The aim of present study was isolation and identification of fungi present on *O. aureus* in the University of Basrah fish ponds.

Materials & Methods

During the period from February until June 2014, a total of 30 fish samples of *Oreochromis aureus* were collected from University of Basrah fish ponds. Sampling of infected fish was carried out by collecting the fish in polythene bags. These were bought to the laboratory in living condition. Purification of cultures was done by preparing the cultures on Potato Dextrose Agar (PDA) and Glucose Yeast Agar (GYA). To inhibit the bacterial growth 500 µg/ ml each of penicillin and streptomycin was added to PDA and GY agar plates. All the cultures were incubated at temperatures 18±2 °C. Slides were prepared according to (Beakes *et al.*[11]) by taking material from each colony and staining with 0.05% trypanblue in lactophenol. The slides were observed under Digipro-labomed microscope and photographed. The fungi were identified with the help of available fungal identification keys and literature[12].The fish samples were surfaced sterilized with 70% ethanol and rinsed with three changes of sterile distilled water. A 10 g tissue portion of fish was cut from the abdominal region with a sterile forceps, macerated aseptically in a mortar and mixed in 10 ml of sterile peptone water. From this mixture, further tenfold dilutions were made up to 10^3, and 0.1 milliliter of each dilution was plated in triplicate on potato dextrose agar (PDA) supplemented with streptomycin to

inhibit bacterial growth. Plates were incubated at 28 ± 2◦ C and examined daily for 7 days. The mean number of all fungal colonies appearing in the three plates was taken as the average number of colonies per plate for fish. This was used to estimate the number of colonies per gram of fish sample using a known dilution series. The prevalence of fungi (%) was calculated according to the following equation[13].

$$\text{Prevalence (\%)} = \frac{\text{Number of infected fishes}}{\text{Number of examined fishes}} \times 100$$

Results

The species of fungi distributed in 30 samples of *O. aureus* included *Aspergillus* sp. (49% samples), *Alternaria* sp. (25% samples), *Mucor* sp. (37% samples), *Penicillium* sp. (27% samples), *Brachiomyces* sp. (30% samples) and *Ichthyophonus* sp. (20% samples). Details of mycoflora isolated from head, skin, gills, abdomen, caudal fin, dorsal fin and pectoral fin are shown in (Fig. 1 and 2). Gills and abdomen had higher infection than rest of the organs (Table 1). *Aspergillus* sp. and *Mucor* sp. were the most prevalent fungi infecting these fishes (Table 1) and (Fig. 1).

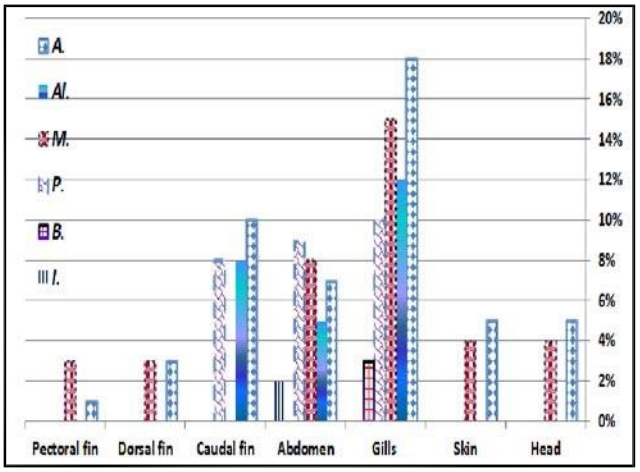

Fig. 1. The prevalence of fungal species: the frequency of isolation (%) from *O. aureus*. A.: *Aspergillus* sp., Al.: *Alternaria* sp., M.: *Mucor* sp., P.: *Penicillium* sp., B.: *Brachiomyces* sp. and L.: *Ichthyophonus* sp.

Table 1. The fungal species isolated from *O. aureus* and fungal colony counts from fish tissue

Fish organ	Fungal species	No. of colonies per gram of fish tissue
Head, Skin, Gills, Abdomen, Caudal fin, Dorsal fin, Pectoral fin	*Aspergillus* sp.	4.1 X 10^3
Gills, Abdomen, Caudal fin	*Alternaria* sp.	2.8 X 10^3
Head, Skin, Gills, Abdomen, Dorsal fin, Pectoral fin	*Mucor* sp.	3.5 X 10^3
Gills, Abdomen, Caudal fin	*Penicillium* sp.	1.3 X 10^3
Gills	*Brachiomyces* sp.	1.4 X 10
Abdomen	*Ichthyophonus* sp.	1.4 X 10

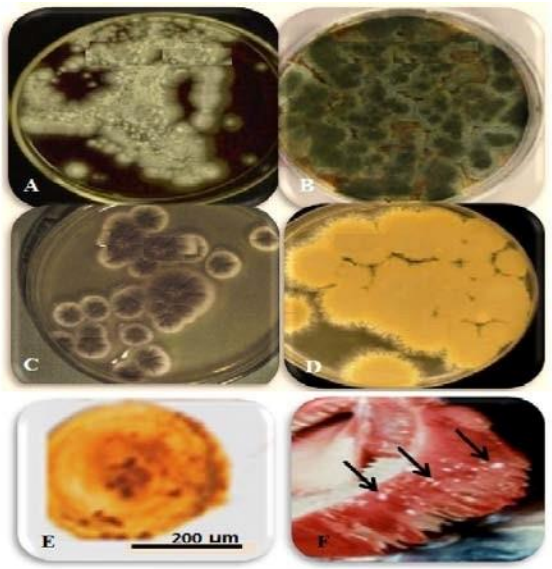

Fig. 2. The fungal species isolated from *O. aureus*: A: *Aspergillus* sp., B: *Alternaria* sp., C: *Mucor* sp., D: *Penicillium* sp., E: *Brachiomyces* sp. and F: *Ichthyophonus* sp.

Fungal infection was studied in *O. aureus*. Six fungi *Aspergillus* sp., *Alternaria* sp., *Mucor* sp., *Penicillium* sp., *Brachiomyces* sp. and *Ichthyophonus* sp. were isolated from the head, skin, gills, abdomen, caudal fin, dorsal fin and pectoral fin of these fish samples. *Aspergillus* sp. was the most prevalent fungus infecting all the organs of *O. aureus*, followed by *Mucor* sp. and *Brachiomyces* sp.

Discussion

The infection observed on gills may lead to serious disease condition, and such fishes cannot be treated and these fishes eventually die[4]. Gill infection may interfere with respiratory function of the fish, However, skin and fin infection are considered less serious as compared to gills[6,14]. These fungi may not be considered as non-pathogenic, but they can be better understood as opportunistic fungi[15]as many of them possess virulence factors, which enable them to cause disease, especially under predisposing conditions[16]. Fin infection is considered less pathogenic as such fishes survive but this infection may lead to complete damage of the fins[17, 18]. The single most affected site was gills. The infection on sensitive areas like gills of fish may lead to serious disease conditions[17].

The poor management of fish ponds increases the chances of fungal infection in fishes[19]. This is indicated by isolation of *Aspergillus* sp. from aquarium water[20]. Source of fungal infection may be the consumption of contaminated feed present in the pond. Moreover, the decomposition of this feed may also add to infection[21]. There might be certain other conditions in the pond which increase the possibility of fungal infection including: poor pond management, injured fish or fish having other diseases, or large amounts of decomposing organic matter in pond[10].

In our study, isolation of *Aspergillus* sp., *Alternaria* sp., *Mucor* sp., *Penicillium* sp., *Brachiomyces* sp. and *Ichthyophonus* sp. from fish samples has given an indication of pond contamination. Source of fungal infection may be the consumption of contaminated feed present in the pond. Moreover, the decomposition of this feed might have also added to infection[22]. There might be certain other conditions in the pond including injured fish or fish having other diseases, or large amounts of decomposing organic matter in pond[23, 24]. Hence, attention must be paid to carry out; good pond and fish health management, through the use of good quality inputs such as feed and water.

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