



Isolation and Identification of Pathogenic Fungi from *Cyprinus carpio* L. (1758) in Al-Nasiriya City

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Abstract

Thirty samples of *Cyprinus carpio* were collected from Al-Nasiriya fish ponds during the period from March until November 2017. Seventeen fish samples showed fungal infection. In this study eight species were identified and the most common were *Aspergillus niger*, *Saprolegnia* sp., *Achlya proliferata*, *Alternaria alternata*, *Penicillium funigulosum*, *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Aspergillus flavus*. *Aspergillus Niger* and *Saprolegnia* sp. were the most prevalent fungi infecting all the organs of *Cyprinus carpio*. Gills and abdomen had higher infection than rest of the organs.

Keywords: Contaminant, Disease, Environment, Fishes, Fungi, Ponds.

Introduction

Most fungi attack the external tissues and only few fungi infect the internal organs of fish. There are relatively few fungi that affect fish. Many fungi cause fish diseases such as *Alternaria* sp. was isolated from *Carassius auratus* [1], *Xiphophorus maculatus* and *Poecilia reticulata*, *Aspergillus*, isolated from *Carassius auratus*, *Mucor* and *Rhizopus* from *Cyprinus carpio* [2]. Mycotic infections associated with Saprolegniaceae are widely reported in freshwater fish. They are rarely found in brackish water [3, 4].

Isolated six fungal species, three as *Aphanomyces invadans*, *Achlya bisexualis*, and *Phialemonium dimorphosporum* from the gills of Channel catfish fry [5]. Isolated 15 fungal species as *Aphanomyces* spp. and *Saprolegnia salmonis* from eggs of *Coregonus lavaretus holsatus*. More than 80 isolates from different kinds of aquatic fungus belong to *Saprolegnia*, *Pythium*, *Thraustotheca*, *Achlya*, *Aphanomyces*, *Dictyuchas* and *Protachlya* were identified as special parasites of temperate fish in India. From all of these *Saprolegnia* and *Achlya* was the most virulent parasite in comparison to the others [6]. *Aspergillus* sp., *Penicillium* sp., *Absidia* sp. and *Pseudallescheria* sp. are reported contaminated trout pellet feed [7, 10].

Studied the fungal infection of rainbow trout eggs in Iran and indicated that *Saprolegnia parasitica* was the most important fungal species causing infestation in salmon hatcheries. *Aspergillus Niger* is human pathogen and environmental contaminant [9, 10]. This leads to internal and external infection in fish [11, 12]. *Penicillium* is a fungus exist in nature (soil and sediments) but did not isolate from fishes as a pathogenic agent, some types of *Penicillium* are able to make pathogenic signs in fish. The study was aimed to investigate the fungal infections in *Cyprinus carpio*.

Material and Methods

During the period from March until November 2017, a total of 30 fish samples of *Cyprinus carpio* were collected from local fish ponds in Al-Nasiriya city-South of Iraq. Sampling of infected fish was carried out by collecting the fish in Polythene bags. These were brought to the laboratory in living conditions. Infected fishes usually have white fluffy appearance and bloody spot at the site of infection. The fungal culture medium plates were prepared by using two different media, (13 g/200mL) Malt extract agar (MEA), and (7.8 g/200mL) Potato extract agar (PEA).

The fish body was divided into two parts; anterior part (head and gills) and Posterior part (all fins and rest of the body). Infected fish tissues were sterilized with 1% alcohol for 5 min and then rinsed with sterilized water. A 10 g tissue portion of fish was cut from the different region with a sterile forceps, macerated aseptically in a mortar and mixed in 10 ml of sterile peptone water. From this mixture, further fourfold 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 chloramphenicol to inhibit bacterial growth.

Plates were incubated at $25 \pm 2^\circ \text{C}$ and examined daily for 7 days. Slides were prepared from each colony and stained with lactophenol. The slides were observed under Digipro-labomed microscope and photographed. The fungi were identified with the help of available fungal identification key of [13]. 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 serial dilutions. The prevalence of fungi (%) was calculated according to the following equation [14]:

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

Results

The species of fungi distributed in (30 samples) of *Cyprinus carpio* included *Saplolegnia sp.* (30% samples), *Achyla proliferata* (23 samples) *Aspergillus sp.* (40% samples), *Alternaria alternata* (27% samples), *Penicillium funigulosum* (25% samples), *Rhizopus stolinifer* (28% samples), *Aspergillus fumigatus* (17% samples) and *Aspergillus flavus* (13% samples). These results were similar with the findings of [1] which showed that three genera of *Aspergillus*, *Rhizopus* and *Mucor* were

isolated eighty five cultured plates from *P.reticulata.*, and also obtained that incidence of *Rhizopus spp.* Was high in *P. reticulata* on caudal fin, head, eye, abdomen, pectoral fins. Details of mycofloran isolated from head, skin, gills, abdomen, caudal fin, dorsal fin and pectoral fin are shown in (Fig. 1). Gills and abdomen had higher infection than rest of the organs (Table 1). *Aspergillus sp.* and *Saplolegnia sp.* were the most prevalent fungi infecting these fishes (Table 1) and Fig.1. Caudal fins were completely damaged by *Aspergillus Niger*.

Table .1: The fungal species isolated from *C. carpio* 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	<i>Aspergillus niger</i>	6.4×10^3
Gills, Abdomen, Caudal fin	<i>Penicillium funigulosum</i>	3.2×10^3
Head, Skin, Gills, Abdomen , Dorsal fin , Pectoral fin	<i>Achyla proliferata</i>	2.9×10^3
Gills, Abdomen , Caudal fin	<i>Rhizopus stolinifer</i>	4.1×10^3
Gills	<i>Saplolegnia sp.</i>	4.9×10^3
Abdomen	<i>Alternaria alternata</i>	3.9×10^3
Dorsal fin	<i>Aspergillus fumigatus</i>	2.3×10^3
Abdomen	<i>A.flavus</i>	1.2×10^3

Fungal infection was studied in *Cyprinus carpio*. six fungi *Aspergillus niger*, *Alternaria alternate*, *Saplolegnia sp.*, *Achyla proliferata*, *Rhizopus stolinifer*, *Penicillium*

funigulosum, were isolated from the head, skin, gills, abdomen, caudal fin, dorsal fin and pectoral fin of these fish samples, but *Aspergillus flavus*, was appear only in

abdomen and *Aspergillus fumigatus* appear in dorsal fin only. *Aspergillus niger*,

Saprolegnia sp. was the most prevalent fungi infecting all the organs of *Cyprinus carpio*.

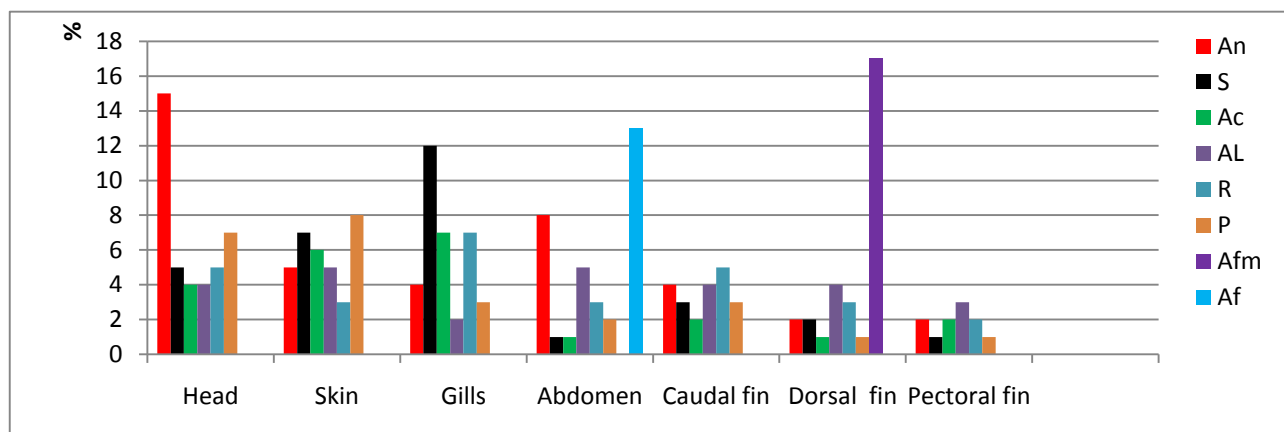


Fig.1: The prevalence of fungal species: the frequency of isolation (%) from *C. carpio*. An.: *Aspergillus niger*, S : *Saprolegnia sp.*, Ac : *Achyla proliferata*, AL : *Alternaria alternate*, R: *Rhizopus stolonifer*, P : *Penicillium funigulosum*, Afm : *Aspergillus fumigatus*, Af : *Aspergillus flavus*

Discussion

The infection observed on gills may lead to serious disease condition, and such fishes cannot be treated and these fishes eventually die [2]. Gill infection may interfere with respiratory function of the fish. 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,

The poor management of fish ponds increases the chances of fungal infection in fishes [16]. This is indicated by isolation of *Aspergillus sp.* From aquarium water [17]. Source of fungal infection may be the consumption of contaminated feed present in the ponds. Moreover, the decomposition of this feed may

also add to infection [18]. 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 [19]. Fungal load increases significantly during storage period of feed at high moisture levels in ground and tree nuts [20].

Fish feed stored under tropical conditions is contaminated with *Aspergillus flavus*, hence the toxins produced by the fungus may be deposited on feed pallets. If such contaminated feed is consumed by the fish, it may cause acute deleterious effects, which may lead to mass mortalities [21, 22].

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