



## Full Length Article

# Incidence of Fungal Infection of Freshwater Ornamental Fish in Pakistan

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## Abstract

An investigation was conducted on fungal diseases of three imported freshwater ornamental fish *Carassius auratus* (L.), *Xiphophorus maculatus* (Günther) and *Poecilia reticulata* (Peters). A total of 45 fish specimens, were dissected. Different body parts of fish were infected and showed clinical signs. Clinical picture of *C. auratus* showed that scales were eroded and haemorrhage and lesions were on abdomen. Dorsal and caudal fin were disintegrated. In *X. maculatus* eyes and mouth was covered with fungal hyphae. The clinical picture of *P. reticulata* showed that gills were whitish due to the presence of fungal hyphae and caudal fin also had cotton wool like margin. The fungi isolated from the infected areas on the fish were cultured on different media, Malt extract agar (MEA), Sabouraud dextrose agar (SDA) and Potato dextrose agar (PDA). The fungus was allowed to grow for 5–7 days at 28–30°C. White, grey, black, brown, green and orange colors of colonies were observed. The slides were prepared and stained with 0.05% Trypanblue in lactophenol. The fungal infection in *C. auratus*, *X. maculatus*, and *P. reticulata* were (80%), (80%) and (70%), respectively. Five genera of fungi were identified. Species of *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium* and *Alternaria* were isolated from *C. auratus*. Species of *Aspergillus*, *Rhizopus*, *Mucor* and *Alternaria* were isolated from *X. maculatus*. Species of *Aspergillus*, *Mucor* and *Rhizopus* were isolated from *P. reticulata*. Incidence of *Aspergillus* spp. was (47%), *Rhizopus* spp. (23.3%), *Mucor* spp. (10.3%), *Penicillium* spp. (8%) and *Alternaria* sp. (1.3%). Incidence of *Aspergillus* spp. was highest in the infected fish sample. Posterior part of the fishes had significantly higher ( $\chi^2 = 0.4$ ,  $P = 0.02$ ) infection as compared to anterior part of the fishes. This study showed that fungi isolated from different freshwater ornamental fishes are considered as normal mycoflora but they can cause infection. © 2014 Friends Science Publishers

**Keywords:** Fungal infection; *Aspergillus*; *Rhizopus*; *Mucor*; *Carassius auratus*; *Xiphophorus maculatus*; *Poecilia reticulata*

## Introduction

National and International transference are the main causes of parasites dissemination in ornamental fish and other native fish. The presence of parasites in this kind of activity are reported in China (Kuo *et al.*, 1994), Germany (Moravec *et al.*, 1999), Australia (Evans and Lester, 2001), South Africa (Mouton *et al.*, 2001), France (Michel *et al.*, 2002), Korea (Kim *et al.*, 2002), Norway (Levsen *et al.*, 2003) and Sri Lanka (Thilakarathne *et al.*, 2003). Conroy *et al.* (1982) observed that ornamental fish during shipment and at holding facilities are affected by bacterial and mycotic infections, as well as parasitic infestation. Most fungi attack the external tissues and only few fungi infect the internal organs of fish. There are relatively few fungi that affect fish. Mycotic infections associated with Saprolegniaceae are widely reported in freshwater fish. They are rarely found in brackish water (Hussein *et al.*, 2002).

Vandersea and Litaker (2007) isolated six fungal species, three as *Aphanomyces invadans*, *Achlya bisexualis*, and *Phialemonium dimorphosporum* from the gills of Channel catfish fry. Czczuga *et al.* (2004) isolated 15

fungal species as *Aphanomyces* spp. and *Saprolegnia salmonis* from eggs of *Coregonus lavaretus holtsatus*. 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 (Sati and Khulbe, 1991). *Aspergillus* sp., *Penicillium* sp., *Absidia* sp. and *Pseudallescheria* sp. are reported contaminated trout pellet feed (Diaz *et al.*, 2009; Cutuli *et al.*, 1991; Alinezhad *et al.*, 2011).

Firoozbakhsh *et al.* (2005) isolated *Branchiomyces*, *Phoma* and *Exophiala* from *Cyprinus carpio*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* in north Iran. Ebrahimzadeh *et al.* (2007) 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 (Alinezhad *et al.*, 2011; Saleemi *et al.*, 2012). This leads to internal and external infection in fish (Firoozbakhsh *et al.*,

2005; Ebrahimzadeh *et al.*, 2007). *Penicillium* is ubiquitous fungi in nature 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 three imported aquarium freshwater ornamental fishes.

## Materials and Methods

The experimental samples were obtained from the pet shops of local markets in Lahore city and brought to laboratory. These were examined for the fungal infection. The fishes were kept in aquariums containing freshwater and oxygen supply at room temperature 25 -30°C. Fish were not fed during their maintenance in the laboratory. Total length (TL) and body weight (BW) was measured and health status of every fish was observed (Table 1). Infected fishes usually have white fluffy appearance and bloody spot at the site of infection. The fungal culture medium plates were prepared by using different agars (13 g/200mL) Malt extract agar (MEA), (10 g/200mL) Sabouraud dextrose Agar (SDA) and (7.8 g/200mL) Potato extract agar (PEA). The fish body was divided into two parts; anterior part (head, eyes 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. The fungus from infected body part of fish taken and inoculated on prepared agar plates. The inoculated plates placed at 28-30°C and result noted on the 7<sup>th</sup> day and different colonies observed. Slides were prepared from each colony and stained with 0.05% trypanblue in lactophenol. The slides were observed under Digiopro-labomed microscope and photographed. The fungi were identified with the help of available fungal identification key of Klick (2002).

## Statistical Analysis

Data obtained from infection sites from posterior and anterior part were statistically analyzed by Chi-square test.

## Results

A total of 45 ornamental fishes were examined (Table 1). In present study five fungal genera *Aspergillus* spp. (47%), *Rhizopus* spp. (24.3%), *Mucor* spp., (10.3), *Penicillium* spp. (8%) and *Alternaria* spp. (1.3%) were identified from *C. auratus*, *X. maculatus* and *P. reticulata* (Tables 2, 3 and 4).

### Fungal Infection of *C. auratus*

Clinical symptoms of *C. auratus* showed that caudal fins had acquired white edges; scales were eroded near caudal peduncle, tip of dorsal fin edges shredded usually with light color edges, similarly, anal fin edges disintegrated. Scales were eroded from the abdomen; haemorrhages and lesion were present all over the body surface with whitish edges which had fungal hyphae (Fig. 1A) and this sample had 80% infection. Disintegration began at the tip of caudal fin

and other fins. This infection moved towards the body. Typical fungal lesions were present on surface of the skin and were superficial, not penetrated deep into the muscles. Five fungal genera *Aspergillus* spp., *Rhizopus* spp., *Mucor* spp., *Penicillium* spp. and *Alternaria* spp. were identified from *C. auratus* (Fig. 1C, 1D, 1E and 1F).

Mixed infections were seen with different combination of fungal genera in *C. auratus*, *Aspergillus* spp., *Mucor* spp. and *Rhizopus* spp.<sup>1</sup>; *Penicillium* spp. and *Aspergillus* spp.<sup>2</sup>; *Mucor* spp. and *Alternaria* spp.<sup>3</sup>. From one hundred cultures plates, incidence of *Aspergillus* spp. was (51%), *Rhizopus* spp., (27%), *Mucor* spp., (12%), *Penicillium* spp., (8%) and *Alternaria* spp., (2%) in *C. auratus* (Table 2). The most prevalent genus was *Aspergillus* spp. Abdomen, dorsal fin and caudal fin were the most infected organs for *Aspergillus* spp. attack. Specifically *Alternaria* spp. was isolated from the infected eye of fish. *Penicillium* spp. was only seen in *C. auratus*. Posterior part ( $\chi^2= 0.3$  P=0.02) of the fish had significantly higher infection (53%) (P= 0.03) as compared to anterior part of the fish.

### Fungal Infection of *X. maculatus*

Clinical symptoms of *X. maculatus* showed that anterior part of the infected fish was under severe attack than the posterior part. Caudal fin had cotton like margins with bloody lesions. Gills were whitish with fungal attack.

Four fungal genera were isolated in *X. maculatus*. From seventy prepared culture plates *Aspergillus* spp. (49%, Fig. 2C and D), *Rhizopus* spp. (13%), and *Mucor* spp. (6%) and *Alternaria* spp. (2%) were isolated in *X. maculatus* (Table 3). Different combination of fungal genera were seen *Aspergillus*, *Rhizopus* and *Mucor*; *Aspergillus* and *Alternaria*<sup>2</sup>. Abdomen and caudal fins were badly infected by *Aspergillus* spp. Posterior part of the fish had significantly higher infection ( $\chi^2=0.39$ , p=0.02) as compared to anterior part of the fish.

### Fungal Infection of *P. reticulata*

Clinical symptoms of *P. reticulata* showed that the caudal fin was completely eroded; scales of the fish were eroded from anterior to posterior part. Eyes and mouth were completely covered with fungal hyphae; pectoral and pelvic fins damaged were damaged too (Fig. 1B).

Three genera *Aspergillus*, (Fig. 2A and B), *Rhizopus* (Fig. 2E and F), and *Mucor* spp., were isolated from *P. reticulata*. From eighty five cultured plates, incidence of *Aspergillus* spp. (39%), *Rhizopus* spp. (33%) and *Mucor* spp. were (13%) in *P. reticulata* (Table 4). Combinations of fungal genera were, *Rhizopus* and *Mucor*; *Aspergillus* and *Rhizopus*<sup>2</sup>. Incidence of *Rhizopus* spp. was high in *P. reticulata* on caudal fin. Head, eye, abdomen, pectoral fins and caudal fins were completely damaged by *Aspergillus* spp. Posterior part of the fish had significantly higher infection ( $\chi^2=0.6$ , p=0.03) as compared to anterior part.

**Table 1:** Fungal infection in fish

Fish species	No. of fish	No. of infected fish	Fish Infection	Mean TL (cm)	Mean weight (g)	Site of infection in fish
<i>Carassius auratus</i>	25	20	80%	11.8 ±4.1	21.32 ±8.3	Head, eye, gills, abdomen, caudal fin
<i>Xiphophorus maculatus</i>	10	8	80%	3.7 ±2.9	1.47 ±1.0	Eye, gills, abdomen, caudal fin
<i>Poecilia reticulata</i>	10	7	70%	4.1 ±3.3	2.65 ±2.1	Mouth, Head, eye, gills, abdomen and all fins

**Table 2:** Fungi isolated from different organs of *Carassius auratus*

Fish organs	No. of plates	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Rhizopus</i> spp.	<i>Mucor</i> spp.	<i>Alternaria</i> spp.
Head	10	9	X	1	x	X
Eyes	12	6	5	X	x	1
Abd	15	7	X	8	x	X
Gills	8	6	X	2	x	X
Drf	16	X	x	7	9	X
Pvf	5	2	X	X	2	1
Pcf	9	5	1	3	x	X
Cdf	15	6	2	6	1	X
Anf	10	10	X	X	x	X
Total	100	51	8	27	12	2

**Table 3:** Fungi isolated from different organs of *Xiphophorus maculatus*

Fish organs	No. of plates	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Rhizopus</i> spp.	<i>Mucor</i> spp.	<i>Alternaria</i> spp.
Head	8	6	X	2	X	X
Eyes	10	7	X	3	X	X
Abd	12	8	X	2	X	2
Gills	8	6	X	2	X	X
Drf	5	5	X	X	X	X
Pvf	6	4	X	X	2	X
Pcf	4	1	X	X	3	X
Cdf	12	8	X	3	1	X
Anf	5	4	X	1	X	X
Total	70	49	0	13	6	2

**Table 4:** Fungi isolated from different organs of *Poecilia reticulata*

Fish organs	No. of plates	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Rhizopus</i> spp.	<i>Mucor</i> spp.	<i>Alternaria</i> spp.
Head	16	10	X	4	2	X
Eyes	12	12	X			X
Abd	10	4	X	2	4	X
Gills	10	2	X	4	4	X
Drf	8	2	X	4	2	X
Pvf	5	1	X	3	1	X
Pcf	6	2	X	4	x	X
Cdf	12	5	X	7	x	X
Anf	6	1	X	5	x	X
Total	85	39	0	33	13	0

**Note:** Abbreviation used: Abd= abdomen; drf=dorsal fin; pcf=pectoral fin; pvf= pelvic fin; cdf=caudal fin; Anf= anal fin

## Discussion

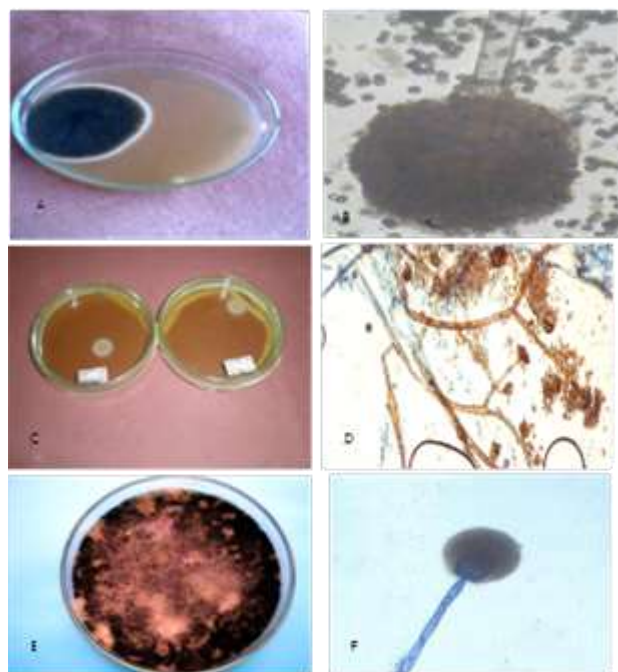
Freshwater imported ornamental fishes *Carassius auratus*, *Xiphophorus maculatus* and *Poecilia reticulata* were examined for fungal infection. Thirty five fishes showed infection out of forty five, which comprised 80% fungal incidence. Five fungal genera *Aspergillus* spp., *Mucor* spp., *Rhizopus* spp., *Penicillium* spp. and *Alternaria* spp. were isolated from these fish. Mixed fungal infection was observed with three combinations as *Aspergillus* spp., *Mucor* spp. and *Rhizopus* spp; *Penicillium* spp. and *Aspergillus* spp.; *Mucor* and *Alternaria* sp. in *C. auratus*. Two combinations as *Aspergillus* spp., *Rhizopus* spp. and *Mucor* spp.; *Aspergillus* sp. and *Rhizopus* spp. were

observed in *Poecilia reticulata*.

The most prevalent genus was *Aspergillus* spp. which was isolated from *C. auratus*; *X. maculatus*. The prevalence of *Rhizopus* spp. was high in *P. reticulata*. *Alternaria* spp. was isolated from both *C. auratus* and *X. maculatus* while *Penicillium* spp. was isolated from *C. auratus* only.

Fifty one percent of *Aspergillus* spp. infection was observed from *C. auratus*. Posterior part of the fish was badly infected with *Aspergillus* attack. Abdomen of the *C. auratus* was infected with *Rhizopus* spp. Dorsal fin of *C. auratus* was mostly infected with *Mucor* spp. *Penicillium* spp. was isolated from eyes, pectoral and anal fin. *Penicillium* spp. was only isolated in *C. auratus*.

Forty nine percent infections were due to *Aspergillus*



**Fig. 2A:** A Colony of *Aspergillus* sp. on SDA isolated from caudal fin of *P. reticulata*. **B:** *Aspergillus* sp. With its round reproductive head and black conidia. **C:** A colony of *Mucor* sp. on MEA isolated from gills of *X. maculatus*. **D:** *Mucor* sp. with its sporangiophores. **E:** A Colony of *Rhizopus* sp. on PEA isolated from pelvic fins of *P. reticulata*. **F:** *Rhizopus* sp. with its pin head sporangium

spp. was isolated from *X. maculatus* from head, eyes, abdomen, gills and all fins. Caudal fin of the fish was infected with *Rhizopus* spp. *Mucor* spp. was isolated from the pectoral, pelvic and caudal fin, while *Alternaria* spp. was isolated from the abdomen only.

Three fungal genera (*Aspergillus* spp., *Rhizopus* spp. and *Mucor* spp.) were isolated from head, gills, abdomen, dorsal fin and pelvic fin. Head and eyes were the most specific sites for *Aspergillus* attack. *Rhizopus* spp. was isolated from caudal fin, while *Mucor* spp. was isolated from gills and abdomen of the *P. reticulata*.

The posterior part of the fish had significantly higher ( $\chi^2 = 0.4$ ,  $P = 0.02$ ) infection as compared to anterior part of fish (35% infection). Infected fishes had whitish fungal hyphae, lesion and haemorrhage on infected sites. The most infected sites were head and eyes. The infection on these sensitive areas of the fish may lead to serious disease condition (Iqbal and Mumtaz, 2013a, b). The treatment of infection is impossible, if the infection penetrate from eye to head because it may cause complete blindness and eventually the fishes die (Srivastara, 2009). Infection of fins in fishes is less pathogenic but the infection may lead to complete damaged of the fins (Iqbal *et al.*, 2012a).

Refai *et al.* (2010) reported 2081 fungal isolates from



**Fig. 1A:** Eroded scale of *Carassius auratus*. **B:** Eroded caudal fins of *Poecilia reticulata* and whitish fungal hyphae on fins and mouth. **C:** A colony of *Penicillium* sp. on PDA isolated from *C. auratus*, **D:** *Penicillium* sp. showing brush like caudal arrangement of fruiting head. **E:** A colony of *Alternaria* sp. on MEA isolated from *C. auratus*. **F:** *Alternaria* sp. branched acropetal chain of multicelled conidia, conidia is ovoid in shape

diseased and apparently healthy fish samples. Isolated moulds belonged to the following genera: *Saprolegnia*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Scopulariopsis*, *Paecilomyces* and *Curvularia* from *Oreochromis* sp. and *Clarias gariepinus*. Shahbazian *et al.* (2010) isolated 17 species of fungi from the rainbow trout eggs. Five fungal species isolated belonged to the saprolegniaceae family. Other fungi were *Fusarium oxysporum*, *F. nipoense*, *Fusarium* sp., *penicillium citrinum*, *Aspergillus treuse*, *Aspergillus clavatus*, *Cladosporium* sp., *Alternaria* sp., *Helmintosporium* sp. and *Mucor* sp. In another study Fadaeifard *et al.* (2011) isolated eight genera from eggs and brood stock of rainbow trout and the most common were *Penicillium* sp., *Acremonium* sp., *Alternaria* sp., *F. solani*, *Aspergillus* sp., *Mucor* sp., *Saprolegnia* sp. and *Cladosporium* sp. Iqbal *et al.* (2012a) observed two fungal genera *Saprolegnia* and *Achlya* from grass carp *Ctenopharyngodon idella*, and *Catla catla*. Iqbal *et al.* (2012b) investigated fungal infection in four species of carps i.e., *C. auratus*, *Hypophthalmichthys molitrix*, *Labeo rohita* and *Ctenopharyngodon idella*. Infected fishes showed clinical signs such as fungal growth on various organs of fish, hemorrhages on body surface and abdominal

distention. They isolated five fungal species, *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Blastomyces* spp. and *Rhizopus* spp.

*Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp. are normal mycoflora are able to cause infections (Refai *et al.*, 2010). Though most fungi regarded as opportunistic pathogen but few of them cause diseases such as Saprolegniasis, Aspergillosis, Scopulariopsis, Paecilomycosis and Penicillium infection (Refai *et al.*, 2010). Environmental variables influence the intensity of aquatic fungal infections (Fadaeifard *et al.*, 2011). These fungal species are presumably infectious and spread through the contamination of fish feed (Saleem *et al.*, 2012).

In conclusion, isolated fungi from diseased and apparently healthy fish *C. auratus*, *P. reticulata* and *X. maculatus* are considered normal mycoflora but these are responsible to produce diseases under particular environmental conditions. Though these are normal mycoflora but need more attention from the health point of view of ornamental fish.

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