Fungal Infections in Some Economically Important Freshwater Fishes

Zafar Iqbal*, Uzma Sheikh and Rabia Mughal

Department of Zoology, University of the Punjab, Lahore, 54590, Pakistan

*Corresponding author: dr.zafariqbal.pu@gmail.com

ARTICLE HISTORY
Received: September 12, 2011
Revised: January 22, 2012
Accepted: February 18, 2012

Key words:
Carassius auratus
Ctenopharyngodon idella
Fungal infections
Hypophthalmichthys molitrix
Labeo rohita

ABSTRACT
Aim of this study was to investigate fungal infections in four species of carps including goldfish, *Carassius* (*C.* *auratus* L.; silver carp, *Hypophthalmichthys* (*H.*) *molitrix* Richardssons; rahu, *Labeo* (*L.*) *rohita* Hamilton and *Ctenopharyngodon* (*C.*) *idella* Valenciennes. Nine specimens of each species were studied for the presence of fungal infections. Infected fishes showed clinical signs such as fungal growth on skin, fins, eyes, eroded fins and scales, hemorrhages on body surface and abdominal distension. The specimens from infected organs of fish were inoculated on each, malt extract, Sabouraud dextrose and potato dextrose agars. The fungal colonies of white, black, green, grey and brown colors were observed in the agar plates. Slides were prepared and stained with 0.05% Trypan blue in lactophenol. *C. auratus* showed the highest infection rate (44.4%) followed by *H. molitrix* and *L. rohita* (11.1% each). Five fungal species viz. *Aspergillus* (33.3%), *Penicillium* (22.2%), *Alternaria* (27.7%), *Blastomyces* spp (11.1%) and *Rhizopus* (5.5%) were isolated. Posterior part of the fish had significantly (P=0.05) higher (62.5%) infection as compared to anterior part (37.5%). The caudal fin with 31.25% infection was the single most affected area. This study showed that most of the fungi isolated from fishes are considered as normal mycoflora, yet many fungi can cause natural infections in ponds and aquarium.

INTRODUCTION

Freshwater fishes are an important protein source for people of many countries (Hussain et al., 2011; Rubbani et al., 2011). However, globally fish from freshwater and marine sources are in severe decline, driven in large part by economic and human population growth (Limburg et al., 2011). Fish farming in various parts of the world has increased many folds in the last decade. As a result, fish culture has now become commercially an important industry worldwide. In the Punjab province of Pakistan, five species of carps including *H. molitrix*, *C. idella*, *L. rohita*, *Catla catla* Hamilton and *Cirrhinus mirgala* Hamilton are cultured. The establishment of 7829 fish farms (area 45650 acres) in private sector is an indicator of rapid growth of fisheries sector (Khan et al., 2011). The growth of fish culture has also raised issues of fish health. Bacterial hemorrhagic septicemia, lemaecasis, saprolegniasis and anoxia are the most commonly occurring fish diseases in pond fishes in Punjab (Iqbal et al., 2001).

Ornamental fish keeping has become an increasingly popular hobby worldwide. The trade of ornamental fish is a multi-million dollar industry now. Twenty species of ornamental fishes are imported from Southeast Asian countries into Pakistan (Ahmad, 1996). These fishes are transported alive and sold to the hobbyists by the pet shops. Fungi are known to attack fish eggs, fry, fingerlings and adult fish. Water molds infections cause losses of freshwater fishes and their eggs in both natural and commercial fish farms (Bangyeekhun and Sylvie, 2001). The fungal diseases occur in brood stock and all life stages of fish and eggs. Fungal infection cause low productivity of fry and low production in fish culture (Kwanprasert et al., 2007). The mortality rate due to fungal infection may reach some time up to 80-100% in incubated eggs (Chukanhom and Hatai, 2004). According to Akande and Tobor (1992) post-harvest handling of fishes may also result in infection with microorganisms such as bacteria and fungi. The ubiquitous fungi are part of the normal mycoflora of fresh and estuarine ecosystems and have a worldwide distribution. However, there are certain fungi which cause fish diseases. Shahbazian et al. (2010) isolated *Penicillium expansum*, *Penicillium citrinum*, *Aspergillus terruse*, *Aspergillus cli-
vatus; Alternaria spp; Saprolegnia parasitic, Saprolegnia laponica, Saprolegnia ferox and Saprolegnia hypogyna and 7 other species of fungi from infected eggs of rainbow trout, Oncorhyncus mykiss in Iran. However, Fadaeifard et al. (2011) isolated 8 species of fungi from eggs and brood stock of rainbow trout O. mykiss. These isolates were Penicillium spp, Acremonium spp, Alternaria spp, Fusarium solani, Aspergillus spp, Mucor spp, Saprolegnia spp. and Cladosporium spp. Primary infections in fishes and fish eggs by oomycetes are also reported (Walser and Phelps, 1993). Although, infection as a result of microbial contamination does not usually result in disease but environmental stress may upset the balance between the potential pathogens and their hosts. Under such conditions the chances of infection increases. This study was aimed to investigate the mycoflora associated with apparently healthy as well as diseased ornamental and culturable fishes.

MATERIALS AND METHODS

Nine goldfish, C. auratus were collected from a pet fish shop in Lahore and nine specimens each of culturable carps, grass carp (C. idella), silver carp (H. molitrix) and rahu (L. rohita) were obtained from Fish Research Farms, University of the Punjab, Lahore, Pakistan. The fishes were transported immediately to laboratory in sterile polyethylene bags in aerated aquarium/pond water. The fishes were kept separate in glass aquaria with continuous air supply at ambient temperature. Total length (TL) and weight (Wt) of each specimen was measured and health status of every individual fish was also observed. The fish body was divided into two parts; Anterior part (head, eyes and gills) and posterior part (all fins and rest of the body) to note the infection site and data thus obtained was analyzed by Chi-square test.

For culturing of fungal specimens, three different types of media including malt extract agar (MEA), Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) were prepared and streptomycin sulphate was supplemented to each preparation of media to avoid bacterial contamination. The body surfaces of all the fishes under study were disinfected by dipping each fish in 1% formaldehyde for 1 to 5 minutes followed by 70% alcohol and finally in sterile water in which it was thoroughly rinsed. The fungal isolates were collected from infected organs of fish with sterile needle and inoculated on malt extract (Oxoid, UK), Sabouraud dextrose (Oxoid, UK) and potato dextrose (M906-India) agars. The agar plates were incubated at 28-30°C and fungal growth was observed after 4-7 days. The fungal colonies of various colors were observed in the agar plates. For microscopic examination, slides were prepared from each colony and stained with 0.05% Trypan blue 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 (Willoughby, 1994).

RESULTS AND DISCUSSION

A total of 36 fishes of four species were examined (Table1). Fungal infection was observed in C. auratus, H. molitrix and L. rohita. However, no fungal infection was observed in C. idella (Table 1). The clinical picture of infected C. auratus showed fungal growth on head, gills, eyes and fins. Additionally, infected fishes had eroded scales and hemorrhages over body surface and moderate body distension. Tips of caudal fin were eroded in H. molitrix. Early infection was seen on caudal fin of L. rohita. Posterior part of the fish had 62.5% infection had significantly (χ²=3.38; P=0.05) more infection than anterior part (37.5%) infection and hence, the attack on both anterior and posterior part of the fish was not equal. The attack on the posterior part was always recorded higher because of having 85-90% more surface area. The single most affected site was caudal fin (31.25%). The infection on head, gills and eyes of fish may lead to serious pathological conditions as extensive growth of fungal hyphae in eyes may cause complete blindness and from eyes may penetrate into brain and in such condition the treatment is impossible and eventually the fishes die (Srivastava, 2009). Fin infection is considered less pathogenic as such fishes survive but this infection may lead to complete damage of the fins.

Table 1: Fungal species isolated from different organs of fishes.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Organ</th>
<th>Fungal colonies</th>
<th>Fungi isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. auratus</td>
<td>CDF</td>
<td>a (1)</td>
<td>Penicillium spp.</td>
</tr>
<tr>
<td></td>
<td>PCF</td>
<td>a (2)</td>
<td>Aspergillus spp.</td>
</tr>
<tr>
<td></td>
<td>PVCF</td>
<td>a &amp; b (2)</td>
<td>Alternaria spp.; Blastomyces spp.</td>
</tr>
<tr>
<td>C. idella</td>
<td>Eye</td>
<td>a &amp; b</td>
<td>Aspergillus spp.; Alternaria spp.</td>
</tr>
<tr>
<td></td>
<td>Gills</td>
<td>a (1)</td>
<td>Alternaria spp.</td>
</tr>
<tr>
<td></td>
<td>Head</td>
<td>a (3)</td>
<td>Aspergillus spp.; Penicillium spp.</td>
</tr>
<tr>
<td>C. auratus</td>
<td>CDF</td>
<td>a (1)</td>
<td>Rhizopus spp.</td>
</tr>
<tr>
<td></td>
<td>Ab</td>
<td>a (1)</td>
<td>Penicillium spp.</td>
</tr>
<tr>
<td>C. idella</td>
<td>CDF</td>
<td>a (1)</td>
<td>Aspergillus spp.</td>
</tr>
<tr>
<td></td>
<td>Gills</td>
<td>a (2)</td>
<td>Blastomyces spp.</td>
</tr>
<tr>
<td>H. molitrix</td>
<td>CDF</td>
<td>a (1)</td>
<td>Alternaria sp.</td>
</tr>
<tr>
<td></td>
<td>Head</td>
<td>a (2)</td>
<td>Alternaria spp.</td>
</tr>
<tr>
<td></td>
<td>Ab</td>
<td>a (1)</td>
<td>Aspergillus spp.</td>
</tr>
<tr>
<td>L. rohita</td>
<td>CDF</td>
<td>A</td>
<td>Aspergillus spp.</td>
</tr>
</tbody>
</table>

Note: fungal colony a (3) means, 3 colonies of which one ‘a’ was processed for identification; colony a&b (2) means, 2 colonies and both were processed. Abbreviations of organ studied: Pcf = pectoral fin; Pf = pelvic fin; Ann = anal fin; CdF = caudal fin; Ab = abdomen.

Five fungal genera viz. Aspergillus spp. (33.3%); Penicillium spp. (22.2%); Rhizopus spp. (5.5%) (Fig. 1, 2, 3); Blastomyces spp. (11.1%) and Alternaria spp. (27.7%) were isolated from C. auratus. Two genera Aspergillus spp. and Alternaria spp. were isolated from H. molitrix. From L. rohita only Aspergillus spp. was isolated (Table 2). Mixed fungal infection was also observed in C. auratus, in three combinations as Alternaria spp., Blastomyces spp., Aspergillus spp., Alternaria spp. and Aspergillus spp. Penicillium spp. The most prevalent genus was Aspergillus spp. It was isolated from C.
Fig 1: A. Colonies of Rhizopus on MEA, (fish 4, C. auratus). B, C, Rhizopus (from plate A) showing long branched sporangophore with sporangium bearing spores.

Fig 2: A. Colonies of Aspergillus on PDA, (fish 5, C. auratus). B. Aspergillus spp. (isolated from plate A) with round head and black conidia.

Fayioye et al. (2008) isolated five different species of fungi including Fusarium, Aspergillus, Rhizopus, Mucor, and Penicillium from 8 edible smoke-dried freshwater fishes. Junaid et al. (2010) isolated 7 fungal species from stockfish in Nigeria and these included A. flavus, A. fumigatus, A. niger, Trihophyton verrucosum, Rhizopus Mucor and Penicillium spp. and among these Mucor spp. showed the highest occurrence. In another study, fungi of eight different genera; Saprolegnia, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Scopulariopsis and Curvularia were isolated from two fish species, Oreochromis spp. and Claris gariepinas (Refai et al., 2010). Shabazian et al. (2010) isolated Penicillium expansum, Penicillium citrinum; Aspergillus terruse, Aspergillus clivatus; Alternaria spp. and 11 other fungal species from infected eggs of rainbow trout. Moreover, Fadaeifard et al. (2011) reported the occurrence of different fungal species of genera including, Penicillium, Acremonium, Alternaria, Fusarium, Aspergillus, Mucor, Saprolegnia and Cladosporium from the eggs and brood stock of rainbow trout.

Shabana (2002) reported Alternaria eichhorniae in Nile tilapia, Oreochromis spp. A fatal behavioral disorder of carp was attributed to cerebral infection with Alternaria spp. probably following exposure to adverse environmental conditions (Sharaburin and Bazderkina,
Blastomycosis is caused by Blastomyces dermatitidis. This infection occurs by inhaling fungal spores and it is increasingly recognized in immuno-compromised hosts (Pappas et al., 1993). Fungi like Aspergillus and Rhizopus spp. are opportunistic pathogens (Refai et al., 1990). Findings of Refai et al. (2010) as normal mycoflora and these spp. may be regarded as opportunistic pathogens (Refai et al., 2004) as many of them possess virulence factors which enable them to cause disease (Refai et al., 2010), especially under favorable predisposing conditions. Ecological differences play an important role in species diversity of fungi that develop on both fish and eggs (Hussein et al., 2001). Interaction of physiochemical factors generally has influence on the diversity of water molds (Paliwal and Sati, 2009).

Lack of good aquarium keeping in pet shops and fish farms increases the chances of fungal infection in fishes. The basic health management practices may be easily overlooked due to dearth of trained personal or resources. This study indicates that although most fungi isolated from fishes are considered as normal mycoflora, yet they can cause infections. This is confirmed by the presence of fungal hyphae in the lesions on the body of the fish. These findings point our attention to the possible role of fungi in affecting both ornamental and culurable fishes.

Acknowledgement: We are thankful to Dr. Abdul Nasir, Associate Professor, Department of Botany, University of the Punjab, Lahore for authentication of fungal species identified in the present study.

REFERENCES


