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RESEARCH ARTICLE

Fungal Infections in Some Economically Important Freshwater Fishes

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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* Richardsons; 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.

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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 mrigala* 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, lernaeasis, 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 citrinium; Aspergillus terruse, Aspergillus cli-*

vatus; Alternaria spp*; Saprolegnia parasitic, Saprolegnia lapponica, Saprolegnia ferax* and *Saprolegnia hypogyna* and 7 other species of fungi from infected eggs of rainbow trout, *Oncorhynchus 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, *Acreomonium* 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 aquariums 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 (M096-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% had significantly (χ^2=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.

Table1: Infection level and site of infection in fishes.

Fish Species	Fish Infection	Mean TL(cm)	Mean Wt (g)	Site of infection in fish
C. auratus	(44.4%)	10.75±3.32	19.46±8.10	Head, eyes, gills, fins, abdomen
H.molitrix	(11.1%)	19.78±5.33	146.33±99.80	Head, Abdomen, Caudal fin
L.rohita	(11.1%)	23.56±3.61	183.89±105.71	Caudal fin
C. idella	(0%)	21.50±3.41	127.44±56.76	No infection

Table 2: Fungal species isolated from different organs of fishes.

Fish species	Organ	Fungal colonies	Fungi isolated
C. auratus	Head	a (1)	*Penicillium* spp.
	CdF	a (2)	*Aspergillus* spp.
C. auratus	PcF	a & b (2)	*Penicillium* spp.
	PvF and AnF	a & b (2)	*Alternaria* spp.; *Blastomyces* spp.
	Eye	a & b	*Aspergillus* spp.; *Alternaria* spp.
	Gills	a (1)	*Alternaria* spp.
	Head	a (3)	*Aspergillus* spp.; *Penicillium* spp.
C.auratus	CdF	a (1)	*Rhizopus* spp.
	Ab	a (1)	*Penicillium* spp.
C.auratus	CdF	a (1)	*Aspergillus* spp.
	Gills	a (2)	*Blastomyces* spp.
H.molitrix	CdF	a (1)	*Alternaria* sp.
	Head	a (2)	*Alternaria* spp.
	Ab	a (1)	*Aspergillus* spp.
L.rohita	CdF	A	*Aspergillus* spp.

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; PvF= pelvic fin; Anf = 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.*

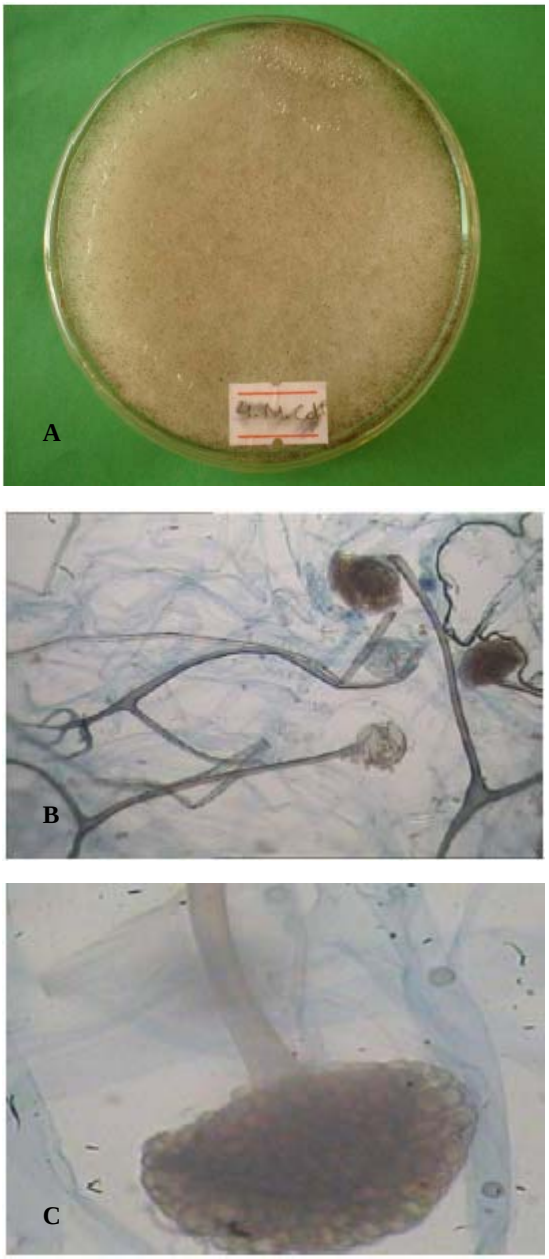

Fig 1: A, Colonies of *Rhizopus* on MEA,(fish 4,*C.auratus).***B**, **C,** *Rhizopus* (from plate–A) showing long branched sporangophore with sporangium bearing spores.

auratus; H. molitrix and *L. rohita. Alternaria* spp. was isolated from *C. auratus; H. molitrix* and *Penicillium* spp., *Blastomyces* spp., and *Rhizopus* spp. were isolated from *C. auratus* only (Table 2).

Aspergillomycosis has been principally described in African fish, especially the tilapia *Oreochromis* sp. (Olufemi, 1983). A number of *Aspergillus* species such as *A. flavus, A. japonicus*, and *A. terreus* are involved in this infection. These species presumably cause infection via entry into the fish through contaminated feed. Jalilpoor *et al.* (2006) reported infection of *Aspencer percicus* eggs with *Penicillium* spp., *Fusarium* spp., *Mucor* spp. and *Saprolegnia* spp. which caused 7 and 22% mortality of these eggs.

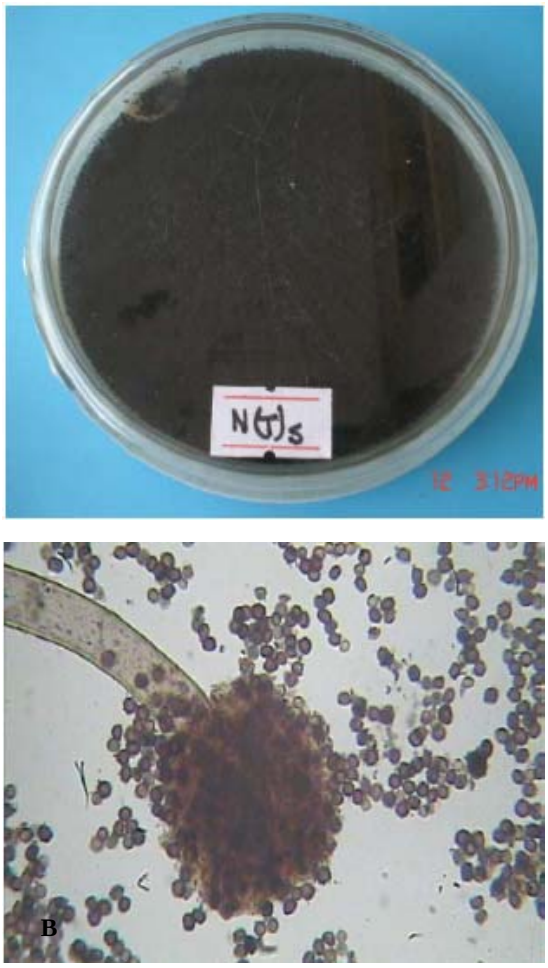

Fig 2: A, colonies of *Aspergillus* on PDA, (fish 5, *C.auratus*). **B.** *Aspergillus* spp. (isolated from plate A) with round head and black conidia.

Fayioye *et al*. (2008) isolated five different species of fungi including *Fusarium*, *Aspergillus, Rhizopus*, *Mucor*, and *Penicillum* 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 *Penicillum* spp. and among these *Mucor* spp. showed the highest occurrence. In another study, fungi of eight different genera; *Saprolegnia, Aspergillus, Fusarium, Mucor, Pencillium, Rhizopus, Scopulariopsis* and *Curvularia* were isolated from two fish species, *Oreochromis* spp. and *Claris gariepinas* (Refai *et al*., 2010). Shahbazian *et al.* (2010) isolated *Penicillium expansum, Penicillium citrinium; 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*, *Acreomonium*, *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,

1990). 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).

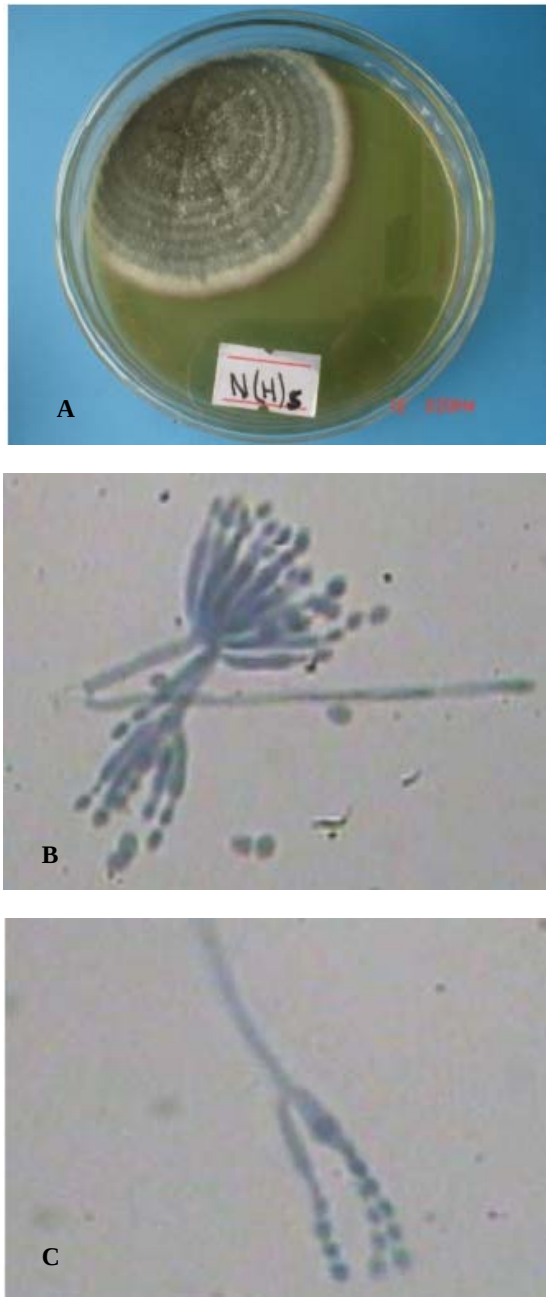

Fig 3: A. Colonies of *Penicillium* on SDA, (fish 1, *C. auratus*). **B, C,** *Penicillium* sp. showing brush like arrangement of fruiting head.

Findings of the present study are comparable to the findings of Refai *et al*. (2010), Shahbazian *et al.* (2010) and Fadaeifard *et al.* (2011). However, Refai *et al*. (2010) has characterized *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp. 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 over looked 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 culturable fishes.

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