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Research Article

Morphological and Pathological Effects of Aspergillosis in Silver Carp, *Hypophthalmichthys molitrix*

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Authors' Contributions
SS conducted the experiments, complied results and wrote paper. ZI designed the experiments and supervised the paper writing. ANK supervised the laboratory work and identified the fungi.

Keywords
Aspergillosis, Silver carp, *Aspergillus terreus*, Ponds

Abstract | Fungal diseases are very common in freshwater fishes. Aspergillosis is a fungal disease of fish. This study was designed to examine a fungal infection in silver carp, *Hypophthalmichthys molitrix*. The infected fish showed clinical signs such as: eroded gills, damaged fins and lesions all over the body. The fish specimens showed mild to severe infection. The infection caused by fungus was confirmed by isolating the pathogenic fungus from affected skin, gills, fins, heart, liver, kidney and intestine of fish and culturing it on four different culture media; Malt extract agar (MEA), Corn meal agar (CMA), Sabouraud dextrose agar (SDA) and Potato dextrose agar (PDA). The inoculated culture plates were incubated for 5-8 days at 28-32 °C. The fungal growth in the form of colonies of different shapes and colour appeared in agar plates. Four *Aspergillus* spp.; *Aspergillus terreus* (8.40%), *A. flavus* (17.06%), *A. fumigatus* (24.02%) and *A. niger* (50.52%) were isolated from fish. *Aspergillus niger* showed high infection and it was recorded on all 200 fish samples. Fins were the most affected parts with 39.10% infection. Intestine was the least affected part with 6.59% infection. The major reason of aspergillosis in silver carp is the use of unhealthy and unhygienic feed given to silver carp reared in earthern ponds. Due to aspergillosis fish may not be recommended for human consumption. If proper health management practices are adopted on fish rearing facilities, then the chances of incidence of aspergillosis in silver carp may be minimized.

Novelty Statement | This study has highlighted fungal infection in a commercial fish silver carp. The impact of fungal infection on fish and aquaculture industry is discussed. New data on fungal infection is added and contributed.

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Introduction

Fish is good source of animal protein throughout the world. Fish consumption is 11kg/capita/annum in world (Ayub, 2009). It is an important source of selenium and omega-3 fatty acids and also has lower levels of fats. Consumption of fish oil also benefits to enhance cardiovascular health and early perceptive development (Turyk *et al.*, 2012). Fish meat is also involved in the impediment of restenosis after angioplasty (Jenkin *et al.*, 2009). Among the saprophytic moulds, *Aspergillus* sp. plays an important role in the diseases of human, plants and animals. A number of *Aspergillus* species are found in soil, air and water (Ogorman, 2008). Initially mycoses were observed due to Oomycete especially *Saprolegnia*, but now aspergillosis is documented in freshwater fishes (Iqbal *et al.*, 2014; Podeti and Benarjee, 2016). Association between the fish mycoses and *Aspergillus* spp. are reported in India (Chauhan *et al.*, 2014). The causative agent of aspergillosis was considered as *A. terreus* and *A. flavus* and it was reported

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in *Labeo calbasu*. *Aspergillus* spores are stored in the mucus of fishes. *Aspergillus niger* and *A. flavus* spores were found in the mucus of *H. molitrix* (Balasubramanian *et al.*, 2012). The spores might become pathogenic, when these spores are established in skin and sometime invade in epidermis. *Aspergillus niger* and *A. fumatigus* hyphae were observed on wounds and lesions on skin of *Channa punctatus* (Podeti and Benarjee, 2015). Firouzbakhsh *et al.* (2005) reported *A. niger* from gills of common carp (*Cyptinus carpio*), grass carp (*Ctenopharyngodon idella*) and silver carp. The infection leads to external and internal mycosis in these fishes. *Aspergillus* is not found only on the skin of fish but it is also found on the gills and intestine of fish (Salawudeen *et al.*, 2017). *Aspergillus niger, A. fumigatus* and *A. flavus* are reported from *Channa striatus* (Podeti and Benarjee, 2016); *L. calbasu* (Lone *et al.*, 2018); *Oreochromis niloticus* and *Clarias gariepinus* (El-Tawab *et al.*, 2020). Silver carp, is a popular commercial fish reared in ponds in Pakistan. However, little information is currently available on various aspects of fish disease and health management especially fungal infection in this fish. The aim of the present study was to study aspergillosis in the silver carp reared in earthen ponds.

Materials and Methods

Two hundred silver carp fish were obtained from Punjab University Research Fish Farm in Spring and Summer seasons in 2015-2016. These fishes were taken to lab in sterilized bags and shifted into fish tank and maintained for two days in Laboratory conditions. Health status of fishes was observed and body measurement were done. Body weight (46.24±38.29g) and total length (18.09±5.01cm) were observed. Silver carps were sterilized with 1% formaldehyde, 70% alcohol and sterilized distilled water for 5-10 minutes respectively. This was done to disinfect the fish to avoid secondary infection with airborne *Aspergillus* spores. For isolation of *Aspergillus* four type of medium were used i.e., Malt extract agar (MEA), Corn meal agar (CMA), Sabouraud dextrose agar (SDA) and Potato dextrose agar (PDA) were used in routine cultivation of *Aspergillus* species (Ellis *et al.*, 2007).

Streptomycin sulphate 250 mg was used as an antibiotic in each preparation of media to diminish bacterial infectivity. Inoculation was done with inoculating needle from infected parts of fish such as: fins, gills, skin, intestine, heart, liver and kidney of fish in Laminar flow. Inoculated plates were incubated at 28-32 ºC and fungal growth was observed after 5-8days. The material from *Aspergillus* colony was taken on clean glass slide and stained with 0.05% Trypanblue in Lectophonel and observed under microscope. Morphological identification was done with the help of Ellis *et al.* (2007) and Nyongesa *et al.* (2015).

Result

All the specimens of silver carp were infected with *Aspergillus*. *Aspergillus* spp. infection was noticed in the form of scales erosion and damaged fins. Lesions were noticed on many samples but deep wounds were not observed in any fish (Figure 1).

Figure 1: Infected silver carp show lesion on body and damage caudal fins.

Four species of *Aspergillus* were isolated i.e., *Aspergillus niger*, *A. fumigatus*, *A. flvaus* and *A. terreus*. *Aspergillus niger* was more prominent with 50.52% infection followed by *A. fumigatus* (24.02%), *A. flavus* (17.06%), A. *terreus* (8.40%) (Table 1). When organ-wise infection was observed, fins showed highest infection (39.1%) and intestine showed least infection (6.59%) (Table 2). All the four species of *Aspergillus* showed different colonies color. *Aspergillus niger*, *A. fumigatus A. flavus* and *A. terreus* showed black, grey, green and skin color respectively on culture medium (Figures 2, 3, 4, 5).

Table 1: Percentage infection of different *Aspergillus* sp. in silver carp.

Sr. No.	*Aspergillus* sp.	No. of plates	% age infection
1	*Aspergillus niger*	944	50.52%
2	*Aspergillus fumigatus*	449	24.02%
3	*Aspergillus flavus*	319	17.06%
4	*Aspergillus terreus*	157	8.40%

Table 2: Organ wise fungal infection in silver crap.

S. No.	Organ	No. of plates	%age infection
1	Skin	303	16.38
2	Gills	235	12.7
3	Fins	723	39.1
4	Intestine	122	6.59
5	Heart	160	8.65
6	Liver	148	8
7	Kidney	158	8.54

Discussion

Aspergillosis was studied in silver carps. The isolated *Aspergillus* species were identified as *A. niger*, *A. fumigatus*, *A. flavus*, and *A. terreus*. *Aspergillus niger* was the most prominent *Aspergillus* sp. followed by the other three.

Fins were the most affected area which showed 39.10% infection. Infection on vital organs like gills, heart, liver, kidney and intestine was low but seems to be more serious and sometime may be fatal.

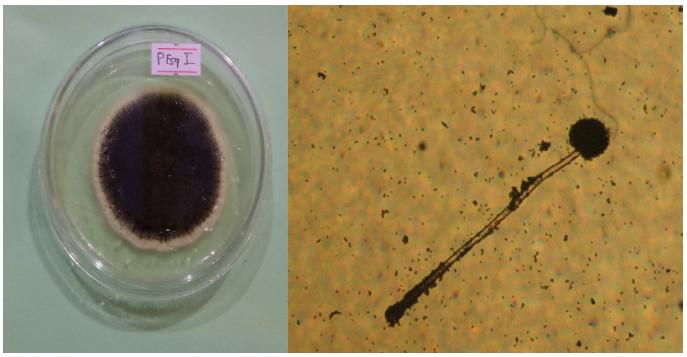

Figure 2: *Aspergillus niger* (black colony and conidiophore).

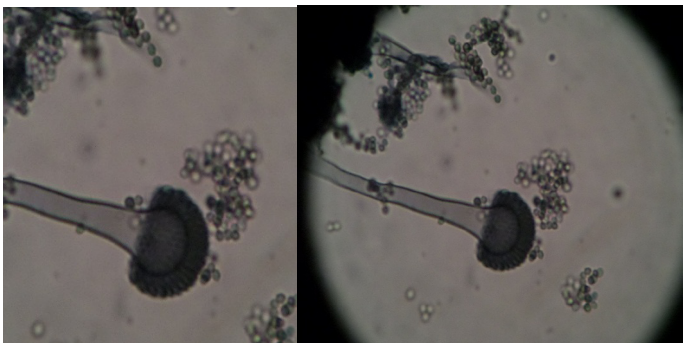

Figure 3: *Aspergillus fumigatus* (grey colony and conidiophore).

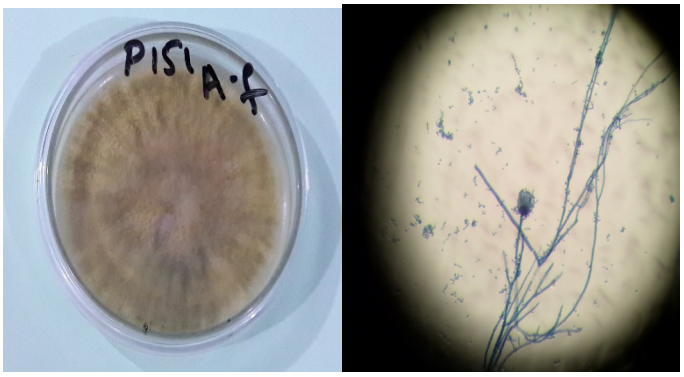

Figure 4: *Aspergillus flavus* (Green colony and coiniophore).

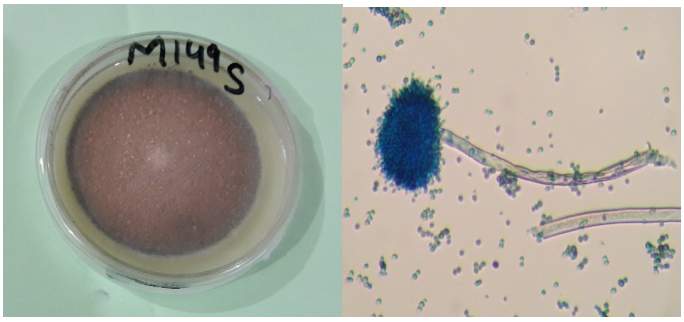

Figure 5: *Aspergillus terreus* (skin and green colony and coiniophore).

Aspergillus infection in gill resulted in damage of secondary lamellae. It may be source of respiratory distress in fish (Iqbal *et al*., 2012a). Iqbal *et al.* (2012b) reported fungal infection in *Ctenopharyngodon idella* and *Catla catla*. This infection was related to such factors that cause environmental unsuitability. High stocking density, excessive organic fertilizer in ponds and incompatible water quality for fish health were included in environmental unsuitability. *Aspergillus* infection in ponds fishes may be due to intake of unhygienic feed present in the fish ponds. Decomposition and decay of contaminated feed also lead to more severe infection. (Iqbal and Saleemi, 2013). Iqbal *et al*. (2014) reported high infection in the head area of silver carp because this part first interacts with the *Aspergillus* spores during feeding and swimming.

Diversity of fungal species varies with the season of the year among freshwater fishes. It is more prevalent in the winter. Younis *et al.* (2020) reported *A. flavus*, *A. fumigatus* and *A. niger* from skin, fins, gills, eyes, liver, kidney and spleen of *O. niloticus* and *C. gariepinus* in winter. These fungal species were more prominent in winter as compared to spring and summer. In winter low temperature is responsible for fungal infection in fishes. Pachade *et al.* (2014) isolated different fungal species throughout the whole year. *Aspergillus niger* and *A. flavus* showed more infection from November to January.

Aspergillus showed maximum virulence while other fungal genera i.e. *Rhizopus* and *Alternaria* showed minimum virulence in *L. rohita, C. catla, C. marulius* and *C. striatus* (Kumari and Kumar, 2015). Ali (2015) investigated fungal infection on head, caudal fin and abdomen of *C. carpio*, *C. carpio regularis* (mirror carp) and *H. molitrix* from Suliamania Province, Iraq. *Cyprinus carpio* showed higher incidence of fungal infection (55%) as compared to other two species which showed 22.5% and 22% infection respectively.

The ratio of mycotic infection was observed 62% and it is considered bigger hazard to the aquaculture industry (Abbas *et al*., 2016). *Aspergillus flavus* were isolated and identified from *H. molitrix, C. auratus, C. carpio, Liza abu, Barbus luteus, Aspius varax* and *Mugil cephltus.* Systematic mycosis results revealed that the percentage infection in fish was 62% as compared to other yeast.

Mycobiota associated with fish and their environment was described by Abdel-Sater *et al.* (2017). Eleven genera and twenty five fungal species were isolated and identified from broomtail wrasse (*Cheilinus lunulatus*), Crocodile fish (*Cymbacephalus beauforti*), Rabbit fish (*Siganus rivulatus*), Sergeant major (*Abudefduf saxatili*), Doublebar bream (*Acanthopagrus bifasciatus*), Klunzinger's wrasse (*Thalassoma rueppellii*), Blacktip mojarra (*Gerres oyena*), Picnic sea bream (*Acanthopagrus berala*). *Aspergillus* sp.

was isolated from the skin, gills and liver of fishes. Gills and skin showed relatively higher fungal infection than liver. Shamsan and Al-Jobory, 2018 isolated *A. awamori*, *A. candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *A. parasiticius*, *A. sulphureus* and *A. terreus* from sun-dried fishes of Yemen. Recently *Aspergillus* spp. were isolated from seven fish species, *Wallago attu*, *H. molitrix*, *L. rohita*, *C. mrigala*, *C. idella*, *C. carpio* and *C. catla* from River Ravi (Iqbal and Khatoon, 2019).

The presence of aspergillus in silver carp sample is of a great significance in view of food safety and quality. *Aspergillus* spp. are common in air and soil, and their presence in fish samples might contain metabolites produced by them, that may make the fish consumption hazardous to human health. Similarly, Mitchell (2007) mentioned that the potency of these metabolites is not affected by cooking and may cause severe or fatal damage to the liver and kidney.

Aspergillus produce a group of toxic waste called aflatoxins that are major reason in the spoilage of food. If these toxic wastes assemble in fish tissues it is fatal for human consumption (Adebayo-tayo *et al*., 2008; Junaid *et al*., 2010). *Aspergillus* spp. associated with fish mycoses (Chauhan *et al*., 2014). Aspergillus infections are not as dangerous as other fungus species such as *Saprolegnia*. It only affects those fishes which are under unfavorable environmental conditions. *Aspergillus* seems to be opportunist fungus; hence proper husbandry practices must be adopted on the fish farms. The study of aspergillosis, pathogenicity of *Aspergillus* species and histopathology of infected fishes needs our urgent attention to further understand this pathogenic fungus which is affecting warm water fish culture in Punjab, Pakistan.

Conflict of interest

The authors have declared no conflict of interest.

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