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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...
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. fumigatus* 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.](image)

Four species of *Aspergillus* were isolated i.e., *Aspergillus niger*, *A. fumigatus*, *A. flavus* 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.**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th><em>Aspergillus</em> sp.</th>
<th>No. of plates</th>
<th>%age infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>A. niger</em></td>
<td>944</td>
<td>50.52%</td>
</tr>
<tr>
<td>2</td>
<td><em>A. fumigatus</em></td>
<td>449</td>
<td>24.02%</td>
</tr>
<tr>
<td>3</td>
<td><em>A. flavus</em></td>
<td>319</td>
<td>17.06%</td>
</tr>
<tr>
<td>4</td>
<td><em>A. terreus</em></td>
<td>157</td>
<td>8.40%</td>
</tr>
</tbody>
</table>

**Table 2: Organ wise fungal infection in silver carp.**

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

**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.

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. robota, 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 lutes, Aspius varax and Mugil cephitus. 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 (Abudelfaf saxatil), Doublebar bream (Acanthopagrus bifasciatus), Klunzinger’s wrasse (Thalassoma rueppellii), Blacktip mojarra (Gerres oyena), Picnic sea bream (Acanthopagrus beralia), 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. parasiticus*, *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. robota*, *C. nigrala*, *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 opportunistic 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.

Reference


Kumari, R. and Kumar, D.C., 2015. Fungal infection in


