



## Morphological identification of fungus isolated from silver carp, *Hypophthalmichthys molitrix* from three locations of Punjab, Pakistan

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**Abstract**-Infections caused by fungi have increased in recent years in freshwater fish. The present study was aimed to isolate the fungal genera in a commercial fish, silver carp, *Hypophthalmichthys molitrix* reared in some earthen ponds in the province of Punjab, Pakistan. Based on the morphological analysis eleven fungal genera were isolated and identified. These fungal species were; *Aspergillus* sp., *Alternaria* sp., *Curvularia* sp., *Chaetomium* sp., *Fusarium* sp., *Mucor* sp., *Neurospora* sp., *Penicillium* sp., *Rhizopus* sp., *Simplicillium* sp. and *Trichoderma* sp. Four new genera *Curvularia* sp., *Chaetomium* sp., *Simplicillium* sp. and *Trichoderma* sp. first time isolated from silver carp. The infected fish showed eroded scales, skin darkening, swollen eyes, erythema on skin, eyes, operculum and near anus and granuloma on anal fin. The infectivity level on different organs of fish was changeable such as: Fins showed the highest infection in all three fish sampling locations i.e., 38.36%, 40.56%, 42.29% infection from location 1, 2 and 3 respectively. Least infected organ was intestine. Silver carp showed fungal infection occurred through improper environment having unhygienic feed. Fungal infection in silver carp decreases the nutritional value of fish flesh. Hence this situation suggested that silver carp is not good for human health if reared in contamination water.

**Keywords:** silver carp, *Curvularia*, *Trichoderma*, ponds, unhygienic feed

### I. INTRODUCTION

Fish are continuously exposed to the microorganisms present in water and sediments. These organisms influence the microflora on the skin surface, gills and digestive tract of fish in different water bodies. Fungal infection in fishes is the serious cause of losses of fish and their products. Sometime mortality rate reach upto 100% due to fungal diseases in fishes (Chukanhom and Hatai, 2004). The most familiar fungal infections are those caused by *Saprolegnia* and other water molds (Yanong, 2003). Fungal infection is also known as mycoses. A number of diseases are present in freshwater are because of fungi. At least one species of fungus are caused disease in the all life stages of fish (Neish, 1997). Fungal infection are observed in eggs of fish (Czeczuga *et al.*, 2005; Ebrahimzadeh *et al.*, 2007; Melaku *et al.*, 2017) fry and fingerling stages (Vandersea and Litaker, 2007; Kwanprasert *et al.*, 2007) and adult fish (Bangyeekhun and Sylvie, 2001). Winter saprolegniasis can infect and kill the eggs, fry, fingerlings and adults of fishes (Durborow *et al.*, 2003). Abbas *et al.*, 2016 Studied the systemic mycological isolates from *Aspius vorax*, *Barbus grypus*, *B. Xanthopterus*, *B. Luteus*, *B. Sherpeyi*, *C. carpio*, *C. auratus*, *Liza abu*, *Mugil cephalus* and *H. molitrix*.

Fungus apparently infect the fish body and also penetrate into their internal organ and cause damage. Marzouk *et al.* (2003) observed the fungal growth on the fish externally and internal organs. Hyphae embedded in the tissues of diseased fish shows the similar pattern as observed in the fungus growth in the culture medium (Vandersaet *et al.*, 2006). Vandersea and Litaker (2007) reported six fungal species from the gills of channel catfish fry. Ebrahimzadeh *et al.* (2007) investigated that rainbow trout eggs are contaminated with *Saprolegnia parasitica* and it was most important fungal species causing infestation in salmon hatcheries. Different species of *Saprolegnia* were observed in the commercialable fishes (Mastan *et al.*, 2008; Mastan *et al.*, 2012 and Mastan 2015). Aspergillomycosis was reported in freshwater fishes, such as *Labeocalbasu* (Chauhan *et al.*, 2014). The causative agent of this diseases is *A. flavus*, *A. terreus* and *A. japonicus*. Abeyayo-Tayo *et al.* (2008) isolated *A. flavus*, *A. fumigatus* and *A. terreus* from skip jack tuna fish (*Katsuwonus pelamis*).

Water mould variety is depending upon on the communication of physiochemical parameters. Any defecate in management of pond will increases the happening of fish diseases in the pond (Pailwal and Sati, 2009). Fungus are also observed in economically important freshwater fishes of Asia (Zahura *et al.*, 2004; Siddique *et al.*, 2009; Chauhan, 2012; Chauhan, 2013; Kumari and Kumar, 2015; Salawudeen *et al.*, 2017).

Diseases interrupt the growth of fish and result in poor economical production. Pathogenic fungi infected the fish which result in diminishes the nutritional value of fish. Incidence of Fungal infection observed in silver carp (*H. molitrix*) reared in earthen ponds. *Rhizopus*, *Mucor*, *Penicillium* and *Aspergillus* were isolated from *H. molitrix* (Iqbal *et al.*, 2014). Chauhan *et al.* (2014) isolated *A. fumigates*, *A. niger* and *A. sydowii* from *C. striatus*, *C. mrigala*, *C. batrachus*, *L. rohita*, *M. aculeatus*, *M. seenghala*, *P. ticto* and *Trichogaster fasciatus*. The objectives of this study were to isolate and identify the silver carp fungal genera on morphological basis on the three locations of Punjab, Pakistan.

## II. MATERIALS AND METHODS

Fish sample were collected from three locations of Punjab. From each location about 200 fishes were used for isolation of fungus. Location 1 is Punjab University Fish Research Farm, Lahore, Location 2 is Himalyia Fish Hatchery, G.T. Road, Muridkay and Location 3 is Manawan Fish Hatchery, Lahore. The fish were brought in aerated water in sterilized polyethylene bags to Fish Disease and Health Management laboratory, Zoology department Punjab University Lahore. Body measurement of each fish was done. Fish were examined thoroughly with the help of magnifying glass for any infection on the body of the fish, fins and the head region. The fungal infection was observed on the body of fish, fins, eyes, operculum, buccal cavity, head region and the gills. The occurrence of fungal infection in each fish and in every sample was observed and recorded. Red patches on skin, mouth and operculum and scales were totally removed in the region of caudal peduncle. For sterilization of fish, dipped it in 1% formalin, 70% alcohol and sterilized distilled water for 5-10 minutes respectively. Prepared medium (Sabouraud Dextrose Agar, Corn Meal Agar Malt Extract Agar and Potato Dextrose Agar) were used. To avoid bacterial interference in agar plates added 250mg antibiotic (streptomycin sulphate). The fungal genera were isolated from fins, gills, buccal cavity, operculum, eyes and skin with sterilized inoculated needle. Laminar flow air cabinet was used to prevent airborne germs spore. The agar plates were put at 28-32 C° in the incubator. After 3-8 day fungal colony color, texture and diameter were noted. Trypanblue in Lactophenol was used for the staining of fungus. The stained slides were observed and photographed. Morphological identification of fungus was done on the basis of Nyongesa *et al.*, (2015); Samson *et al.*, (2014) for *Aspergillus* sp., Visagie *et al.*, (2014) for *Penicillium* sp., Leslie and Summerell (2006); Kosiak *et al.*, (2005) for *Fusarium* sp., Madrid *et al.*, (2014) for *Curularia* sp., Wang *et al.*, (2016); Aghdam and Fotouhifar, (2016) for *Chaetomium* sp., Liu and Cai, (2012); Zare and Gams, (2001) for *Simplicillium* sp., Kumari and Kumar, (2015) for *Neurospora* sp., Ellis, *et al.*, (2007) for *Trichoderma* sp. and Keet *et al.*, (2010) for *Mucor* sp. and *Rhizopus* sp.

## III. RESULTS:

Six hundred specimens were examined from three locations to investigate fungal infection. Body weight (grams), body depth (cm), total length (cm), standard length (cm) and fork length (cm) were recorded (Table.1.1). Out of 600 fishes, 550 fishes were unhealthy and infected. Only 50 fishes looked healthy. The typical clinical signs of diseased fishes were: ruptured dorsal fin and lesions on skins and body, caudal peduncle, granuloma on anal fin.

**Table.1.1: Morphometric data of *H. molitrix* from three locations**

Locations	Total no. of fishes	Body weight (grams)	Body depth (cm)	Total length (cm)	Standard length (cm)	Fork length (cm)
Location 1	200	46.86±19.147	4.24±0.647	17.09±2.172	13.60±1.917	14.00±2.01
Location 2	200	46.90±17.173	4.23±0.664	16.88±2.095	13.55±1.666	14.60±1.840
Location 3	200	46.15±22.082	4.22±0.682	16.95±2.378	13.35±2.015	14.43±2.239



Fig: 1.1: whole body of *H. molitrix* covered with red lesions

Fungal genera were isolated from the different organ of fishes. Gills, eyes, skin, fins, heart, liver, kidney and intestine were used for the isolation of fungi. Fins showed the highest infection in all three fish sampling locations i.e., 38.36%, 40.56%, 42.29% infection from location 1, 2 and 3 respectively. Least infected organ was intestine. Fish from location 1 showed 6.82%, Location 2 showed 5.83% and location 3 showed 2.74% infection. Other organs also showed infection as shown in Table.1.2.

**Table.1.2: Organ wise infection and fungal colonies in silver carp**

S. No	organ	Total no. of plates	Percentage infection in three locations		
			Location 1 (% infection)	Location 2 (% infection)	Location 3 (% infection)
1	Eyes	1028	9.06	7.62	10.03
2	Gills	1026	11.26	14.12	16.82
3	Skin	1029	14.12	17.06	10.23
4	Fins	1032	38.36	40.56	42.29
5	Heart	1022	8.96	7.42	6.36
6	Liver	1030	7.26	4.36	5.82
7	Kidney	1026	4.16	3.04	3.71
8	intestine	1029	6.82	5.83	4.74

#### **Fungal genera isolated from silver carp**

Eleven fungal genera were isolated and identified up to species level. The macroscopic and microscopes features of all the eleven genera are discussed one by one.

#### **1- Genus *Aspergillus***

This genus was observed in fishes collected from location 1, 2 and 3. The percentage infection was 66.84%, 69.84%, and 67.12% in location 1, 2 and 3 respectively. Location 2 was the most infected site for *Aspergillus* sp. Macroscopic observation showed that the colony surface of was black, green, grey green, blue green, yellow green, white, brown and yellow color. Colonies showed smooth circular margins (Fig.1.2), while some colonies showed irregular margins. Reverse side showed white to pale color. Microscopic observation showed that the Conidia of *Aspergillus* were dark brown, round and having rough surface. Conidiophores was septate and hyaline and showed darker side towards vesicle. Conidial head was brown and biserial having Phialides (Fig.1.3).

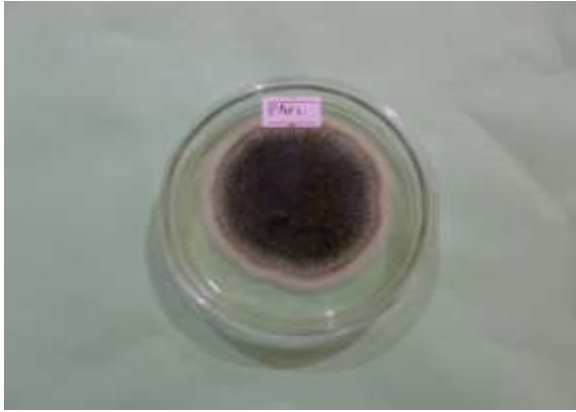


Fig 1.2.: *Aspergillus* colony isolated from *H. molitrix*

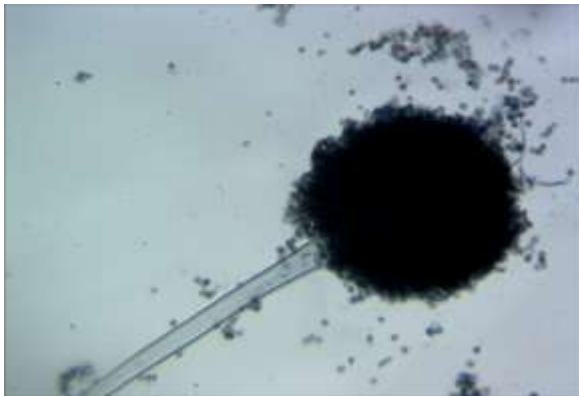


Fig 1.3: Conidiophores and hyphae of *Aspergillus* sp. isolated from *H. molitrix*

## 2- Genus *Alternaria*

This genus was observed in fishes obtained from all three Locations i.e., 1, 2 and 3. The percentage infection was 1.19%, 1.68% and 1.67% in location 1,2 and 3 respectively. Location 2 was the slightly high infected site for *Alternaria* sp. than location 3. Macroscopic observation showed that the Colony surface colour showing dirty brown colour having white margins (Fig.1.4). Reverse side showing brown colour. Microscopic observation showed that the Conidia of *Alternaria* sp. tear drop like, brown, smooth walled and produced singly (Fig.1.5). Hyphae were septate and brown in color. Conidiophores was septate and frequently producing in zigzag pattern.



Fig 1.4: *Alternaria* colony isolated from *H. molitrix*

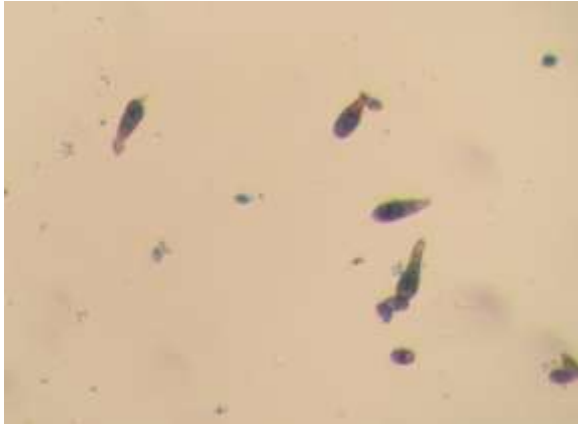


Fig 1.5: Conidia of *Alternaria* sp. isolated from *H. molitrix*

### 3- Genus *Chaetomium*

This genus was observed in fish from two locations 1 and 3. The percentage infection was 0.79% and 1.01% in location 1 and 3 respectively. Location 1 was the most infected site for *Chaetomium* sp. Macroscopic observation showed that the colony surface of *Chaetomium* sp. was grayish in appearance. Margins of colony was irregular (Fig.1.6). Reverse side was black showing red pigmentation. Microscopic observation showed that the hyphae were hyaline and septate. Ascoma was circular with frequent brownish, unbranched, septate and curling hairs. Ascospores were brown and lemon shaped (Fig.1.7).



Fig 1.6: *Chaetomium* colony isolated from *H. molitrix*

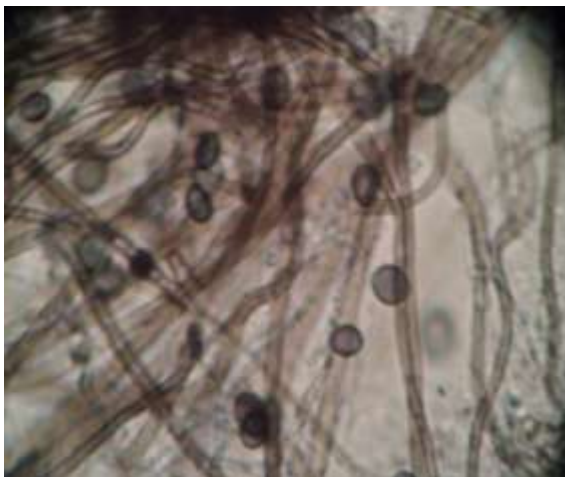


Fig 1.7: Ascospores of *Chaetomium* sp. isolated from *H. molitrix*

### 4- Genus *Curvularia*

This genus *Curvularia* was observed in fishes from Locations 1 and 2. The percentage infection was 0.99% and 1.33% in location 1 and 2 respectively. Location 2 was the most infected site for *Curvularia* sp. Macroscopic observation showed that the *Curvularia* produced rapidly growing, smooth colony on medium. From the front, the color of the colony was lighter gray initially and turns to black as the colony

matures. Margins of colony showed white boundary. Colony showed concentric rings (Fig.1.8). From the reverse side, it showed dark brown. Microscopic observation showed that the brown and septate hyphae, brown conidia and conidiophores are visible. Conidiophores are simple and branched. It is brown, pyriform and multiseptate. The septa are transverse and split each conidium into four cells (Fig.1.9).



Fig 1.8: *Curvularia* colony isolated from *H. molitrix*



Fig 1.9: Conidia and hyphae of *Curvularia* sp. isolated from *H. molitrix*

#### 5- Genus *Fusarium*

This genus was observed in silver carp examined from location 1, 2 and 3. The percentage infection was 7.97%, 5.65% and 7.67% in location 1, 2 and 3 respectively. Location 1 was the most infected site for *Fusarium* sp. Macroscopic observation showed that the colony surface of *Fusarium* was white color having smooth margins (Fig.1.10). Reverse side showed whitish yellow shade. Microscopic observation showed that the microconidium was absent. Macroconidium were dorsiventral curvature (Fig.1.11). They usually contain 3-5 divisions. Chlamydospores were in the form of chain having four round cell.



Fig 1.10: *Fusarium* colony isolated from *H. molitrix*



Fig 1.11: Macroconidium of *Fusarium* sp. isolated from *H. molitrix*

#### 6- **Genus *Mucor***

This genus *Mucor* was observed in experimental fishes from Location 1, 2 and 3. The percentage infection was 1.90%, 2.50% and 1.88% in location 1, 2 and 3 respectively. Location 2 was the most infected site for *Mucor* sp. Macroscopic observation showed that the colony surface colour was black and white with fluffy appearance (Fig.1.12). Reverse side showed dark colour. The fungal colony covered the whole plate within 5<sup>th</sup> day of inoculation. It showed very rapid growth. Microscopic observation showed that the hyphae of *Mucor* were sparsely septate and broad. Rhizoid was absent. Conidiophores were inverted umbrella shape (Fig.1.13).



Fig 1.12: *Mucor* colony isolated from *H. molitrix*



Fig.1.13: Conidiophores of *Mucor* sp. isolated from *H. molitrix*

#### 7- **Genus *Neurospora***

This genus was observed in silver carp sampled from location 1, 2 and 3. The percentage infection was 18.59%, 5.26% and 6.33% in location 1, 2 and 3 respectively. Location 1 was the most infected site for

*Neurospora* sp. Macroscopic observation showed that the colony surface of *Neurospora* sp. was light orange with fluffy appearance. Colony growth was very rapidly. Its colony covered the whole plate within 3<sup>rd</sup> day of inoculation (Fig.1.14). Reverse side shown white colour. Microscopic observation showed that the Septate hyphae were seen mostly under microscope. Hyphae were hyaline and smooth walled. Conidiophores were poorly differentiated and some time seen singly. Mature hyphae divided into rectangular cells were connected by disjunctors. These rectangular cells are known as an arthroconidia (Fig.1.15).



Fig 1. 14: *Neurospora* colony isolated from *H. molitrix*



Fig 1.15: Septate hyphae and arthroconidia of *Neurospora* sp. isolated from *H. molitrix*

#### 8- Genus *Pencillium*

This genus was observed in experimental fishes obtained from location 1, 2 and 3. The percentage infection was 3.46%, 6.26%, 4.22% in location 1, 2 and 3 respectively. Location 2 was the most infected site for *Pencillium* sp. Macroscopic observation showed that the colony surface colour was grey (Fig.1.16) and yellowish white. Reverse side of plate showed black and white colour. Microscopic observation showed that the Hyphae were septate and clear. Blue conidia were circular and found in the foam of chain showing brush like appearance (Fig.1.17). Phialides extend directly from the hyphae and monoverticillate conidiophores.



Fig 1.16: *Pencillium* colony isolated from *H. molitrix*



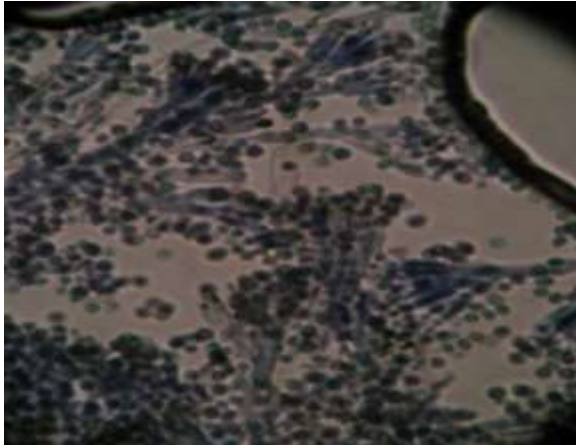


Fig 1.17: Brush like appearance conidiophores of *Pencillium* sp. isolated from *H. molitrix*

#### 9- Genus *Rhizopus*

This genus was observed in silver carp sampled from Location 1 and 2. The percentage infection was 1.02% and 2.80% in location 1 and 2 respectively. Location 2 was the most infected site for *Rhizopus* sp. Macroscopic observation showed that the colony surface colour was white with fluffy appearance (Fig.1.18). Reverse side showed white colour. It showed rapid growth, full plate growth was observed after 5<sup>th</sup> day of inoculation. Microscopic observation showed that the hyphae of *Rhizopus* was non septate. Rhizoids, conidia and conidiophores were visible. Conidiophores were round with flattened bases, unbranched and brown in colour (Fig.1.19).



Fig 1.18: *Rhizopus* colony isolated from *H. molitrix*

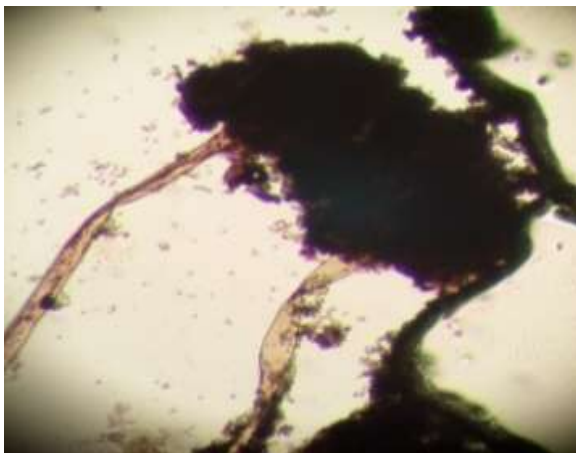


Fig 1.19: Conidiophores of *Rhizopus* sp. isolated from *H. molitrix*

#### 10- Genus *Simplicillium*

This genus was observed in silver carp from Location 1 and 2. The percentage infection was less than 1% (0.99% and 1.85%) in location 1 and 2 respectively. Location 2 was the most infected site for *Simplicillium* sp. than location 1. Macroscopic observation showed that the colony surface of *Simplicillium* was blackish white having fluffy appearance (Fig.1.20). Reverse side showed white color.

*Simplicillium* sp. showed rapid growth. Colony covers the whole plate by the 5<sup>th</sup> day of inoculation. Microscopic observation showed that the conidia of *Simplicillium* were produced diagonally forming short chains, round to oval shape. Phialides were long, solitary and slender. Hyphae were aseptate and long. Rhizoids were also observed (Fig.1.21).



Fig 1.20: *Simplicillium* colony isolated from *H. molitrix*

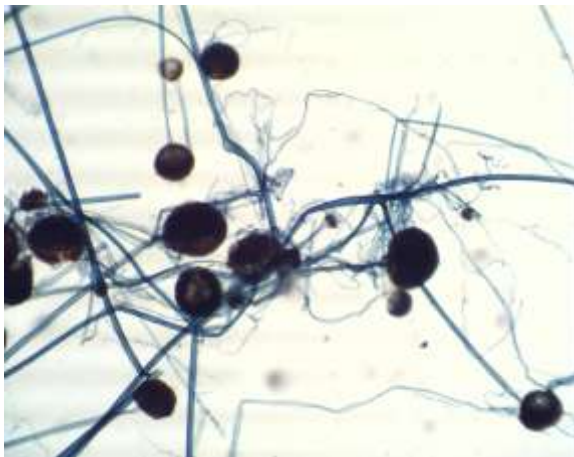


Fig 1.21: Conidiophores and hyphae of *Simplicillium* isolated from *H. molitrix*

#### 11- Genus *Trichoderma*

This genus *Trichoderma* was observed in fish from two locations 2 and 3. The percentage infection was 2.76% and 9.14% in location 2 and 3 respectively. Location 3 was the most infected site for *Trichoderma* sp. Macroscopic observation showed that the colony surface of *Trichoderma* was green and white with concentric rings appearance (Fig.1.22). Reverse side of colony showed whitish color. Microscopic observation showed that the hyphae were hyaline and wider. Conidia were green, smooth walled and circle. Conidiophores were long and relatively divided at right angle. Phialides were flask shaped having cylindrical base. Chlamydo spores were hyaline, smooth walled and terminal (Fig.1.23).

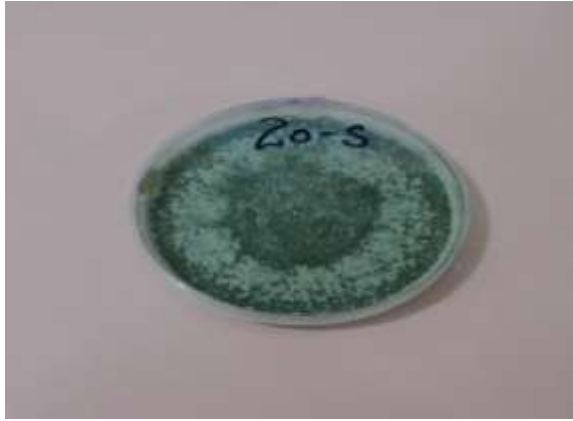


Fig 1.22: *Trichoderma* colony isolated from *H. molitrix*

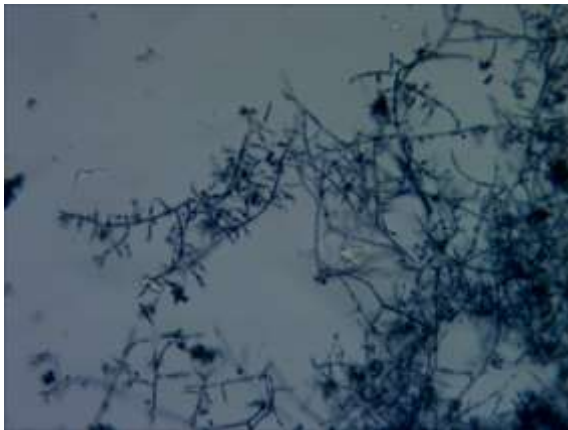


Fig.1.23: Conidiophores of *Trichoderma* sp. isolated from *H.*

#### IV. DISCUSSION:

Fungal infection was observed in silver carp sampled from earthen ponds from three different locations in Punjab. Eleven fungal genera were isolated from silver carp and the most prominent genus was *Aspergillus* sp. Other genera were *Alternaria*, *Trichoderma*, *Chaetomium*, *Curvularia*, *Fusarium*, *Mucor*, *Neurospora*, *Penicillium*, *Rhizopus* and *Simplicillium*.

The fungus noticed from gills and eyes leads to severe infection. If fungal infection is on eyes, it may result in blindness of fish. If fungal hyphae penetrate into the brain and such fish cannot survive and may lead to death (Iqbal and Saleemi, 2013). Iqbal *et al.*, (2014) observed *Penicillium*, *Rhizopus*, *Aspergillus* and *Mucor* from the anterior part of the silver carp. Higher infection was noticed on the anterior part because these parts interact more with the fungal spores during feeding and swimming behavior. High stocking density associated with unsuitable water quality in the ponds may be the reason for fungal infection in pond fish. *Saprolegnia* sp. and *Achlya* sp. were observed in *C. idella* and *C. catla* (Iqbal *et al.*, 2012). Fungal infection on gills disturbs the respiratory system of the fish.

Diversity of fungal species varies with the season of the year among freshwater fish. It is more prevalent in the winter. Pachadeet *et al.*, (2014a) isolated different fungal species in the whole year. *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *Alternaria alternate*, *Curvularia lunata*, *Saprolegnia parasitica*, *Rhizopus stolonifer* and *Mucor mucedo* showed more infection in November to January. In winter, low temperature is responsible for fungal infection in fish. *Aspergillus*, *Saprolegnia*, *Fusarium* and *Achlya* were reported from *C. catla*, *Channabatrachus*, *C. punctatus*, *Myxus cavasius*, *M. seenghala* and *Trichogaster fasciatus* (Chauhan, 2012). Chauhan *et al.*, (2013) reported *Alternaria*, *Aspergillus*, *Penicillium* and *Fusarium* associated with fungal infection in fish. *Curvularia lunata*, *Fusarium*, *Aspergillus* and *Penicillium* were isolated from *Channapunctatus* (Bloch) (Malathi and Rajendran, 2012).

Aspergillomycosis is a fungal disease caused by *Aspergillus* species, which was reported in *Oreochromis* sp. (Olufemi *et al.*, 1983) and *Labeocalbas* (Chauhan *et al.*, 2014a). *Aspergillus* infection developed from conidiophores present in the body and on the skin of fish. Refeiet *et al.*, (2010) investigated some fungal genera from *Clarias gariepinus* and *Oreochromis* species. Thirteen fungal genera; *Curvularia*, *Saprolegnia*, *Paecilomyces*, *Aspergillus*, *Scopulariopsis*, *Fusarium*, *Paecilomyces*, *Mucor*, *Rhizopus*,

*Penicillium*, *Candida*, *Torulopsis* and *Rhodotorula* were responsible to cause natural infection in *C. gariepinus* and *Oreochromis* sp. Incidence of *Aspergillus*, *Fusarium*, *Trichoderma* and *Penicillium* were observed in *C. striatus* and *C. punctatus*. *Channapunctatus* (17.39%) was more infected as compared to *C. striatus* (13.16%) (Podeti and Benarjee, 2015b). *Achyla* and *Aspergillus* showed maximum virulence while *Verticillium*, *Rhizopus* and *Alternaria* were shown minimum virulence in *L. rohita*, *C. catla*, *C. marulius*, *C. striatus* and *N. chital* (Kumari and Kumar, 2015). Ali, (2015) investigated the fungus on the site of head, caudal fin and abdomen of silver carp (*H. molitrix*). He observed the pathogenic fungi of *C. carpio*, *C. carpio regularis* (mirror carp) and *H. molitrix* of Suliamania Province, Iraq. *Cyprinus carpio* showed higher incidence of fungal infection 55% as compared to other two species which showed 22.5% and 22% respectively. Five fungal genera were isolated and identified i.e., *Blastomyces*, *Rhizopus*, *Penicillium*, *Aspergillus* and *Candida*.

The ratio of mycotic infection was observed 62% and it is considered bigger hazard to the aquaculture industry (Abbas *et al.*, 2016). Some fungal species such as *Cryptococcus* sp., *Rhizopus* sp., *A. flavus*, *Blastomyces dermatitidis*, *Candida pseudotropicalis*, *C. krusei*, *C. quillermondii* were isolated and identified from *H. molitrix*, *C. auratus*, *C. carpio*, *Liza abu*, *Barbus luteus*, *B. sherpeyi*, *B. xanthopterus*, *B. grypus*, *B. sherpeyi*, *Aspius varax* and *Mugil cephalus*. Systematic mycosis results revealed that the percentage infection in fish was 62% further divided into 7% moulds and 55% yeasts. *Alternaria*, *Aspergillus*, *Saprolegnia*, *Cladosporium* and *Penicillium* isolated from *C. gariepinus* and *O. niloticus* (Younis *et al.*, 2020).

Aquatic fungal flora was isolated from eggs and brood stock of African catfish (*Clarias gariepinus*). *Saprolegnia*, *Mucor*, *Alternaria*, *Rhizopus*, *Microsporium*, *Trichophyton* and *Penicillium* were collected from water, body surface and eggs of catfish. *Trichophyton* was prominent genus which showed 13.08% infection (Melaku *et al.*, 2017). The fungal infection observed in the skin and gills of *Clarias gariepinus* were *Rhizopus* sp., *Penicillium* sp., *Trichophyton* sp., *Mucor* sp., *Aspergillus* and some yeast isolates. Skin and gills showed fungal infection in healthy and diseased fishes. 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 saxatilis*), Doublebar bream (*Acanthopagrus bifasciatus*), Klunzinger's wrasse (*Thalassoma rueppellii*), Blacktip mojarra (*Gerres oyena*), Picnic sea bream (*Acanthopagrus beral*). *Alternaria alternata*, *Aspergillus*, *Cladosporium*, *Exophiala*, *Fusarium oxysporum*, *Nigrospora sphaerica*, *Penicillium aurantiogriseum*, *Purpureocillium lilacinum*, *Pseudallescheria* sp., *Pythium* sp., *Rhizoctonia solani* and *Stemphylium botryosum* were isolated from the skin, gills and liver of fishes. Gills and skin showed relatively higher fungal infection than liver. Freshwater marine fishes also showed fungal infection. *Aspergillus flavus*, *A. niger*, *Penicillium* sp., and *Fusarium* sp., and *R. stolonifer* reported from fish (Ikram and Shoab, 2019).

Incidence of the mycotic infections was observed in cultured Gilthead seabream (*Sparus aurata*). About one hundred fishes were used to isolate fungi such as the *Aspergillus* sp., *Paecilomyces* sp., *Fusarium* sp., *Alternaria* sp., *Aphanomyces* sp., *Geotrichum* sp., *Cladosporium* sp., *Helminthosporium* sp., *Ichthyophonus* sp. and *Nigrospora* sp. Yeast isolates such as *Rhodotorula* sp., *Torulopsis* sp., *Candida* sp. and *Cryptococcus* sp. were also reported (Abdel-Latif *et al.*, 2015). Job *et al.*, (2016) isolated the *Saccharomyces*, *Fusarium*, *Rhizopus*, *Acremonium*, *Aspergillus*, *Penicillium*, *Mucor*, *Rhodotorula*, and *Schizosaccharomyces* from smoked dried fishes. *Penicillium* and *Fusarium* showed highest infection. Atawodiet *et al.*, (2017) reported *Aspergillus*, *Trichophyton*, *Mucor*, *Penicillium* from African Mudfish, *Clarias gariepinus*. *Aspergillus*, *Helminthosporium*, *Fusarium*, *Trichoderma*, *Penicillium* and *Alternaria* isolated from fish species of River Ravi i.e., *Wallago attu*, *H. molitrix*, *L. rohita*, *C. mrigala*, *C. idella*, *C. carpio* and *C. catla* (Iqbal and Khatoon, 2019). Our findings are comparable to Iqbal *et al.*, 2012; Iqbal and Saleemi, 2013; Chauhan, 2013; Iqbal *et al.*, 2014; Kumari and Kumar, 2015; Iqbal and Khatoon, 2019. Our study indicates that most fungi are responsible to cause infection in silver carp under unfavorable environmental condition. It is suggested that health management practices must be acquired while rearing carps in the ponds and hatcheries of Punjab, so that likelihoods of fungal infection can be diminished.

## REFERENCES

1. Abbas MS, Khalaf JM and Yassein SN, 2016. Isolation and identification of systemic mycological isolates from fishes samples that obtained from local markets in Baghdad, Iraq. World J Exp Biosci 4:143-146.
2. Abdel-Sater MA, Abdel-Hadi AM, Abdul-Raouf UM, *et al.*, 2017. Mycobiota associated with some Red Sea fish, shellfish and their environment at Hurgada, Egypt. J Basic Appl Mycol 8: 9-23.

3. Abdel-Latif HMR, Khalil RH, El-hofi HR, *et al.*, 2015. Epidemiological investigations of Mycotic infections of cultured Gilthead seabream, *Sparus aurata* at Marriott Lake, Egypt. *Int. J. Fish. Aquac* 2:05-13.
4. Adebayo-Tayo BC, Onilude AA and Patrick UG, 2008. Mycoflora of Smoke Dried Fishes Sold in Uyo, Eastern Nigeria. *World J. Agric. Sci* 4:346 – 350.
5. Ali HH, 2015. Isolation and identification of pathogenic fungi from carp fish in Suliamania province. *G.J.B.B* 4: 356-363.
6. Atawodi JC, Yola IA, Kawo AH, and Abdullahi B, 2017. Fungi associated with African mudfish (*Clarias gariepinus*, Burchell 1822) in selected fish farms and dams in Zaria and its environs, Kaduna State, Nigeria. *BAJOPAS* 10: 642-646.
7. Bangyeekhun E, and Sylvie MA, 2001. Characterization of *Saprolegnia* sp. isolates from channel catfish. *Dis. Aquat. Organ* 45: 53-59.
8. Chauhan R, 2012. Study on certain fungal diseases in culturable and non-culturable species of fishes of Upper Lake, Bhopal. *J. chem. biol* 4:1810-1815.
9. Chauhan R, Lone S, Beigh H, and Tabbsum G, 2013. Mycotic studies of some fresh water fishes with emphasis on *Achyla* spp. *International Journal of Research in Fisheries and Aquaculture* 3:165-169.
10. Chauhan R, Nisar Z, and Baig AH, 2014a. Studies on Aspergillomycosis in *labeocalbasu* found infected with *Aspergillus flavus* and *A. terreus*. *WJPPS* 37: 1842-1848.
11. Chauhan R, Lone SA, and Beigh AH, 2014b. Pathogenicity of three species of aspergillus (*A. fumigates*, *A. niger*, *A. sydowii*) on some fresh water fishes. *Life sci. leaf* 48:65-72.
12. Chukanhom K, and Hatai K, 2004. Freshwater Fungi Isolates from Eggs of The Common Carp (*Cyprinus carpio*) in Thailand. *Myosci* 45: 42-48.
13. Czczuga B, Bartel R, Kiziewicz B, *et al.*, 2005. Zoospore fungi growing on the eggs of sea trout (*Salmo trutta m. trutta* L.) in river water of varied trophicity. *Pol J Environ Stud* 14:295-303.
14. Durborow MR, Wise DJ, and Teerhune JS, 2003. Saprolegniasis (winter fungus) and Branchiomycosis of commercially cultured channel catfish. Southern Regional Aquaculture Center. USA. SRAC Pub. 4700.
15. Ebrahimzadeh MH, Hoseinifard AS, Khosravi M, *et al.*, 2007. Isolation and identification of saprophytic fungi from Rainbow trout infected eggs in farms of Mazandaran Province, Iran. *Iranian J. Vet. Res* 62: 163–168.
16. Ellis D, Davis S, Alexious H, Handke R, and Bartley R. 2007, Description of Medical Fungi, 2<sup>nd</sup>ed: South Australia
17. FAO, 2009. *State of the World Fisheries and Aquaculture*. FAO, Rome.
18. Ikram N, and Shoaib N, 2019. Detection of Mycoflora from Marine Fishes of Karachi Fish Harbour and Ormara Region (Pakistan). *Pak. J. Zool* 51: 1967-1970.
19. Iqbal Z, Sheikh U, and Mughal R, 2012. Fungal infection in some economically important fresh water fishes. *Pak. Vet. J.*, 32: 422–426.
20. Iqbal Z, and Saleemi S, 2013. Isolation of pathogenic fungi from a freshwater commercial fish, *Catla catla* (Hamilton). *Sci. Int* 25:851-855.
21. Iqbal Z, Najam U, and Saleemi S, 2014. Fungal Infection In Silver Carp, *Hypophthalmichthys Molitrix* (Valenceinnes) Reared In Earthen Pond. *Sci. Int* 26: 261-266.
22. Iqbal Z, and Khatoon Z, 2019. Fungal infection in commercially important fishes of Ballokihedworks, River Ravi, Punjab, Pakistan. *Int. J. Biol. Res* 7: 47-55.
23. Job MO, Agina SE, and Dapiya HS, 2016. Occurrence of Aflatoxigenic Fungi in Smoke-dried Fish Sold in Jos Metropolis. *Br. Microbiol. Res. J* 11: 1-7.
24. Junaid SA, Olarubofin F, and Olabode AO, 2010. Mycotic contamination of stockfish sold in Jos, Nigeria. *J Yeast and Fungal Res*, 1: 136–141.
25. Kumari R, and Kumar DC, 2015. Fungal infection in some economically important fresh water fishes in Gandak river near Muzaffarpur region of Bihar. *Int. J. Life Sci. Pharma Res* 5:1-11.
26. Kwanprasert P, Hangavant C, and Kitanchaoen N, 2007. Characteristics of *Achylabixexualis* Isolated from Eggs of Nile Tilapia (*Oreochromis niloticus* Linn.). *KKU Res J.*, 12: 195-202.
27. Malathi K, and Rajendran K, 2012. Isolation of fungi and bacteria from various tissues of freshwater fish *Channa punctatus* (Bloch). *Asian J. Microbiol. Biotechnol. Environ. Sci* 18: 553-556.
28. Marzouk MSM, Rezeka S, and El-Gamal MH. 2003. Some mycological investigations on cultured tilapia in Kafr El Sheikh Governorate. *Kafr El Sheikh Vet. Med. J* 2:97-114.
29. Mastan SA. 2008. Incidences of dermatomycosis in fishes of Larpur reservoir, Bhopal (M.P.). *J. herbal med toxicol* 2:37-40.
30. Mastan SA, Reddy MRK, and Lakshmi DS. 2012. Oomycete infection in freshwater fishes. *Int. J. Fish. Aquaculture* 4:186-190.

31. Mastan SA. 2015. Fungal infection in freshwater fishes of Andhra Pradesh, India. *Afr. J. Biotechnol* 14:530-534.
32. Melaku H, Lakew M, Alemayehu E, Wubie A, and Chane M. 2017. Isolation and Identification of Pathogenic Fungus from African Catfish (*Clarias gariepinus*) Eggs and Adults in National Fishery and Aquatic Life Research Center Hatchery, Ethiopia. *Fish Aqua J* 8:1-5.
33. Neish GA. 1997. Observations on Saprolegniasis of adult sockeye salmon, *Oncorhynchus nerka* (Walbaum). *J. Fish. Biol* 10: 513-522.
34. Podeti KR, and Benarjee G. 2015a. Studies on haematological and histological mycosis variations of *Channapunctatus* (Bloch) found infected with *Aspergillus fumigatus* and *Aspergillus niger* spp. exhibited EUS characteristics. *WJPPS* 4: 1233-1246.
35. Podeti KR, and Benarjee G. 2015b. Incidence of mycoflora infections in *Channapunctatus* and *Channa striata* of Bhandham Lake of Warangal, Telangana, India. *World J. Pharm. Res* 4: 587-596.
36. Pailwal P, and Sati SC. 2009. Distribution of Aquatic fungi in relation to physicochemical factors of Kosi River in Kumaun Himalaya. *Nat Sci* 7: 70-74.
37. Pachade GR, Bhatkar NV, and Hande DV, 2014a. Incidences of Mycotic infections in *Channapunctatus* of Wadali Lake, Amravati, MS, India. *Int. Res. J. Biological Sci* 3: 47-50.
38. Pachade. G. R., Bhatkar. N.V. and Hande D. V., 2014b. Mycotic diversity of edible fishes in relation to physico-chemical properties of Wadali Lake, Amravati (M.S.) India. *Trends in life sciences* 3: 2319-5037.
39. Refai MK, Laila A, Mohamed AM, Kenawy M and Shimaa EMA. 2010. The Assessment of Mycotic Settlement of Freshwater Fishes in Egypt. *J. Amer. Sci* 6: 595-602.
40. Salawudeen MT, Kazeem HM, Raji MA, Oniye SJ, Kwanashie CN, Ibrahim MJ. 2017.
41. Isolation and identification of fungi from apparently healthy and diseased *Clarias gariepinus* from freshwater in Zaria, Kaduna State, Nigeria. *Microbiol Res Int* 5: 8-15.
42. Siddique MMR, Basher MA, Hussain MA, and Kibria ASM. 2009. Fungal disease of freshwater fishes in nature district of Bangladesh. *J. Bang. Agric. Uni* 7: 157-162.
43. Shahbazian N, Ebrahimzadeh MHA, Soltani M, *et al.*, 2010. Fungal Contamination in Rainbow Trout Eggs in Kermanshah province propagations with emphasis on saprolegnaceae. *Iran. J. Fish Sci* 9: 151-160.
44. Tidwell HJ and Allan LG. 2001. Fish as food: Aquaculture's contribution. *EMBO report*, 2: 958-963.
45. Vandersea MW, and Litaker RW. 2007. *Aphanomyces invadans* and Ulcerative Mycosis in Estuarine and Freshwater Fish in Florida. *J. Aquat. Anim. Health* 19: 14-26.
46. Vandersea MW, Litaker RW, Yonish B, and *et al.*, 2006. Molecular assays for detecting *Aphanomyces invadans* in ulcerative mycotic fish lesions. *AEM* 72: 1551-1557.
47. Younis GA, El-Kheir A, Esawy M, and *et al.*, 2020. Conventional Identification of Pathogenic Fungi Isolated from Fresh Water Aquarium Fish (*O. niloticus* and *C. gariepinus*) Combined with Molecular Identification of *Saprolegnia parasitica* in Egypt. *Adv. anim. vet. Sci* 8: 77-88.
48. Yanong RPE. 2003. Fungal diseases of fish. *Vet Clin Exot Anim* 6 :377-400.
49. Zahura UA, Chowdhury MBR, and Faruk MAR. 2004. Fungal infection in freshwater fishes of Mymensingh, Bangladesh. *Indian J. Fish* 51: 61-68.