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Mycotic studies of fishes collected from Upper Lake with emphasis on *Labeo* Spp.

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Abstract
The present study was aimed to investigate the fungi found associated with mycotic infected fishes collected from Upper Lake. Infected fishes were observed weekly in the fish catch from December - 2018 to May-2019. Infected fishes showed the symptoms of eroded scales, ulcerations on the skin, cottony growth on body and on eyes. In laboratory mycological examination of fishes have been done with 98 fishes belong to eight different species *viz*. *Labeo rohita, Labeo calbasu, Ctenopharyngodon idella, Channa gachua, Chanda ranga, Hypopthalmichthys molitrix, Puntius sophore and Puntius ticto*. Most affected fish species was *Puntius sophore* (20.4%). From the infected fishes 228 isolates were cultured and maximum number of isolates (38) have been collected from *Labeo rohita* in which many parts were found infected. Identification of isolates revealed the presence of eight different genera of fungi *viz*. *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp. and *Rhizopus* sp. *Aspergillus* was found to be most prevailing genera contributing (32.8%) of the total isolates and minimum isolates recorded were from Curvularia (2.6%).Temperature range 28-30 °C found to be supportive with other factors for the growth of fungi and causing mycotic infections.

Keywords: Upper Lake, mycotic infections, fish, higher group fungi and *Aspergillus*

Introduction
Fungal infections in aquaculture may cause severe diseases and mortality events leading to economic losses. They can rather be considered opportunistic fungi as many of them have virulence factors which enable them to cause disease particularly under favourable predisposing conditions. Mycotic infections become more invasive with the changes in environmental factors like temperature and salinity. Along with zoosporic fungi, there are many conidial fungi found associated with fish diseases. Some of these genera involved are *Aspergillus* (Salem *et al*., 1989b) [21], *Fusarium* (Bisht *et al*., 2000) [1]. Iqbal *et al*. (2012) [7] isolated *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., from infected fishes. Refai *et al*. (2010) [20] reported *Aspergillus, Fusarium, Mucor, Penicillium* and *Rhizopus* from infected fresh water ornamental fishes.
There are so many reports on zoosporic fungi (Oomycetes) in fishes but work on Eurotiomycetes (*Aspergillus*) infection in fishes have been sporadic. Due to increase in organic pollutants, occurrence and parasitic activity of aquatic fungi is also expected more. Therefore, present study has been aimed to isolate and identify the fungi found associated with mycotic infected fishes and to find most prevailing fungi.

Material and Methods
Collection of fish samples for the isolation of fungi
For this study fishes were collected weekly from Upper Lake for the period of six months from December 2018 to May 2019. The fishes were examined in living condition and the diseased fishes were sorted out and brought to the laboratory in sterilized polythene bags for fungal examination.

Laboratory examination of infected fishes
The fishes brought to the laboratory in sterilized polythene bags and kept in aquaria with continuous aeration. Fishes were examined grossly with naked eyes and by using magnifying

glass to find out fungal infection. After identification fish were photographed and some of the fishes were identified with the help of keys, in laboratory and photographed.

Isolation of fungi from infected fishes

Isolation of fungi from infected fishes was carried out by taking small pieces of muscles about 2 mm in diameter from different portions of the body. They were then washed thoroughly with sterilized distilled water to remove the unwanted microorganisms adhered on the surface. These tissues were then inoculated over the plates containing different agar media. Alternatively, small pieces of mycelia, taken out from infected parts of fish body, were washed thoroughly with distilled water. They were then placed in petridishes containing 20-30 ml agar media.

From the fish's fungi was also isolated by using streaking method. These petridishes were incubated at 28.0 °C to 30.0 °C temperature. Forceps, petridishes and water used were sterilized thoroughly before use.

For the isolation of fungi innocula were taken from different parts of fish body like fins, skin, gills and eyes. After taking innocula from outer surface fishes were dissected out to study the infection in internal organs. From internal organs like liver and kidney innocula were taken by wire loop and cultures were prepared by streaking methods. All the cultures were prepared on different agar media and incubated in BOD incubator.

To avoid bacterial contamination all, the glass wares, instruments and media were sterilized, along with all aseptic conditions, Streptomycine sulphate 100 mg/ml were used in media. Inoculation was done in Laminar flow in sterilized conditions. The agar plates were incubated at 28±2 °C for the growth of cultures. Growth of colony was observed in 3-4 days. For full growth of colony, plates were kept for 6-8 days for incubation. For full growth of colony plates were kept for 8-10 days for incubation. Purification of cultures was done by methods of Johnson (1956) [10].

Slide preparation of fungal isolates

For identification of fungi and microphotography slides were prepared by taking small hyphal tuft on the slide, spread with water, stained with Lactophenol cotton blue,and covered after pouring a drop of glycrine. Slides were observed under microscope and photographs were taken. Identification of fungi was carried out on the basis of keys of Nelson *et al.* (1983) [13], Raper and Fennel (1965) [18], Refai *et al*. (2004) [19], Willoughby (1994) [23], Khulbe (2001) [11] and Srivastava (2009) [22]. Fishes were identified by the keys of Jhinghran (1982) [8] and Qureshi and Qureshi (1988) [17].

Media used in the study

Cultures were prepared on Sabourauds Dextrose Agar (SDA), Potato Dextrose Agar (PDA), Corn Meal Agar (CMA) and Yeast Glucose Medium (YG).

Results and Discussion

In the present study a total number of 98 fishes were examined for fungal isolation. Infected fishes showed the symptoms of eroded skin, lesions, cottony growth and patches on body which were supported by findings of Chauhan (2013) [4]. These infected fishes belong to eight different species *viz. Labeo rohita*, *Labeo calbasu*, *Ctenopharyngodon idella* (*grass carp*), *Channa gachua*, *Hypopthalmichthys molitrix* (*silver carp*), *Puntius sophore* and *Puntius ticto.* Among the eight species of fishes maximum infected fishes were found to be, *P sophore* (22) followed by *P.ticto* (20), *C.ranga* (18), *L.rohita* (11), *H.molotrix* (9), *C.gachua* (8), *L.calbasu* (6) and minimum infected specimens of *C.idella* (4) Table-1.

Table 1: Total no. and percentage of infected species of fishes found during the study period.

S. No.	Fish species	Local name	No. of infected fishes	% of infected fishes
1.	*Labeo rohita* (Ham.)	Rohu	11	11.22
2.	*Labeo calbasu* (Ham.)	Rohu	6	6.12
3.	*Ctenopharyngodon idella* (Val.)	Grass carp	4	4.08
4.	*Chanda ranga* (Ham.)	Lal chanda	18	18.36
5.	*Hypopthalmichthys molitrix* (Val.)	Silver carp	9	9.18
6.	*Channa gachua* (Ham.)	Snake head	8	8.16
7.	*Puntius sophore* (Ham.)	Punti	20	20.40
8.	*Puntius ticto* (Ham.)	Punti	22	22.44

It was observed that in fish catch maximum infected fishes were small sized fishes but in *Labeo rohita* most of the body parts were found attacked by fungi. Similar symptoms of fungal infection in fresh water fishes have been reported by Hatai and Hashiai (1992) [5] and Hatai *et al*. (1994) [6], Iqbal and Mumtaz (2013) [9], Chauhan *et al*. (2014) [3].

From the collected infected fishes, a total no. of 228 isolates were prepared from the inocula taken from different organs of fishes. Maximum number of isolates (38) have been cultured from the inocula taken from *L.rohita*, from *H. molitrix* (34) isolates were collected, (32) each from *L. Calbasu* and *C.gachua*, (29) from *P.ticto* and (24) from *C.ranga* (21) from *p.sophore* and minimum (18) isolates have been collected from *C.idella* (Fig-1).

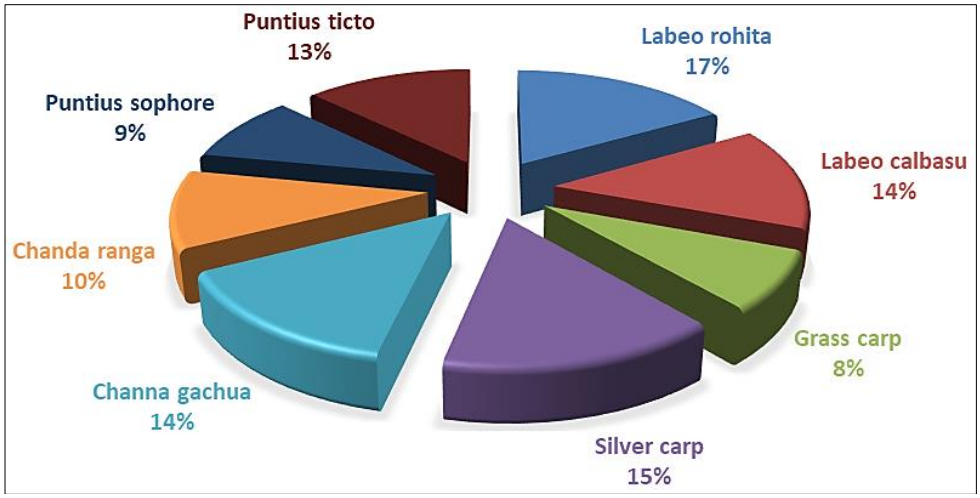

Fig 1: Total percentage of isolates from collected species of infected Fishes.

Identification of isolates prepared from infected fishes revealed the presence of eight different genera of pathogenic fungi, *viz*. *Alternaria* sp. *Aspergillus* sp. *Cladosporium* sp. *Curvularia* sp. *Fusarium* sp. *Mucor* sp. *Penicillium* sp. and *Rhizopus* sp. Among all the isolated eight genera *Aspergillus* was found to be most prevailing genera contributing (32.8%) isolates followed by (15.3%) isolates of *Cladosporium*, (13.1%) isolates of *Penicillium*, (10.9%) *Alternaria*, (9.6%) *Mucor*, (9.2%) of *Rhizopus*, (6.14%) of *Fusarium* and minimum (2.6%) isolates were contributed by *Curvularia.* which is in support with the findings of Olufemi (1983, 1985) [14, 15], Willoughby (1994) [23], Srivastava (2009) [22] and Refai *et al*. (2010) [20], Chauhan (2013) [4], recorded the infection percentage and pathogenicity of *Aspergillus* on fresh water fishes. (Fig-2)

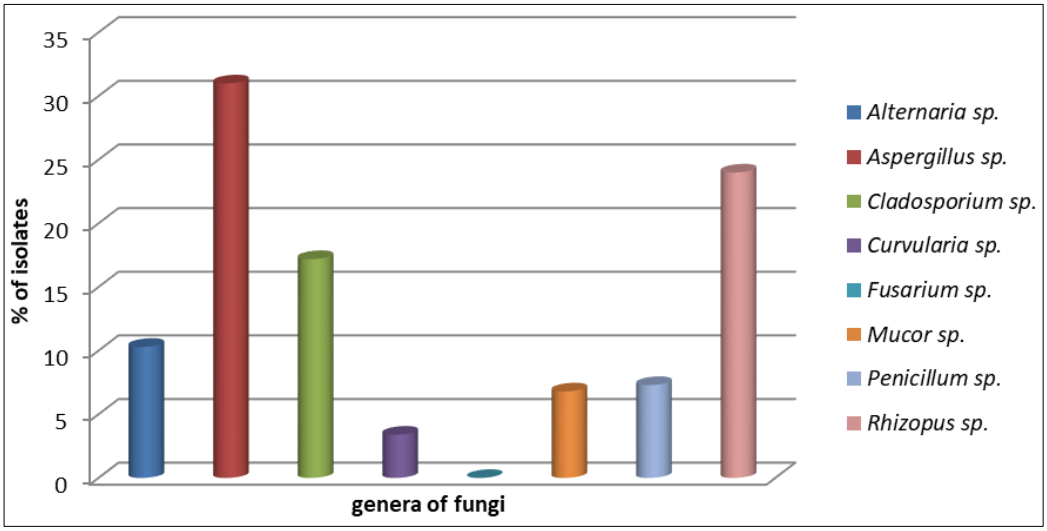

Fig 2: Total percentage of isolates of each genera of fungi, collected from infected fish.

Fungi isolated in the present study are comparable to the findings of Refai *et al.* (2010) [20], Malathi, *et al.* (2012) [12], Chauhan, (2012) [2], (Iqbal *et al.* 2012a and Iqbal *et al* 2013b) [7, 9]. Role of ecology is important factor, which influence the diversity of fungus genera on the fish and their eggs. According to Pailwal and Sati (2009) [16], Chauhan and Qureshi (2012) [2] diversity of water molds depends upon the interaction of physicochemical factors. During the present study maximum infection incidences were reported in the month of March when temperature range between 28-30 °C and other factors also supports fungal growth. Possibly the causes of fungal infections are poor water quality and injury.

Conclusion

The findings of present study revealed that Upper Lake of Bhopal showed extensive concentration of fungal infection in fishes. Among the eight species of fishes studied, maximum infected fishes were found to be, *P sophore* followed by *P.ticto* and *C.ranga.*

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