FUNGAL INFECTIONS ON EDIBLE FISHES FROM WADALI LAKE, AMRAVATI MS

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ABSTRACT:

In the present investigation *S. parasitica* and *A. niger* were found to be the most common water molds responsible for the fungal infections to fresh water fishes. *Saprolegnia* is found to be more virulent for fishes. Initially the infection was in the form of small patches and in advance cases big lesions penetrated up to the muscles. Saprolegniasis is considered as a localized infection and not systemic infection. Generally it is an external infection and can be appeared any where over the body surface especially fins, eyes, gills and ulcerated area on the body. The clinical signs exhibited by the fishes due to *Achlya* infection were characterized by the presence of a brownish cotton wool like growth and small white patches on the head and fins of the affected fishes. Morphotaxonomy of the fungi isolated from Fishes studied here.

INTRODUCTION:

Fishes are one of the most important groups of vertebrates which provide free economic services to human beings in several ways. These are more common and widely distributed almost in all parts of the world in marine, freshwater as well as estuarine ecosystems. The quality and quantity may vary but they are used by human being everywhere. Nearly all fishes fresh water and marine are edible and have been an important sources of protein food. Fishing is one of the oldest professions of man. It has received much attention from the very beginning of the human history, as fish constitute one of the most nutritionally important items for human consumption. Fishes are the primary source of protein along with omega three fatty acids. The fish protein is more easily digested in comparison to that obtained from other sources. One of the best ways to get Omega 3-fatty acids into the diet is to eat fish twice a week.

Nutritional studies have proved that fish proteins rank in the same class as chicken protein and superior to milk, beef protein and egg albumen. Fish proteins comprise of all the ten essential amino acids in desirable strength for human consumption, namely lysin (high concentration), arginine, histidine, leucine, isoleucine, valine, threonine, methionine, phenylalmine and tryptophane. This accounts for the high biological value of fish flesh. Fish therefore becomes a valuable supplement to human diet for people who are habitually taking cereals, starchy roots and sugar as their principle diet. In most fishes, the flesh is white and contains 16 to 29 percent of protein and has a food value of 300 to 1600 calories per pound. Fish oils are rich sources of the soluble fat. An excessive use of fish generally lowers the blood cholesterol level and reduces the risk of coronary heart diseases. As it contains all the ten essential amino acids in desirable quantity for human consumption, it is recommended by cardiologists to use generous quantities of fish in food to obtain adequate protein without taking in excessive fatty acids and lipids.

Representatives of all taxonomic classes of Fungi and the Oomycetes have been reported from aquatic habitats. Fungal species reported from aquatic habitats range from those that are adapted to complete their life cycles in aquatic habitats and are not found outside of the aquatic environment (residents) to those that occur in water fortuitously by being washed or blown in (transients). Knowledge about the occurrence of Fungi in aquatic habitats is important and its survey is essential.

Wong *et al*. (1998) studied more than 600 species of freshwater Fungi with a greater number known from temperate, as compared to tropical, regions. It is suggested that three main groups can be considered which include Ingoldian Fungi, aquatic Ascomycetes and non-Ingoldian hyphomycetes, chytrids and oomycetes. Freshwater Fungi are thought to have evolved from terrestrial ancestors. Many species are clearly adapted to life in freshwater as their propagules have specialized aquatic dispersal abilities. Freshwater Fungi are involved in the decay of wood and leafy material and also cause diseases of plants and animals.

Fungal infections of freshwater fish are common and distributed worldwide and associated with immune suppression (Pickering and Willoughby, 1982a). Fungal diseases are easily recognized by relatively superficial, colony of fluffy growth on the skin and gill of fishes. Fungal infection in fishes causes clinical abnormalities such as skin darkening, exophthalmia, corneal opacity, abdominal distention, ulceration of the skin and cotton wool like growths on various parts of the body (Refai, *et al.*, 2010).

Fungal infections are therefore a sign that fish are in very poor health (Scott, 1961; Shrivastava, 1979; Hatai, 1989; Willougby, 1994; Lu *et al*., 1998; Takuma *et al.*, 2013).

Mastan *et al.*(2012) carried out mycological studies on fishes namely *Channa striatus, Channa punctatus, Clarias batrachus, Labeo rohita, Heteropneustis fossilis* and *Mystus cavasius* with fungal infections of five species viz. *Saprolegnia diclina, S. ferax, S.hypogyana, S. parasitica* and *Achlya Americana*.

RESEARCH METHODOLOGY:

Wadali Lake is located at 20^{0}93”N and 77075”E and at an elevation of 343m in Amravati, Maharashtra (India). It is in the vicinity of Sant Gadge Baba Amravati University campus towards South – East direction of the university in the Pohara range of hills.

The water from the lake is being used for the drinking purpose and fishery activities, where the fishing of *Channa punctatus* (Phool-dhok), *Catla catla* (Katla), *Labeo rohita* (Rohu), *Clarias* species (Mangri), *Wallago attu* (Shivada), *Mystus seenghala* (Singala) is carried out on commercial scale.

Collection of fishes and Sampling techniques:

The healthy and infected *Channa punctatus* and *Clarias* species were collected randomly every week at regular interval from the study area with the help of fishermen. The infected fishes in catch were identified from red spot on their body, excess mucus secretions, damaged and infected gills and their sluggishness. For further investigations like isolation and culture of infective Fungi to know their morphotaxonomy and pathogenecity to fishes, histopathological alterations in various organs and alterations in muscle protein contents the healthy as well as infected fishes were brought to the laboratory immediately after collection. They were acclimatized at laboratory condition in big aquaria (48x18x18) inches for 15 days.

Isolation of fungus:

Following steps were followed for the fungal studies.

1) Potato Dextrose Agar (PDA) media was used as a culture media for the isolation of the fungus.
2) Infected fishes were cut in cross section, using a flamed scalpel.
3) Small block of muscle was removed from the lesion.
4) Blocks of tissue were removed and placed into Petri dishes, washed with 15 ml distilled water.
5) The tissue blocks were transferred into the other set of Petri dishes. The Petri dishes were placed inverted in incubator at 25 °C for 3 days, until a circular fungal mat developed, which were used for subculture of the fungus.
6) A suitable portion of culture of different colonies from PDA was taken out with the help of forceps or needle and put on a slide in 1 or 2 drops of cotton blue on clear slides and examined under a compound microscope.

RESULTS AND DISCUSSION:

The Fungi were isolated from lesions on the skin, gill, kidney and liver of *Clarias* sp. and *Channa punctatus* from Wadali lake. Isolated Fungi were obtained by culturing them on PDA (Potato Dextrose Agar), Corn Meal Agar, Czapex Dox Agar and Water Agar. Following Fungi are reported during the present investigations.

1. *Achlya hypogyna* Coker and Pemberton
2. *Alternaria alternata* (Fr.) Keissler
3. *Aphanomyces invadans* Willoughby, Roberts and Chinabut
4. *Aspergillus flavus* Link ex Gray
5. *Aspergillus niger* Van Tieghem

6. *Cladosporium cladosporides* Link ex Gray
7. *Curvularia lunata* Boedijn
8. *Drechslera hawaiinsis* Ellis
9. *Fusarium oxysporum* Schlechtendahl
10. *Mucor mucedo* Micheli ex Saint-Amans
11. *Rhizopus stolonifer* Ehrenberg ex Corda
12. *Saprolegnia parasitica* Coker

1) Achlya hypogyna

This fungus was isolated from white lesions on the skin and gills of infected *Clarias sp* and *Channa punctatus*. Colonies of *A. hypogyna* on PDA and Corn Meal Agar were observed as white mass initially which got converted into light green and branched hyphae growing in all directions after two days .

The hyphae were coenocytic. Oogonia were developed at the base of hyphae. Oogonia observed lateral, spherical, oval or subglobose.Oogonial wall examined smooth or papillate. Oospores observed were centric or subcentric. The antheridia were hypogynous. The observed characters resemble with *Achlya hypogyna* morphology and sporulations characteristics.

2) Alternaria alternata

Growth of *A.alternata* was observed on the skin of infected *Clarias* sp. and *Channa punctatus* . Upon culturing on the colonies appeared flat, woolly initially and they turn greenish black after 24 hrs.

Upon microscopic examination mycelia appeared septate brown hyphae alongwith septate and brown conidiophores. Conidia were large with transverse as well as and longitudinal septa. They were obclavate singly or in chains, darkly pigmented, muriform with smooth or rough wall. The conidia were broder proximally and tapered distally.

3) *Aphanomyces invadans*

A.invadans colonies were observed on skin and gills of *Clarias* sp. and *Channa punctatus*. Colonies were observed as wooly mycelia on PDA and Czapex Dox Agar.

Mycelia were slow-growing and coenocytic with broad hyphae. Globular sporangia with zoospores were observed which were slender but of same diameter as that of hyphae. Primary zoospores were observed within zoosporangia and encysted in a cluster at the top of the zoosporangia. The primary zoospores were developed inside the sporangium. The primary zoospores were released from the tip of the sporangium.

4) Aspergillus flavus

Infection of *A. flavus* were observed on the skin of *Clarias* sp. and *Channa punctatus*. Creamy to lime green colonies developed on PDA and its texture was woolly to granular.

Under microscope hyphae appeared septate and hyaline. Conidial heads were radiate to loosely columnar with age. Conidiophores were dome-like with smaller heads and flask-shaped with larger heads. Vesicles were globose to subglobose, metulae covering nearly the entire vesicle. Conidia were with smooth to very finely roughened wall and were globose to subglobose in shape.

1) *Aspergillus niger*

Infection of *A.niger* was observed on skin of *Clarias* sp. and *Channa punctatus*. Colonies examined on PDA were initially white which quickly became blackish with conidial production.

Hyphae were septate and hyaline. Yellow to brown conidiophores with thick walls were mostly arising directly from the substratum. They were long, smooth, and hyaline, darker at the apex and terminating in a globose vesicle. Metulae covered the entire vesicle. Conidia were brown to black, very rough and globose.

2) *Cladosporium cladosporioides*.

The fungus was detected from white and red lesions on the skin and gills of *Clarias* sp. and *Channa punctatus*. Colonies on PDA were velvety to powdery, green to black.

Mycelia observed were septate brown. Hyphae observed with erect and pigmented conidiophores, and conidia. Conidial wall was smooth. Conidia appeared smooth pale brown, single celled, ovate, oval elliptical, slightly tapering at one or both ends, however more cylindrical. They were present as chains.

3) *Curvularia lunata*

Colonies of *C. lunata* was examined from dermal region of *Clarias* sp. and *Channa punctatus*. *Curvularia* produced woolly colonies on PDA which turned into olive green after maturity.

Mycelia observed were septate and brown. Conidiophores and conidia were also brown. Conidiophores were simple or branched and were bent at the points where the conidia originate. Sympodial geniculate growth

was observed. The conidia were straight, brown and multiseptate. The septa were transverse dividing each conidium into multiple cells. The central cell was typically darker with a swelling.

4) *Drechslera hawainsis*

Drechslera hawainsis was noted from dermal region of *Clarias* sp. and *Channa punctatus*. Colonies observed on PDA were olive green to black.

Hyphae were septate. Conidiophores were brown to dark brown, erect and parallel to each other. Conidia were multicellular, thick-walled large, solitary, club-shaped, and pale to dark brown in color.

5) *Fusarium oxysporum*

The fungus was detected as white lesions on the skin and gills of infected *Clarias* sp. and *Channa punctatus*. Colonies on PDA were initially white but later became purple (Fig.4.33 E).

Mycelia were septate, branched and formed sporodochia. Conidiophores were short, single and arranged in densely branched clusters. Macroconidia were fusiform, slightly curved, pointed at the tip, mostly three septate. Microconidia were mostly single celled, hyaline, oval, oblong, minute and ellipsoidal produced singly from the tips of the phialides.

6) *Mucor mucedo*

M. mucedo was examined from dermal region of *Clarias* sp. and *Channa punctatus*. Colonies observed on PDA grew rapidly as grayish brown.

Mycelia observed were nonseptate with broad hyphae. Sporangiophores were short, erect and columella brown. Sporangia were rounded gray to black in color and filled with sporangiospores. Sporangiospores were rounded or slightly elongated.

7) *Rhizophus stolonifer*

This fungus was observed from red lesions on the skin of *Clarias* sp. and *Channa punctatus*. Colonies grown on PDA appeared as white cottony spreading rapidly.

Mycelia appeared aseptate and broad under microscope. Sporangiophores were with rhizoids. Sporangia and sporangiospores were also observed. Sporangiophores were brown in color and unbranched, solitary or in clusters. Rhizoids were seen at the point where the stolons meet. Sporangia were seen at the tip of the sporangiophores. Sporangiospores were unicellular, round to ovoid in shape, hyaline to brown in color.

8) *Saprolegnia parasitica*

Infection of *S.parasitica* was observed on from the skin, gills, kidney and liver of *Clarias* sp. and *Channa punctatus*. Colonies of *S.parasitica* observed on PDA were cottony white.

Mycelia were well developed with coenocytic hyphae and sporangia at the tips. The sporangia were cylindrical with many nuclei. Antheridia and oogonia were also observed. The oogonia were globular with thicker wall. Antheridia were club shaped. The globular oospores were also detected.

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Plate-I

Fig.:*Saprolegnia* with terminal Oogonium and lateral antheridium.

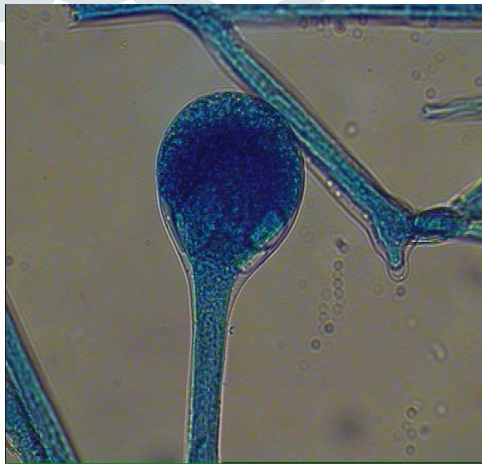

Fig.:*Saprolegnia* with oogonium.

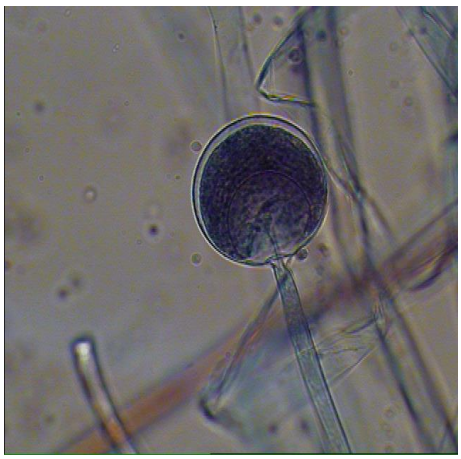

Fig.: *Achyla* with terminal Oogonium

Fig.: *Aphanomyces* with oogonium.

Fig.:*Saprolegnia* with oospore.

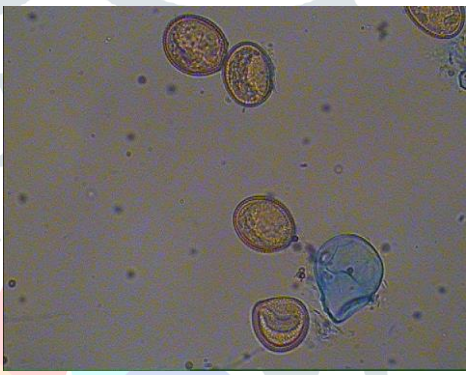

Fig.: *Saprolegnia* with zoospore.

Plate-II

Fig.: *Achyla* with Oospore

Fig.:*Saprolegnia* with mature oospore.

Fig.: *Aphanomyces* with oospore

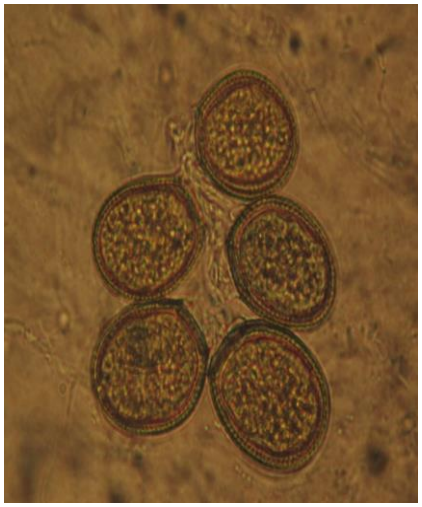

Fig.:*Aphanomyces* with oogonium.

Fig.: *Saprolegnia* with oogonium and antheridium

Fig.: *Saprolegnia* with oogonia.

Plate-III

Fig.: *Saprolegnia* with oogonium

Fig.: *Saprolegnia* with oogonia.

Fig.: *Curvularia* conidia on conidiophore.

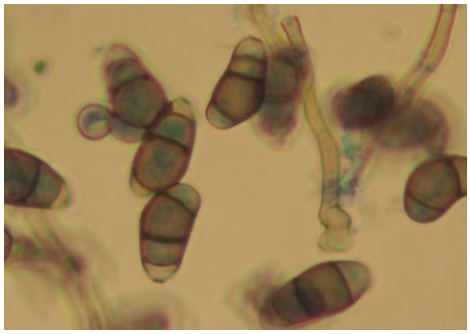

Fig.*: Curvularia* conidia.

Fig.: *Mucor* sporangium.

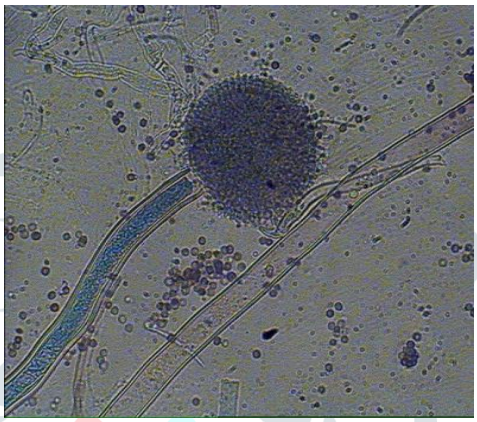

Fig.*: Aspergillus* sporangium.