

## Full Length Research Paper

# Oomycete infections in freshwater fishes

S. A. Mastan\*, M. Radha Krishna Reddy and D. Sri Lakshmi

Post-Graduate Department of Biotechnology, D. N. R. College, P. G. Course and Research Centre, Bhimavaram, 534202 W. G. (Dt.) Andhra Pradesh, India.

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**Mycological studies were carried out on fishes with fungal infection. A total of seventeen isolates of fungi were obtained from diseased fishes which belongs to five species namely *Saprolegnia diclina*, *Saprolegnia ferax*, *Saprolegnia hypogyana*, *Saprolegnia parasitica* and *Achlya americana*. All these fungi were isolated from six different species of fishes viz. *Channa striatus*, *Channa punctatus*, *Clarias batrachus*, *Labeo rohita*, *Heteropneustis fossilis* and *Mystus cavasius*. The parasitic ability of all the seventeen fungi was confirmed by conducting pathogenicity tests under laboratory conditions using healthy fishes of the same species. All the species of fungi were found to be pathogenic to fish, but *Saprolegnia parasitica* was more virulent showing infection within eight hours.**

**Key words:** Oomycetes infection, pathogenicity, freshwater fishes.

## INTRODUCTION

Oomycetes are saprophytic opportunists multiplying on fish that are physically injured, stressed or infected (Pickering and Willoughby, 1982a). Members of this group are generally considered agents of secondary infections arising from conditions such as bacterial infection, poor husbandry practices, and infestations by parasites and social interactions. However, there are several reports of Oomycetes as infectious agents of fish and their eggs. Oomycetes infections occur frequently during winter months in India when the temperature is low. Fungal infections were also reported from Japan, Australia and South-Asian countries (Scott and O'Bier, 1961; Bhargava et al., 1971; Willoughby, 1978; Srivastava, 1980; Sati and Khulbe, 1983, Sati, 1991; Hatai and Hoshiai, 1992; Walser and Phelps, 1993; Khulbe et al., 1994,1995; Kitancharoen et al., 1995; Kitancharoen and Hatai, 1996; Bisht et al., 1996; Hatai and Hoshiai, 1992; Vikas et al., 2005; Mastan, 2008). The present paper reports the occurrence of Oomycete infections in freshwater fishes.

## MATERIALS AND METHODS

### Collection of infected fish samples

Incidences of fungal infection were recorded during the winter

months of September, 2008 to December, 2008, and January, 2009 to February, 2009 from fish culture ponds of west Godavari District, Andhra Pradesh. A total of 1, 270 fishes were screened. The fishes were caught with the help of fisherman in three alternate days in a week. The fungal infected fishes were brought to the laboratory in living condition and kept in glass aquarium of size 90×45×45 cm filled with clean fresh water. The dead as well as living fishes were examined grossly for lesions and ulcerations.

### Mycological examinations

Isolation of fungi from infected fishes was carried out by taking small pieces of muscles about 2 mm in diameter from infected portions of the body. They were then washed thoroughly with sterilized distilled water to remove the unwanted micro organisms 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 placed in a Petri dish containing 20 to 30 ml distilled water and baited on different baits such as hemp and mustard seeds. These Petri dishes were incubated at 15 to 22°C for a week. Pure and bacteria free cultures were prepared by using the methods of Coker (1923) Johnson (1995) and Scott (1961). Identification of fungi was done on the basis of their vegetative and reproductive characters using the monographs of Coker (1923) and Khulbe (1994).

### Pathogenicity studies

In order to demonstrate the pathogenicity of the isolates obtained from the naturally infected fishes, experimental infection trails were

\*Corresponding author. E-mail: [shaikmastan2000@yahoo.com](mailto:shaikmastan2000@yahoo.com).

conducted in the laboratory. Isolated species of fungi such as *Saprolegnia diclina*, *Saprolegnia ferax*, *Saprolegnia hypogyana*, *Saprolegnia parasitica* and *Achlya americana* were tested separately on the fingerlings of different species of fishes, having average size and weight of  $8.16 \pm 0.13$  cm and  $12.5 \pm 0.28$  g, respectively.

The pathogenicity tests were carried out by employing the methodology of Scott and O' warren (1964). Covered glass troughs (12' x 9'), wrapped in aluminum foils were sterilized in hot air oven at 120°C for 24 h. Filtered sterile lake water was filled aseptically into each trough. An aerator was used to aerate the water throughout the experiment. Six fungal inoculated blocks (1.0 cm<sup>2</sup>) of sulfite polymyxin sulfadiazin (SPS) agar/ potato dextrose agar (PDA) medium were placed at different sites in the trough. Six uninoculated blocks of the same agar medium were placed in another trough which was used as control. After 48 h, when spores developed, experimentally injured fishes were placed in these troughs. Four fishes of each species were kept in each trough. All the experiments were conducted at 20.0 to 25.0°C in triplicate sets.

Water samples were collected from infected water bodies for analysis of various physico-chemical parameters such as temperature, conductivity, pH, FC<sub>O2</sub>, dissolved oxygen, total alkalinity, total hardness and chloride as per methods of American Public Health Association (1995).

## RESULTS

In the present study, a total of 17 isolates of fungi were obtained from the fishes investigated. These isolates represent five species and belonged to two genera namely *Saprolegnia* and *Achlya*.

### *Saprolegnia declina*

*S. diclina* was isolated twice from infected fishes. One isolate was collected from *Channa gachua* and one from *Channa striatus*

### *Saprolegnia ferax*

A total of three isolates of *S. ferax* were obtained, one was collected from *C. batrachus*, one from *C. striatus* and one from *C. gachua*.

### *Saprolegnia hypogyana*

One isolate of *S. hypogyana* was collected from infected *C. gachua*.

### *Saprolegnia parasitica*

*S. parasitica* is the most frequently occurring parasite of fish. A total of six isolates of this species were obtained from infected fishes. Three were isolated from *H. fossilis*, two were collected from *Mystus cavasius* and one from *Channa punctatus*.

### *Achlya americana*

A total of five isolates of this species were obtained, two were collected from *H. fossilis*, two from *M. cavasius* and one from *Labeo rohita*. The maximum percentage of infection was recorded as 4.8 in the month of December, 2008, while the minimum percentage of infection was recorded as 0.8 in the month of February, 2009 (Figure 1).

In case of fish species, the highest percentage of infection (0.94%) was reported in *H. fossilis* while lowest infection (0.24%) was reported in *L. rohita* (Figure 2). The experimental infection trails were conducted with fungi isolated from naturally infected fish to test their pathogenicity under laboratory conditions. Each isolate was tested on that particular species of fish from which it was originally isolated. The results are summarized in Table 1.

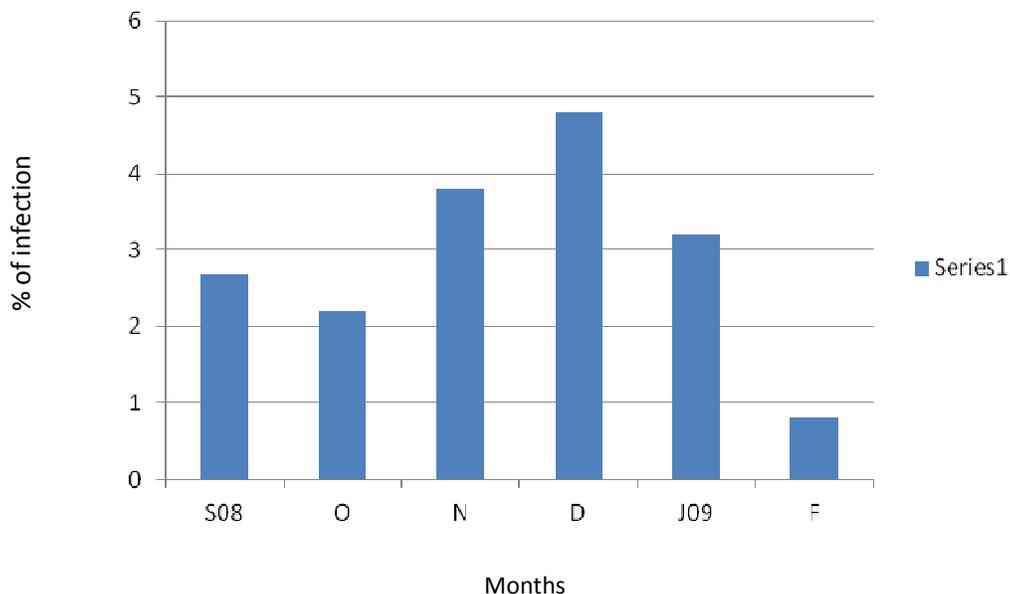
All the isolates of genus *Saprolegnia* are found to be pathogenic to fish. Hyphal growth of fungi was clearly visible at the site of injured areas of experimental fishes within 8 to 48 h after inoculation. All the test fishes died within 24 to 96 h after catching infection (Table 1). It is observed that although all the isolates have the potential to parasitize the fish, *S. parasitica* is more vigorous, showing infection within 8 h (Table 1). A wide range of fluctuations were noticed in various water quality parameters of affected water bodies (Table 2)

The moderate values of physico-chemical parameters are congenial for growth and development of aquatic fungi. The low temperature, moderate D.O, pH and low alkalinity are some of the pre disposing factors for fungal infection.

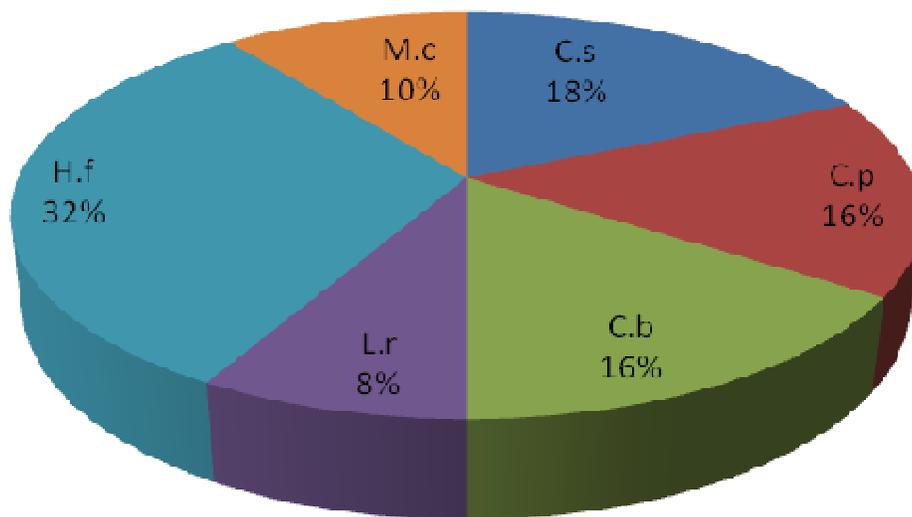
## DISCUSSION

Fungal infection in fish was first reported during the mid-eighteenth century (Arderon, 1748). Later on, some other workers reported several pathogenic fungi from different species of fish and fish eggs (Sati, 1982; Fraser et al., 1992; Roberts et al., 1993; Chinnabut et al., 1995; Willoughby et al., 1995; Khulbe et al., 1995; Mastan, 2008).

In India, the mycological studies were initiated by Chidambaram (1942) who observed red patches on the body of *Osphronemus gouramy* due to *Saprolegnia* species. Tiffney (1939b) was the first to demonstrate the ability of *S. parasitica* (Coker) to parasitize a wide range of fishes and amphibians and emphasized the fact that the injury greatly lowers the resistance of hosts to fungal infections. Vishniac and Nigrelli (1957) conducted laboratory experiments and demonstrated the parasitic ability of sixteen species of aquatic fungi belonging to seven genera of *Saprolegniaceae*. Scott (1964) demonstrated that *S. parasitica*, *S. ferax*, *S. diclina*, *Saprolegnia monoica*, *Achlya bisexualis* and some non fruiting isolates



**Figure 1.** Monthly percentage of fish infection (from September, 2008 to February, 2009). Monthly percentage of fish infection was calculated as: No. of fish infected ÷ No. of total fishes screened × 100.



**Figure 2.** Species-wise percentage of fungal infection showing 1, *Channa striatus* (C.s); 2, *Channa punctatus* (C.p); 3, *Clarius batrachus* (C.b); 4, *Labeo rohita* (L.r); 5, *Heteropneustis fossilis* (H.f); and 6, *Mystus cavasius* (M.c). Species infection percentage was calculated as: No. of specimens of particular species infected ÷ Total no. of fishes screened × 100.

of *Saprolegnia* could parasitize wounded platy fish under controlled conditions. Sati and Khulbe (1983) carried out host range studies with *S. diclina* on nine species of cold water fishes viz. *Barilius bendelisis*, *Carassius auratus*, *Cyprinus carpio*, *Nemachelius rupicola*, *Puntius conchoniensis*, *Puntius ticto*, *Schizothorax palgiostomus*, *Saprolegnia richardsoni* and *Tor tor*. The experimental

infection of *Saprolegnia* on different species of fishes has also been reported by Qureshi et al. (1995). Chinabut et al. (1995) and Hatai et al. (1994) reported the pathogenicity of *Aphanomyces* species on Dwarf gourami. Kitanchroen and Hatai (1996) have conducted experimental infection trials with *Saprolegnia* sp. on Rainbow trout eggs.

**Table 1.** Experimental infection trails with various species of fungi isolated from infected fishes.

S/N	Isolate No.	Fungi inoculated	Experimental fish	No. of fish used	Mycosis evident (h)	Death (h)
1	S.d/1	<i>Saprolegnia diclina</i>	<i>Channa gachua</i>	6	16	24
2	S.d/2	<i>Saprolegnia diclina</i>	<i>Channa striatus</i>	6	24	48
3	S.f/3	<i>Saprolegnia ferax</i>	<i>Clarias batrachus</i>	6	48	96
4	S.f/4	<i>Saprolegnia ferax</i>	<i>Channa striatus</i>	6	36	72
5	S.f/5	<i>Saprolegnia ferax</i>	<i>Channa gachua</i>	6	48	72
6	S.h/6	<i>Saprolegnia hypogyana</i>	<i>Channa gachua</i>	6	48	72
7	S.p/7	<i>Saprolegnia parasitica</i>	<i>Heteropneustis fossilis</i>	6	09	38
8	S.p/8	<i>Saprolegnia parasitica</i>	<i>Heteropneustis fossilis</i>	6	08	36
9	S.p/9	<i>Saprolegnia parasitica</i>	<i>Heteropneustis fossilis</i>	6	08	36
10	S.p/10	<i>Saprolegnia parasitica</i>	<i>Mystus cavasius</i>	6	08	42
11	S.p/11	<i>Saprolegnia parasitica</i>	<i>Mystus cavasius</i>	6	08	42
12	S.p/12	<i>Saprolegnia parasitica</i>	<i>Channa punctatus</i>	6	09	42
13	A.m/13	<i>Achlya americana</i>	<i>Heteropneustis fossilis</i>	6	24	72
14	A.m/14	<i>Achlya americana</i>	<i>Heteropneustis fossilis</i>	6	24	76
15	A.m/15	<i>Achlya americana</i>	<i>Mystus cavasius</i>	6	18	48
16	A.m/16	<i>Achlya americana</i>	<i>Mystus cavasius</i>	6	18	48
17	A.m/17	<i>Achlya americana</i>	<i>Labeo rohita</i>	6	18	48

**Table 2.** Water quality parameters of affected water bodies during study period.

S/N	Parameter	Values in range
1	Water temperature (°C)	17-28
2	Conductivity (uscm)	260-290
3	pH	6.8-8.7
4	FCO <sub>2</sub> (mg/l)	1.0-2.6
5	Dissolved oxygen (mg/l)	5.6-8.5
6	Total Alkalinity (mg/l)	78-193
7	Total hardness (mg/l)	69-170
8	Chloride (mg/l)	10-32

In the present study, mycological examination of infected fishes revealed the presence of sixteen isolates of five species viz. *S. diclina*, *S. ferax*, *S. hypogyana*, *S. parasitica*, and *A. americana*. All the species of *Saprolegnia* are found to be virulent for fishes. This observation is in agreement with the finding of Scott and O'Bier (1962) who reported that among the species of fungi, *S. parasitica* is found to be the most destructive. This finding conforms with the reports of Hatai and Hoshiai (1992) who reported that the infection caused by *S. parasitica* in salmon resulted in mass mortality. Both the scaly and non-scaly fishes were found to be equally susceptible to the species of fungi tested. *S. hypogyana* was isolated from, and tried on *C. striatus*, which also showed its wide range on fishes. The same is also reported by Chauhan and Qureshi (1994). Qureshi et al.

(1999) have conducted pathogenicity studies with various species of *Saprolegnia* on different species of fishes of Central India

#### REFERENCES

- Arderon W (1748). The Substance of a letter from Mr. William Arderon, F.R.S., Phil. Trans. Res. Soc. 45(487):321-323.
- American Public Health Association (1995). Standard methods for the Examination of Water and Waste Water .Washington, DC (16<sup>th</sup> Ed.), pp. 1-1268.
- Bhargava KS, Swarup K, Singh SC (1971). Fungi parasitic on certain fresh water fishes of Gorakhpur. Indian Biol. 3:65-69.
- Bisht GS, Deepa B, Chandra JKRD (1996). Potential threat to reservoir fishery by in kumaun Himalaya, India. Curr. Sci. 71(9):720-722.
- Chauhan R, Qureshi TA (1994). Host range studies of *Saprolegnia ferax* and *Saprolegnia hypogyana*. J. Inland Fish. Soc. India 26(2):99-106.
- Chidambaram K (1942). Fungus disease of Gourami (*Osphromenus goramy*, Lacepede) in a pond at Madras. Curr. Sci. 11:289-290.
- Chinnabut S, Robert RJ, Pearson GR Willoughby LG (1995). Histopathology of snake head, *Channa striatus* (Bloch) experimentally infected with the specific *Aphanomyces* fungus associated with Epizootic Ulcerative Syndrome (EUS) at different temperatures, J. Fish Dis. 18:41-47.
- Coker WC (1923). The *Saprolegniaceae*, with notes on other water molds. University of North California Press, Chapel Hill, North Carolina, p. 201.
- Fraser GC, Callinan RB, Calder LM (1992). *Aphanomyces* species associated with red spot disease; an ulcerative disease of estuarine fish from eastern Australia. J. Fish Dis. 15:173-181.
- Hatai K, Hoshiai G (1992). Saprolegniasis in cultured coho Salmon. Fish Pathol. 11:233-234.
- Hatai K, Nakamura K, Rha K, Yuasa SA, Wada S (1994). *Aphanomyces* infection in the dwarf gourami (*Colisa lalia*). Fish Pathol. 29:95-99.
- Johnson Jr. TW (1995). The Genus *Achlya*, Morphology and Taxonomy. University of Michigan Press. Ann Arbor p. 180.

- Kitancharoen N, Hatai K (1996). Experimental infection of *Saprolegniasis* Spp. in Rainbow trout eggs. Fish Pathol. 31(1):49-50.
- Kitancharoen N, Hatai K, Ogihara R, Aya DNN (1995). A new record of *Achlya klebsiana* from snakehead, *Channa striatus*, with fungal infection in Myanmar. Mycoscience 36:325-238.
- Khulbe RD, Joshi C, Bisht GS (1995). Fungal diseases of fish in Nanak Sagar, Nainital, India. Mycopathology 130:71-74.
- Khulbe RD (1994). A world monograph of parasitic water molds. Almora; shree Almora Book depot. p. 144.
- Mastan SA (2008). Incidences of Dermatomycosis in Fishes of Larpur reservoir, Bhopal, (M.P). J. Herb. Med. Toxicol. 2(1): 37-40.
- Pickering AD, Willoughby LG (1982a). *Saprolegnia*, infection of salmonid fish. In: 50<sup>th</sup> Annual Report, Institutes of freshwater Ecology, Windermere Laboratory, England, pp. 38-48.
- Qureshi TA, Chauhan R, Prasad Y, Mastan SA (1995) Fungi isolated from EUS affected Fishes of Hataikheda reservoir, Bhopal. Indian J. Appl. Pure Biol. 10(2):153-157.
- Qureshi TA, Chauhan R, Prasad Y, Mastan SA (1999). Association of Fungi with Epizootic Ulcerative Syndrome of Fishes. Indian J. Appl. Pure Biol. 14(1):45-49.
- Roberts RJ, Willoughby LG, Chinnabut S (1993). Mycotic aspects of Epizootic Ulcerative Syndrome (EUS) of Asian fishes. J. Fish Dis. 16:169-183.
- Sati SC, Khulbe RD (1983). A host range of *Saprolegnia diclina* Humphery on certain cold water fishes of India. Proc. Nat. Acad. Sci. India 53(8): IV 3.9-3.12.
- Sati SC (1982). Aquatic fungi of Kumaun in relation to fish infection. Ph.D. Thesis, Kumaun University, Nainital, India, pp. 1-189.
- Sati SC (1991). Two fungal parasites of the eggs of *Tor putitora* Himalaya. Curr. Sci. 57:95-96.
- Srivastava RC (1980). Studies in fish mycopathology: A review. Mycosen 23:325-332, 380-390, 462-480.
- Scott WW (1961). A monograph of the genus *Aphanomyces*. Var. Agar. Expt. Stn. Tech. Bull. 151:1-95.
- Scott WW, O' Bier AH (1961). Aquatic fungi associated with diseased fish and fish eggs. Prog. Fish Cult. 24: 3-15.
- Scott W (1964). Fungi associated with fish disease. Dev. Ind, Microbiol., 5:109-123.
- Scott WW, O' Warren CO (1964). Studies of the host range and chemical control of fungi associated with diseased tropical fish. Tech. Bulletin 171, Virginia Agriculture Experimental Station, Blacks burg, p. 24.
- Tiffney WN (1939b). The identity of certain species of the *Saprolegnia parasitica* to fish. J. Elisha Mitchell Sci. Soc. 55:143-151.
- Vishniac HS, Nigrelli RF (1957). The ability of the *Saprolegniaceae* to parasitize platy fish. Zoologica 42:131-134.
- Vikas S, Mastan SA, Qureshi TA (2005). Incidences of *Saprolegniasis* in fishes of Hataikheda reservoir, Bhopal. Indian J. Fish. 52(3):367-370.
- Walser CA, Phelps RP (1993) The use of Formalin and iodine to control *Saprolegnia* infections on Channel catfish, *Talurus punctatus* eggs. J. Appl. Aquat. 3:269-278.
- Willoughby LG, Roberts RJ, Chinnabut S (1995). *Aphanomyces invaderis* sp. Nov., the fungal pathogen of freshwater tropical fish affected by Epizootic ulcerative syndrome. J. Fish Dis. 18:273-275
- Willoughby LG (1978). *Saprolegniasis* of Salmonid fish in Windermere, a critical analysis. J. Fish Dis. 1:51-67.