

Full Length Research Paper

Fungal infection in freshwater fishes of Andhra Pradesh, India

S. A. Mastan

Matrix-ANU Advanced Aquaculture Research Centre, Acharya Nagarjuna University, Nagarjuna Nagar-522 510, India.

Received 6 February, 2012; Accepted 19 January, 2015

A total of 17 isolates of fungi were isolated from diseased fishes which belong to five species namely *Saprolegnia diclina*, *Saprolegnia ferax*, *Saprolegnia hypoglyca*, *Saprolegnia parasitica* and *Achlya americana*. All these fungi were isolated from six different species of fresh water fishes viz. *Channa stratius*, *Channa punctatus*, *Clarias batrachus*, *Labeo rohita*, *Heteropneuste fossilis* and *Mystus cavasius*. The parasitic ability of all the fungal species were confirmed by conducting experimental infection under laboratory conditions using healthy fishes of the same species. All the species of fungi were found to be pathogenic to fish.

Key words: Saprolegnia, pathogenicity, fishes.

INTRODUCTION

The saprolegniaceae are responsible for significant infections, involving both living and dead fish and their eggs, particularly in aquaculture. Oomycetes are sapro-phytic opportunists multiplying on fish that are physically injured, stressed or infected (Pickering and Willoughby, 1982). Members of this group are generally considered as 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 Oomy-cetes as infectious agents of fish and their eggs (Scott and O'Bier, 1961; Bhargava et al., 1971; Willoughby, 1978; Srivastava, 1980; Sati and Khulbe, 1983; Sati, 1991; Walser and Phelps, 1993; Hatai and Hoshiai, 1993; Khulbe et al., 1994; 1995; Kitancharoen et al., 1995; Qureshi et al., 1995; Kitancharoen and Hatai, 1996; Bisht et al., 1996; Rajender and Khulbe, 1998; Qureshi et al., 1999; Vikas et al., 2005; Ramaiah, 2006;

Mastan, 2008; and Vinay, 2008; Refai et al., 2010; Rekha and Qureshi, 2012, 2013; Hatai, 2012; Vickie et al., 2013; Zafar Iqbal and Reshma, 2013). This paper communicates the fungal infection in freshwater fishes.

MATERIALS AND METHODS

Collection of infected fish samples

Incidences of fungal infection were recorded during the winter months of 2008 to 2009 from unnamed water bodies of Akividu and Bhimavaram divisions of West Godavari Dist, A.P.,. A total of 1,270 fishes were screened. The fungal infected fishes were brought to the laboratory in the living condition and kept in glass aquarium, of the size of 90 x 45 x 45 cm filled with clean fresh water. The dead as well as living fishes were examined grossly for lesions and ulcerations.

*Corresponding author. E-mail : shaikmastan2000@yahoo.com.

Author(s) agree that this article remains permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

Table 1. Fungi isolated from infected fishes.

Isolate no.	Name of fungi	Host fish
S.d/1	<i>Saprolegnia diclina</i>	<i>Channa gachua</i>
S.d/2	<i>Saprolegnia diclina</i>	<i>Channa striatus</i>
S.f/3	<i>Saprolegnia ferax</i>	<i>Clarias batrachus</i>
S.f/4	<i>Saprolegnia ferax</i>	<i>Channa striatus</i>
S.f/5	<i>Saprolegnia ferax</i>	<i>Channa gachua</i>
S.h/6	<i>Saprolegnia hypogyana</i>	<i>Channa gachua</i>
S.p/7	<i>Saprolegnia parasitica</i>	<i>Heteropneustis fossilis</i>
S.p/8	<i>Saprolegnia parasitica</i>	<i>Heteropneustis fossilis</i>
S.p/9	<i>Saprolegnia parasitica</i>	<i>Heteropneustis fossilis</i>
S.p/10	<i>Saprolegnia parasitica</i>	<i>Mystus cavasius</i>
S.p/11	<i>Saprolegnia parasitica</i>	<i>Mystus cavasius</i>
S.p/12	<i>Saprolegnia parasitica</i>	<i>Channa punctatus</i>
A.m /13	<i>Achlya americana</i>	<i>Heteropneustis fossilis</i>
A.m14	<i>Achlya americana</i>	<i>Heteropneustis fossilis</i>
A.m/15	<i>Achlya americana</i>	<i>Mystus cavasius</i>
A.m/16	<i>Achlya americana</i>	<i>Mystus cavasius</i>
A.m/17	<i>Achlya americana</i>	<i>Labeo rohita</i>

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 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 viz. Hemp seeds, and Mustered seeds. These Petri dishes were incubated at 15 to 22°C tem for a week. Pure and bacteria free cultures were prepared by using the methods of Coker (1923), Johnson (1956) 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).

Artificial infection trials

In order to demonstrate the pathogenicity of the isolates obtained from the naturally infected fishes, experimental infection trails were conducted in the laboratory. Isolated species of fungi viz. *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 8.16 ± 0.13 cm and 12.5 ± 0.28 gm, respectively. The pathogenecity 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 tem for 24 h. Filtered sterile lake water was filled aseptically in to each trough. An aerator was used to aerate the water throughout the experiment. Six fungal inoculated blocks (1.0 cm²) of 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 temperature in triplicate sets. Water samples were collected from infected water bodies for analysis of various Physico-chemical parameters such as Temperature, Conductivity, PH, FCO₂, Dissolved oxygen, Total alkalinity, Total hardness and Chloride as per methods of APHA (1995).

RESULTS AND DISCUSSION

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* (Table 1). *S. diclina* was isolated twice from infected fishes. One isolate was collected from *Channa gachua* and one from *Channa straitus*. A total of three isolates of *S. ferax* were obtained, one was collected from *Clarias batrachus*, one from *C. straitus* and one from *C. gachua*. One isolate of *S. hypogyana* was collected from infected *C. gachua*. *S. parasitica* is the most frequently occurring parasite of fish. A total of six isolates of this species were obtained from infected fishes. Three isolated from *Heteropneuste fossilis*, two collected from *Mystus cavasius* and one from *Channa punctatus*. 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 to be 4.8 in the month of December; 2008. While, the minimum percentage of infection was recorded to be 0.8 in the month of February, 2009. In case of fish species the highest percentage of infection (1.6) was reported in *H. fossilis* while lowest infection (0.8) was reported in *L. rohita*. The experimental infection trails were conducted

Table 2. Experimental infection trails with various species of fungi isolated from diseased 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 hypoglyana</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 3. Showing Water quality parameters of affected water bodies during study period.

S/N	Parameters	Values in range
1	Water temperature (°C)	17-28
2	Conductivity (usc/cm)	260-290
3	pH	6.8-8.7
4	FCO ₂ (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

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 2. 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 2). It is observed that although all the isolates has potentiality to parasitize the fish but *S. parasitica* is more vigorous showing infection within 8 h (Table 2). A wide range of fluctuations were noticed in various water quality parameters of affected water bodies (Table 3). Fungal infection in fish was first reported during mid – eighteen century (Arderon, 1748). Later, other workers reported several pathogenic fungi from different species of fish and fish eggs. (Sati, 1982; Fraser et al., 1992; Hatai and

Hoshiai, 1992; Roberts et al., 1993; Chinnabut et al., 1995; Willoughby et al., 1995; Khulbe et al., 1995; Mastan, 2008; Rekha and Qureshi, 2012).

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 (1939) 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 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 coldwater fishes such as *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. (2002). 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.

In the present study mycological examination of infected fishes revealed the presence of sixteen isolates of five species viz. *S. diclina*, *S. ferax*, *S. hypoglyana*,

S. parasitica, *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 have reported that the species of fungi, *S. parasitica* is found to be the most destructive. This finding confirms with the reports of Hatai and Hoshiai (1992) who have reported that the infection caused by *S. parasitica* in salmon resulted mass mortality. Both the scaly and non – scaly fishes were found to be equally susceptible to the species of fungi tested. *S. hypoglyana* was isolated from and tried on *C. striatus*, also showed its wide range on fishes. The same is also reported by Chauhan and Qureshi (1994). Qureshi et al. (2000) have conducted pathogenicity studies with various species of *Saprolegnia* on different species of fishes of central India.

Conflict of interests

The author did not declare any conflict of interest.

REFERENCES

- APHA (1995). Standard methods for the examination of water and waste water, Washington DC (16th Ed.), pp. 1-1268.
- Arderon W (1748) The Substance of a letter from Mr. William Arderon, F.R.S., Phil. Trans. Res. Soc. 45 (487): 321-323.
- Bhargava KS, Swarup K, Singh CS (1971). Fungi parasitic on certain fresh water fishes of Gorakhpur. Indian Biol. 3:65-69.
- Bisht GS, Deepa B, Chandra J, Khulbe RD (1996). Potential threat to reservoir fishery in Kumaun Himalaya, India. Curr. Sci. 71(9):720-722.
- Chauhan R, Qureshi TA (1994). Host range studies of *Saprolegnia ferax* and *Saprolegnia hypoglyana*. J. Inland Fish. Soc. India 26(2):99-106.
- Chidambaram K (1942). Fungal disease of Gourami (*Ospromenus goramy*, Lacepede) in a pond at Madras. Curr. Sci. 11:289-290.
- Chinnabut S, Roberts RJ, Willoughby LG, Pearson MD (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 Carolina Press, Chapel Hill, North Carolina, 201p.
- 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 (2012). Diseases of fish and shellfish caused by marine fungi, (ed. by C. Raghu Kumar), Biology of Marine fungi, pp.15-49.
- Hatai K, Hoshiai G (1992). Saprolegniasis in cultured Coho Salmon. Fish Pathol. 11:233-234.
- Hatai K, Hoshiai, GI (1993). Characteristics of two *Saprolegnia* species from Coho Salmon with Saprolegniasis, J Aqu Ani health, 5, 115-118.
- Hatai K, Nakamura K, Rha SA, Yuasa K, Wada S (1994). Aphanomyces infection in the dwarf Gourami (*Colisa lalia*). Fish Pathol. 29:95-99.
- Johnson TW Jr (1956). The Genus *Achlya*, Morphology and Taxonomy. University of Michigan Press, Ann Arbor, 180pp
- Khulbe RD, Bisht, GS Chandra Joshi (1994). Epizootic infection due to *Achlya debaryana* in a catfish. Mycoses 37:61-63.
- Khulbe RD (1994). A world monograph of parasitic water molds. Almora, shree Almora Book Depot, p.144.
- Khulbe RD, Joshi C, Bisht GS (1995). Fungal diseases of fish in Nanak Sagar, Nainital, India. Mycopathology 130:71-74.
- 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.
- Mastan SA (2008) Incidences of Dermatomycosis in fishes of Larpur reservoir, Bhopal, (M.P). J Herbal Med. Toxicol. 2(1):37- 40.
- Pickering AD, Willoughby LG (1982). Saprolegnia infection of salmonid fish. In: 50th 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, 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 and Pure Biol 14 (1):45-49.
- Qureshi TA, Chauhan R, Mastan SA (2002). Experimental infection of saprolegnia species on different species of fishes. J. Nat Cons. 14 (2): 385-388.
- Qureshi, TA, Prasad Y, Mastan SA, Chauhan R (2000). Involvement of fungal and bacterial pathogens in EUS affected fishes. Biotech consortium India Limited, pp. 125-139.
- Rajender KS, Khulbe RD (1998). A survey of impact and evaluation fisheries in Kumaun Himalayas, India with special reference to microorganism. Cur Sci 75.No-12:1303-1308.
- Ramaiah N (2006) A review on fungal diseases of algae, marine fishes, shrimps and corals. Indian J Mar. Sci. 35 (4):380-387.
- Refai MK, Laila A, Mohamed, Amamy M, Kenawy, Shimma El-S.MM (2010).The assessment of mycotic settlement of freshwater fishes in Egypt. J. Am. Sci. 6 (11):21-23.
- Rekha C, Qureshi T (2012). Fungal infection of fishes: Parasitic fungi and its role in fish diseases. Lap Lambert Academic Publishing, p.184.
- Rekha C, Qureshi T (2013).Studies on conidial fungi isolated from some freshwater fishes. Int. J Advance Life Sci (4):227-281.
- 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 (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. Mycoses 57:95-96.
- 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 3.9-312.
- Scott W (1964). Fungi associated with fish disease. Dev. Ind. Microbiol. 5: 109-123.
- Scott WW (1961). A Monograph of the genus *Aphanomyces*. Var. Agar. Exp. St 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 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, 24p.
- Srivastava RC (1980). Studies in fish mycopathology: a review. Mycosen 23:325-332.
- Tiffney WN (1939). The identity of certain species of the *Saprolegnia parasitica* to fish. J. Elisha Mitchell Sci. Soc. 55:143-151.
- Vickie B, Scott P, Edward P (2013). Fish health, fungal infection and plesteria:The role the U.S Geological Survey. U.S. Geological Survey Fact sheet, 114-98
- Vikas S, Mastan SA, Qureshi TA(2005). Incidences of saprolegniasis in fishes of Hataikheda reservoir, Bhopal, Indian. J. Fish. 52 (3):367-37.
- Vinay V (2008).Fungal disease in fish, diagnosis and treatment. Vet. World 2:12-15.
- Vishniac HS, Nigrelli RF (1957). The ability of the saprolegniaceae to parasitize platy fish. Zoologica 42:131-134.
- 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 (1978). Saprolegniasis of salmonid fish in Windermere, a critical analysis. J. Fish Dis. 1:51-67.

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.

Zafar I, Reshma S (2013). Some pathogenic fungi parasitizing two exotic tropical ornamental fishes. Int. J. Agric. Sci. Biol. 15(3):595-598.