



## Original Research Article

# Studies on the effect of *Achlya* species in various fish species cultured in different freshwater systems of Pudukkottai District, Tamilnadu, India

R. Saraswathi<sup>1</sup>, P. Sumithra<sup>1</sup> and R. Sivakami<sup>2\*</sup>

<sup>1</sup>Department of Microbiology, Srimad Andavan Arts & Science College,  
Tiruchirappalli – 620 005, Tamil Nadu, India

<sup>2</sup>Department of Zoology, Arignar Anna Govt. Arts College, Musiri – 621 211, Tamil Nadu, India

\*Corresponding author

## ABSTRACT

### Keywords

*Achlya*  
species,  
Fish,  
Mycotic  
infections,  
Fresh water

Even though fish and fisheries are pivotal in meeting the food security and sustenance of people, in most countries diseases outbreaks have threatened profitable and viable operations throughout the world. Fungal infections are second only to bacterial diseases in economic importance. Among the fungi, *Achlya* species appear to be the most virulent among Saprolegniales. Hence the present study was aimed at assessing their presence and their effect in different aquatic systems in Pudukkottai District of Tamil Nadu. Of the total 314 fishes chosen, a total of 63 species were found with mycotic infections. The percentage of infection was found to vary from 15.3 to 38.09% examination of carps showed they had multiple infections. Among the nine species of fish examined, *Channa striatus* was the most prone to infection of *Achlya* species.

## Introduction

Aquaculture contributes to national productivity, socio-economic development as well as to renewable aquatic resources. Fish and fisheries are pivotal in food security and for meeting the sustenance of people by providing many employment opportunities besides providing a vital contribution to a countries foreign exchange. However, in most countries disease outbreaks have threatened profitable and viable aquaculture operations throughout the world. Today, fungal infections are second only to bacterial diseases in economic importance (Ramaiah, 2006). Further, every freshwater fish is exposed to one species of

fungus during its lifetime (Neish, 1997; Noga, 1996) starting from the embryonic stage through adulthood (Bruno and Wood, 1994).

Among the fungi, the Oomycetes (zoosporic fungi) are found harmful to fish industry as they parasitize fish (West, 2006). Infections caused by *Achlya* have been reported by some workers (Chidambaram, 1942; Gopalakrishnan, 1964). In addition, *Achlya* have also been isolated from EVS affected fishes (McGarey *et al.*, 1990) while several authors have reported its pathogenicity to fish (Khulbe and Sati, 1981; Khulbe, 2000;

Czeczuga *et al.*, 2004; Chauhan, 2012; Chauhan *et al.*, 2013). Further, Prasad and Rajanika (2010) while studying the fungal pathogenicity in fish reported that *Achlya* sp. are more virulent among Saprolegniales. Hence the present study was aimed at assessing the presence of these species along with their histopathological conditions in different aquatic systems situated in Pudukkottai district of Tamil Nadu, India.

## Materials and Methods

A total number of 314 fishes were randomly collected from different water bodies of Kottaiappattinam, Pudukkottai District, Tamil Nadu, India and brought to the laboratory in sterilized polythene bags for further examination. The fishes were kept in aquaria with continuous aeration. The fishes were observed to note external symptoms. To avoid bacterial contamination all the glass-ware, instruments and media were sterilized. Streptomycin sulphate 100 mg/ml were also used in the media. Inoculation was done in Laminar flow in sterilized conditions. The agar plates were incubated at 18 ± 2°C for the growth of culture. Growth of colony was observed in 3-4 days. For full growth of colony, plates were kept for 6-8 days.

## Preparation of Pure Cultures

Pure cultures were prepared by picking up small tuff of mycelium and grown on agar media. For the development of zoospores and reproductive structures, cultures were prepared on baits. Petri plates were filled with 20-30 ml sterilized tap water, small piece of media with fungi were kept in these plates and baited with different baits (Soyabean seeds and jowar seeds) at temperature 18 ± 2°C. weeds were used as baits.

## Identification of the Isolates

All pure cultures were examined for colonial growth, morphological features and microscopical characteristics. For identification, slides were prepared from each colony by taking small tuff of mycelium and stained with Lactophenol cotton blue. The slides were observed under microscope. Identification of fungi was carried out on the basis of keys (Coker, 1923; Johnson, 1956; Khulbe, 1993, 2001). Fishes were identified by the keys of Jhingran (1982) and Qureshi and Qureshi (1983).

## Histological Examination

For histological examination, infected tissue of skin and muscles were fixed in aqueous Bouin's fluid for 48 - 72 hours. The tissue was then processed routinely and prepared into paraffin blocks. The blocks of the tissues were cut at 4-6 µm thickness and stained with Haematoxylin and Eosin (H-E). Standard histological procedures (Roberts, 2001) were followed for histopathological investigations.

## Results and Discussion

In the present study, a total of 314 fishes belonging to seven genera were observed for mycotic infection. Of these, a total of 63 fishes were found with mycotic infections (Table-1). The species-wise infection of fishes are also presented in Table-1. In terms of percentage, the infection was found to vary from 15.3% (*Catla catla* and *Clarias batrachus*) to 38.09% (*Cirrhinus mrigala*).

There were five species of *Achlya* which were isolated from the fishes (*A. prolifera*, *A. americana*, *A. klebsiana*, *A. flagellata* and *A. hypoglyca*). Among the five species of *Achlya*, *A. prolifera* appeared to be the most

common species as they were found in six of the nine species of fish that were examined. Examination of the carps revealed that they had multiple infections. While *L. rohita* was found to be infected with *A. americana* and *A. klebsiana*, *Cirrhinus mrigala* was infected with *A. proliferata* and *A. klebsiana* and *Cyprinus carpio* with *A. proliferata* and *A. americana*. *Mystus carasius* on the other hand was found to be infected with *A.*

*prolifera* and *A. hypogyna*. Among the nine species of fish examined, *Channa striatus* appeared to be the fish that was more prone to the infection of *Achlya* sp. as all the fishes examined showed the presence of *A. hypogyna*, *A. proliferata* and *A. americana*. Among the various *Achlya* sp., *A. flagillata* appeared to be rare as it was found to infect only *C. batrachus*.

**Table.1** Mycotic Infected Fishes Isolated from Lake

| S. No. | Species of Fishes        | Percentage of isolated |
|--------|--------------------------|------------------------|
| 1.     | <i>Catla catla</i>       | 8                      |
| 2.     | <i>Labeo rohita</i>      | 10                     |
| 3.     | <i>Cirrhinus mrigala</i> | 12                     |
| 4.     | <i>Cyprinus carpio</i>   | 14                     |
| 5.     | <i>Channa punctatus</i>  | 10                     |
| 6.     | <i>Channa striatus</i>   | 6                      |
| 7.     | <i>Mystus cavasius</i>   | 20                     |
| 8.     | <i>Clarias batrachus</i> | 9                      |
| 9.     | <i>Mystus vittatus</i>   | 11                     |

**Table.2** List of *Achlya* Species Isolated from Infected Fishes

| S. No. | Fungal Species   | No. of fishes examined | Diseased fishes | % of infected |
|--------|--|------------------------|-----------------|---------------|
| 1.     | <i>A. proliferata</i>  | 26                     | 4               | 15.3          |
| 2.     | <i>A. americana</i> + <i>A. klebsiana</i>                        | 23                     | 5               | 21.7          |
| 3.     | <i>A. proliferata</i> + <i>A. klebsiana</i>                      | 21                     | 8               | 38.09         |
| 4.     | <i>A. proliferata</i> + <i>A. americana</i>                      | 40                     | 13              | 32.5          |
| 5.     | <i>A. americana</i> + <i>A. hypogyna</i>                         | 32                     | 7               | 21.8          |
| 6.     | <i>A. hypogyna</i> + <i>A. proliferata</i> + <i>A. americana</i> | 26                     | 8               | 30.7          |
| 7.     | <i>A. proliferata</i> + <i>A. hypogyna</i>                       | 24                     | 9               | 37.5          |
| 8.     | <i>A. flagellate</i>   | 26                     | 4               | 15.3          |
| 9.     | <i>A. proliferata</i>  | 18                     | 5               | 27.7          |

The most commonly noticeable symptom of infection that was observed in all the fishes was decoloration of the body followed by descaling and exposure of epidermis. In addition, there were patches, lesions and ulcerations which mostly occurred in the head and tail regions.

Histopathological studies of the skin recorded changes in the epithelial condition leading to ulceration in the affected part. Epidermal and dermal cells showed varying degrees of vacuolar degeneration and necrosis. The underlying muscle fibres showed inflammation and necrosis. In some, fungal hyphae were also noticed.

A perusal of literature reveals that *Achlya* sp. have been isolated from fishes by some workers (Chidambaram, 1942; Limsuwan and Chinnabut, 1983; McGarey *et al.*, 1990; Chauhan, 2012; Chauhan *et al.*, 2013). Further, the various species of *Achlya* that were isolated during the present study has also been reported by other workers. In the present study, *A. prolifera* was found to be the most common species. Similar observations were also noticed by Sati (1985), Khulbe (2000) and Chauhan *et al.* (2013). Thus among the various species, *A. prolifera* has the maximum host range.

In the present study, among the nine fish species, *C. striatus* appeared to be more prone to multiple infection. However, reports reveal that *M. caracius* and *M. seenghala* are the most affected species (Shrivastava and Shrivastava, 1976, 1977; Qureshi *et al.*, 1995; Chauhan and Benkhede, 2013). This might be attributed to site specific conditions that prevailed in the system.

Histological studies showing the ulceration, vascular degeneration and necrosis noticed in the present study have also been observed by others in *Achlya* species infections

(Roberts *et al.*, 1993; Vishwanath *et al.*, 1998; Chauhan and Qureshi, 2012; Chauhan *et al.*, 2013). Thus it can be concluded that the lake system understudy has considerable amount of fungal species which can infect fish and affect aquaculture prospects.

## References

- Barclay, C.D., Moore, D.M., Lander, S.R. and Legge, R.L. (1990). Head denaturation inetics of lignin peroxidases from *Phanerochaete chrysosporium*. *Enzyme Microb. Technol.*, 12: 778-782.
- Bergey (1984). *Bergey's manual of systematic Bacteriology*. Williams and Baltimore: USA. p. 516.
- Boer, C. G., Obici, L., Souza, C. G. and Peralta, R. M. (2004). Decolourization of synthetic dyes by solid state cultures of *Lentinula (Lentinus) edodes* producing manganese peroxidase as the main lignolytic enzyme. *Bioresour. Technol.*, 94: 107-112.
- Brodkorb, T.S. and Legge, R.L. (1992). Enhanced biodegradation of phenanthrene in oil tar-contaminated soils supplemented with *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* 58: 3117-3121.
- Brooks, J.L. (1988). The role of fungi in the sphagnum peat wastewater treatment system. Ph.D. Thesis, University of Maine, Orono, Maine.
- Campos, R., Kandelbauer, A., Robra, K.L.H., Arthur, C.P. and Gubitz, G.M. (2001). Indigo degradation with purified lactases from *Trametes hirsute* and *Sclerotim rolfsii*. *J. Biotechnol.*, 8: 131-139.
- Cappuccino, J.G. and Sherman, N. (1992). Microbiology; A Laboratory manual (3<sup>rd</sup> ed.) J. Rockland Community College, Suffern: New York. p.45.
- Chung, K.T. and Stevens, S. Jr. (1993). Decolourization of azo dyes by environmental microorganisms and

- helminthes. *Environ. Toxicol. Chem.*, 12: 121-131.
- Ellis, M. B. (1976). *Dematiaceous Hypomycetes*. Commonwealth Mycological Institute Pub., Kew Surrey, England. pp. 17.
- Enhca, G. (1994). The role of microorganism in environmental decontamination. Contaminants in the environment, a multidisciplinary assessment of risk to man and other organisms. *J. Exp. Mar. Biol. Ecol.*, 25: 235-246.
- Gillman, J.C. (1957). *A manual of soil fungi*. Oxford and I.B.H. Publishing company (Indian reprint), Calcutta, Bombay, New Delhi., p. 250.
- Heinfling, A., Bergbauer, M. and Szewzyk, U. (1997). Biodegradation of azo and phthalocyanin dyes by *Trametes versicolor* and *Bjerkandera adusta*. *Appl. Microbiol. Biotechnol.*, 48: 261-266.
- Josey, D. P., Beynon, J. L., Johnston, A.W.B. and Beringer, J.E. (2008). Strain identification in rhizobium using intrinsic antibiotic resistance. *J. Gen. Microbiol.* 10: 1365-2672.
- Kamitsuji, H.Y., Watanabe, T. and Kuwahara, M. (2005). Mn is dispensable for the production of active MnP<sub>2</sub> by *Pleurotus ostreatus*. *Biochem. Biophys. Res. Comm.*, 327: 871-876.
- Maas, R. and Chaudhari, S. (2005). Adsorption and biological decolorization of azo dye reactive red in semicontinuous anaerobic reactors. *Process Biochem.*, 40: 699-705.
- Madhu, G. and Pillai, K.A. (1994). Biological treatment of effluent from a nitrogenous fertiliser complex. *Indian science Annual*, 22: 64-70.
- McMullan, G., Robinson, T., Marchant, R. and Nigam, P. (2001). Remediation of dyes in textile effluent: a critical review on current treatment technologies with proposed alternatives, *Biosource Technol.*, 77: 247-255.
- Namdhari, B. S., Rohilla, S. K., Salar, R. K., Gahlawat, S. K., Bansal, P. and Saran, A. K. (2012). Decolorization of Reactive Blue MR using *Aspergillus* species Isolated from Textile Waste Water. *Phykos*, 1: 24-29.
- Pala, A. and Toket, E. (2002). Color removal from cotton textile industry wastewater by an activated sludge system with various additives. *Water Res.*, 36: 2920-2925.
- Ramesh, J. V. S. and Singh, S. P. (1993). Yearly variation in certain physicochemical parameters of pond at eastern Doon Valley Uttar Pradesh. *J. Zoo.*, 12: 75-77.
- Ritmann, B.E., Jacson D.E. and Strock, S.L. (1988). American public health potential for treatment of hazardous organic chemical with biological process. *Hydrobiol.*, 15:64.
- Sekaran, G. and Mariappan, M. (1994). Treatment of salt laden wastewater from tanning industry. *Indian J. Environ. Prot.*, 14: 801-806.
- Shaul, G.M., Holdworth, T.J., Dempsey, C.R. and Dostal, K.A. (1991). Fate of water soluble azo dyes in the activated sludge process. *Chemosphere*. 22:107-119.
- Shimna, P.P. (2012). Microbial diversity and abundance of ponds of subtropical India. Ph.D. Thesis, Bharathidasan University, Tiruchirappali, India. pp.190.
- Somasegaran, P. and Hoben, H. (1985). In: *Methods in Legume Rhizobium Technology*, University of Hawaii, NIFTAL”, Project and Micren, Dept. of Agronomy and Soil., pp. 1-300.
- Subramanian, C. V. (1971). *Hyphomycetes - An account of Indian species except Cercosporae*. ICAR, New Delhi.
- Swamy, J., and Ramsay, J.A. (1999). The evaluation of white-rot fungi in the decolourization of textile dyes. *Enzyme Microb. Technol.*, 24: 130-137.
- Wood, W.A. and Kellogg, S. T. (1988). Biomass, cellulose and hemicelluloses methods. *Enzymol.*, 160: 623-634.