

Isolation, identification and Pathogenicity of some species of Genus *Achlya* on some fresh water fishes of Bhopal (M.P.)

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Abstract

Water moulds (oomycetes) of the order Saprolegniales, such as *Achlya*, *Saprolegnia* and *Aphanomyces* species, are accountable for overwhelming infections on fish in aquaculture, fish farms and hobby fish tanks. Present study was conducted to isolate, identify and to determine the pathogenicity of some species of Genus *Achlya* on some fresh water fishes of Bhopal. The study has been conducted for six months i.e., December 2013 to May 2014. During the course of study six species of *Achlya* viz., *A. americana*, *A. apiculata*, *A. flagellata*, *A. hypogyana*, *A. klebsiana* and *A. prolifera* were isolated from 15 locally available species of fresh water fishes. Among fungal isolates from these fishes, 40% were found prone to *Achlya prolifera* and minimum (5%) were found to be affected by *Achlya apiculata*. All the six fungi were found to be pathogenic to fish and caused mortality except *Achlya apiculata* and *Achlya klebsiana*, these two only presented signs of infection but no mortality. Total 126 infected fishes were examined for the isolation of different species of *Achlya*. Among them 42 fishes were found infected with *Achlya* species. Maximum percentage of infection was observed in *Mystus* spp. *M. cavasius* (21%) and *M. seenghala* (12%).

Fungal infections of fresh water fishes by Oomycetes (water molds) are widespread and represent the most important fungal group affecting wild and cultured fish. Oomycetes are traditional saprophytic opportunists, multiplying on fish that are physically injured, stressed or infected³¹. Oomycetes are in general considered as agents of secondary infection arising from circumstances such as bacterial infection,

injuries associated with handling and grading, infestation by parasites and public interface. Nevertheless there are more than a few reports of Oomycetes as primary infection agents of fish Willoughby 1978; Pickering and Christie¹⁷. Oomycete (water molds) are the most significant fungi affecting cultivated fish and are measured by some next only to bacterial disease in terms of economic importance to aquaculture¹³. Infections caused

by *Achlya* have been reported by Vishniac and Nigrelli³⁰; Scott and O'Bier²²; Limsuwan and Chinabut,¹² also reported reported *Achlya* and *Saprolegnia* from EUS affected fishes. Lim and Testorake,¹³ and McGarey *et al.*,¹⁴ isolated several oomycetes from the ulcers of fishes including *Achlya* spp. Some other workers reported *Achlya* pathogenic to fishes are Qureshi *et al.*,²⁰ Srivastava,²⁹ Khulbe and Sati,¹⁰ Khulbe,⁸ Czczuga *et al.*,⁵ Sosa *et al.*,²³ Chauhan²; Chauhan and Benkhede³ and Hussein *et al.*, 2013. Prasad and Rajanika,¹⁸ reported *Achlya* spp. more virulent among *Saprolegniales*.

Fishes showing external symptoms of infection were randomly collected from different water bodies of Bhopal and brought to the laboratory in sterilized polythene bags for further examination. The fishes were kept in aquaria with continuous aeration, which were observed to note external symptoms. All the glass wares, instruments and media were sterilized along with all aseptic conditions. Streptomycin sulphate 100mg/ltr was used in media to avoid bacterial contamination

Isolation of fungi from infected fishes was carried out by taking small pieces of skin with little 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 micro-organisms adhered on the surface. These tissues were then inoculated over the plates containing different agar media. Small pieces of mycelia were also taken out from infected parts of fish body which were washed thoroughly with distilled water and placed in petridishes containing 20-30 ml distilled water and baited

on different baits viz. soyabean seeds, mustard seeds, til seeds, and fish skin. These petridishes were incubated in BOD incubator at 15.0°C to 22.0°C temperature. Forceps, petridishes and water used were sterilized thoroughly before use.

All pure cultures were examined for colonial growth, morphological features and microscopical characteristics. For identification, slides were prepared from each colony by taking small tuft of mycelium and stained with lactophenol cotton blue which was observed under microscope. Identification of fungi was carried out on the basis of keys given by Coker⁴, Johnson,⁶ Khulbe^{7,9}. Fishes were identified by using the keys of Qureshi & Qureshi¹⁹.

To determine the pathogenicity of six species of *Achlya*, identified as *A. americana*, *A. apiculata*, *A. flagellata*, *A. hypogyana*, *A. klebsiana* and *A. prolifera*, same fish species were used from which particular strain of fungi were isolated. After identification, pure cultures were prepared and maintained on Corn Meal Agar at 15-20°C and zoospore suspension was prepared for inoculation. The spores were taken gently from 8-10 days old colony by sterile loop and transferred aseptically in a test tube containing sterile distilled water. The fungal zoospore suspension was counted by haemocytometer and suspension was diluted to reach 125 zoospores/ml for all the mentioned species of *Achlya* used for pathogenicity test.

Experimental set up was carried out in triplets and healthy fishes with average weight of 35-40 gm were collected and kept for seven days in aquaria of 10L capacity with

continuous aeration and fed with artificial feed, for acclimatization. The experimental troughs were set up in triplets, aerated continuously and temperature was maintained between 15-20 °C. Injected fishes were observed for seven days. Now the experimental fishes were injected intramuscularly with 0.2 ml zoospore suspension. Infection and mortality was recorded. The dead fishes were sampled for observation and re-isolation.

Isolation and identification:

During present study total 126 fishes were examined for fungal infection from which 42 fishes were found infected with zoosporic fungi *Achlya*, from the infected species of fishes 6 species of *Achlya* were isolated namely *Achlya americana*, *A. apiculata*, *A. flagellata*, *A. hypogyana*, *A. klebsiana* and *A. prolifera*. It was found that most of the isolates were found in combination, among six species of *Achlya* isolated maximum percentage was of *A. prolifera* (40%) and minimum was of *A. Apiculata* (5%) (Fig.1), *A. americana* was isolated from eight different species of fishes, *A. apiculata* from two different species of fishes, *A. flagellate* from one species of fish, *A. hypogyana* from four species of fishes, *A. klebsiana* from one species of fish, and *A. prolifera* from ten different species of fishes (Table-1). Among the infected fish species maximum infection was observed in *Mystus* spp. namely *M. cavasius* (21%) and *M. seenghala* (12%).

Pathogenicity studies of six isolated species of Achlya :

For identification fungal cultures were grown on soybean seed (Fig.4), *A. prolifera* was fully grown on corn meal agar with in the

period of six days (Fig.2).

Pathogenicity of Achlya americana: Achlya americana (Fig. 8: Showing Achlya americana Oogonia) was tested on seven species of fishes from which it was isolated. Results of the experiment showed that infection was observed externally in all the fishes tested and mortality of almost all the fishes within 96 hours except *Nandus nandus* in which one specimen survived and in *Clarias batriachus* only one fish died among the three tested fishes (Table-2).

Pathogenicity of Achlya apiculata: Achlya apiculata was tested on five species of fresh water fishes. Out of five species of fishes only *Trichogaster fasciatus* developed external lesions in 48 hours but no mortality was recorded (Table-2).

Pathogenicity of Achlya flagellata: Achlya flagellata (Fig. 7: Showing gammae, oogonia and zoosporangia of Achlya flagellate) was tested on *Puntius sarana*, in all fishes external infection was observed within 24 hours and death of all the fishes occurred within 96 hours of time (Table-2).

Pathogenicity of Achlya hypogyana: Achlya hypogyana (Fig. 6: Showing Achlya hypogyana zoosporangia with encysted zoospores on its mouth) was tested on three different species of fishes. In *Labeo rohita* (Fig.5) and *Nandus nandus* infection was observed within 48 hours and death of four fishes two from each group was found and in *Clarias batrachus* infection was observed in 72 hours along with death of one fish in time period of 96 hours (Table-2).

Pathogenecity of Achlya klebsiana: *Achlya klebsiana* was inoculated in *Channa punctatus*, out of three only one fish showed external lesions after 96 hours but no mortality was recorded (Table-2).

Pathogenecity of Achlya prolifera: *Achlya prolifera* was tested on 10 different species of fishes which was found naturally infected with the given species showed. Among 10 species of fishes in seven species *Catla catla*, *Channa striatus*, *Cirrhinus mrigala*, *Mystus cavasius*, *Mystus seenghala* (Fig.3), *Puntius ticto* and *Trichogaster fasciatus* showed infection and external lesions were observed within the period of 24 hours, in *Clarias batrachus* and *Mastacembalus armatus* infection was observed in 48 hours and only in *Notopterus notopterus* external infection was observed in 72 hours. All the fishes inoculated with this species died within the period of 96 hours (Table-2).

In the present study mycological investigation of fishes revealed the presence of six species of *Achlya*, viz. *A. americana*, *A. apiculata*, *A. flagellata*, *A. hypogyana*, *A. klebsiana* and *A. prolifera*, isolated from different species of fresh water fishes. The observations are supported by findings of Scott and O'Bier²². It was found that *A. prolifera* was most prevalent species which have been supported by the findings of Khulbe⁸. *A. americana* was isolated from *Cirhanus mrigala*, which is in agreement with the observations of Shrivastava²⁹. *A. prolifera* has been reported from *C. striatus*, *C. mrigala* and *C. catla* by Khulbe⁹. Present findings are supported by above reports by isolating *A. prolifera* from similar species of

fishes. During the present study maximum host range was found in *A. prolifera*, capable of parasitizing a wide range of fishes. These observations are in agreement with the reports of Shrivastava and Shrivastava²⁷. It was found that *M. cavasius* and *M. seenghala* were most affected species. These findings are supported by reports of Qureshi *et al.*,²⁰.

During the present study *Achlya americana* was found pathogenic to seven species of fresh water fishes viz. *Channa punctatus*, *Cirrhinus mrigala*, *Clarias batrachus*, *Mystus cavasius*, *Mystus seenghala*, *Nandus nandus*, and *Trchogaster fasciatus*. Present findings are supported by the findings of Khulbe and Seti,¹¹ who reported *Achlya americana* as a pathogen of *Cyprinus carpio var. communis*, *Puntius conchoniis*, *Puntius ticto*, *Schizothorax plagiostomus*, *S.richardsoni*, and *Tor tor*.

Achlya apiculata was found pathogenic to five species of fresh water fishes viz. *Channa punctatus*, *Mystus seenghala*, *Mystus cavasius*, *Hypophelimeicthes molitris* and *Trichogaster fasciatus*.

Achlya flagellata was found pathogenic to fresh water fish *Puntius sarana*, the present findings correlate with the findings of Srivastava and Srivastava²⁵, who revealed that it could parasitize *Colisa fasciatus*, *C.lalia*, *Puntius sophora*, *Anabas testudines*, *Channa punctatus* and *Chanda ranga*.

Achlya hypogyana was found pathogenic to three fresh water fishes viz; *Clarias batrachus*, *Labeo rohita* and *Nandus*

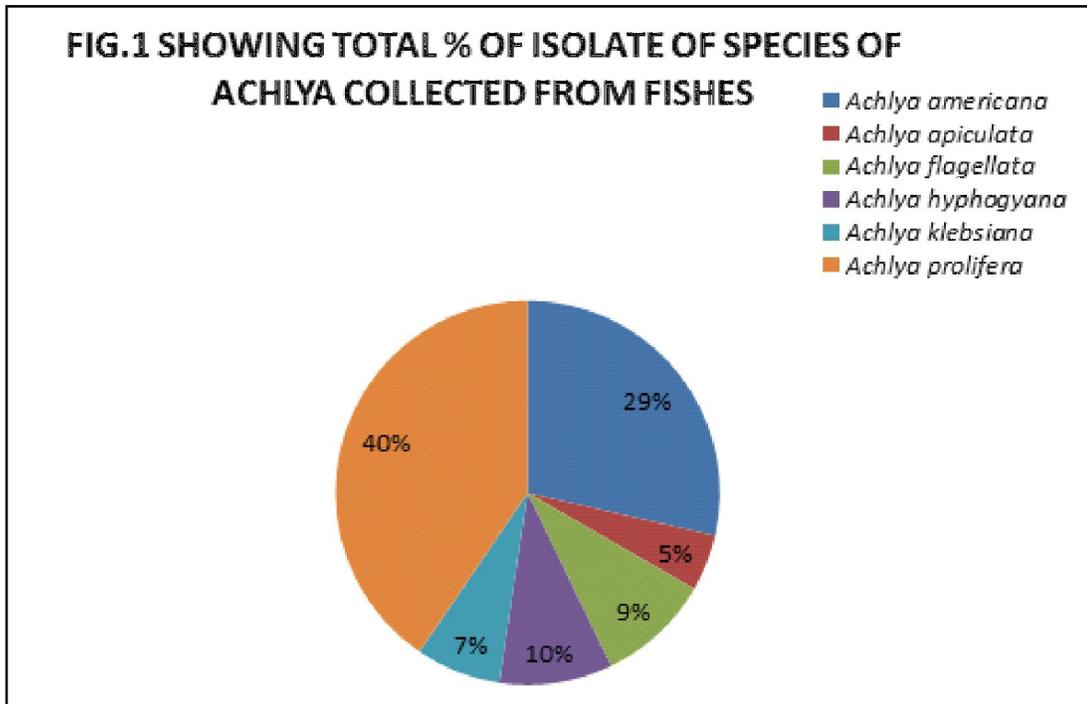


Table-1: Showing various species of *Achlya* isolated from infected fishes.

S.No	Isolated <i>Achlya</i> species	Various infected fishes
1	<i>Achlya Americana</i> (Humphrey)	<i>Channa punctatus</i> , <i>Mystus cavasius</i> , <i>Mystus seenghala</i> , <i>Clarias batrachus</i> , <i>Garva gotly</i> , <i>Cirrhinus mrigala</i> , <i>Tor tor</i> , <i>Notopterus notopterus</i>
2	<i>Achlya apiculata</i> (de Bary)	<i>Puntius ticto</i> , <i>Myustus seenghala</i>
3	<i>Achlya flagellate</i> (Coker)	<i>P. sarana</i> , <i>Myustus cavasius</i>
4	<i>Achlya hypoglyana</i> (Coker & Pemberton)	<i>Labeo rohita</i> , <i>Notopterus notopterus</i> , <i>Clarias gariepinus</i> , <i>Nadus nadus</i>
5	<i>Achlya klebsiana</i> (Pieters)	<i>Channa punctatus</i> , <i>Catla catla</i>
6	<i>Achlya prolifera</i> (Nees)	<i>Catla catla</i> , <i>Channa striatus</i> , <i>Myustus cavasius</i> , <i>Myustus seenghala</i> , <i>Notopterus notopterus</i> , <i>Clarias batrachus</i> , <i>Cirrhinus mrigala</i> , <i>Myustus armatus</i> , <i>Puntius ticto</i> , <i>Carrasius auratus</i>

Table-2: Showing pathogenicity of six species of *Achlya* on some fresh water fishes of Bhopal

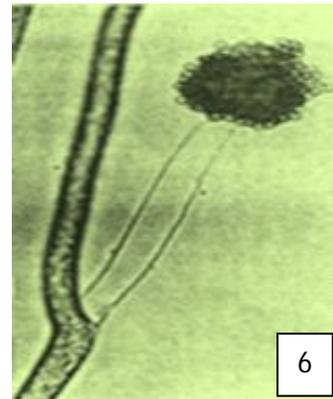
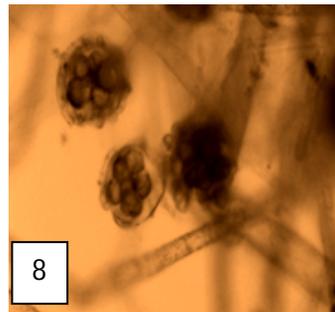
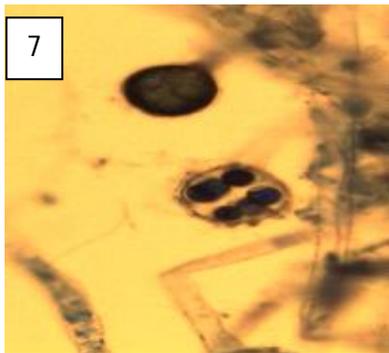
S.No	Fungi Inoculated	Challenged Fish	No. of fish used	No. of fish infected	Infection in hours	Mortality % with 96 hours	Survivality after 96 hours	Resolution
1	<i>Achlya americana</i>	<i>Channa punctatus</i>	3	3	24	100	0	+
		<i>Channa mrigala</i>	3	3	48	100	0	+
		<i>Clarias batrachus</i>	3	3	96	33	67	+
		<i>Mystus cavasius</i>	3	3	24	100	0	+
		<i>Mystus seengla</i>	3	3	48	100	0	+
		<i>Nandus nandus</i>	3	3	48	67	33	+
		<i>Tricogaster fasciatus</i>	3	3	24	100	0	+
2	<i>Achlya apiculata</i>	<i>Channa punctatus</i>	3	Nil	Nil	0	100	-
		<i>Mystus seengla</i>	3	Nil	Nil	0	100	-
		<i>Mystus cavasius</i>	3	Nil	Nil	0	100	-
		<i>Hypothalmithix molitrix</i>	3	Nil	Nil	0	100	-
		<i>Tricogaster fasciatus</i>	3	1	48	0	100	+
3	<i>Achlya hyphogyana</i>	<i>Clarias batrachus</i>	3	3	72	33	67	+
		<i>Labeo rohita</i>	3	3	48	67	33	+
		<i>Nandus nandus</i>	3	2	48	67	33	+
4	<i>Achlya flagellata</i>	<i>Puntius sarana</i>	3	3	24	100	0	+
5	<i>Achlya klebsiana</i>	<i>Channa punctatus</i>	3	1	96	0	100	+
6	<i>Achlya prolifera</i>	<i>Catla catla</i>	3	3	24	100	0	+
		<i>Channa striatus</i>	3	3	24	100	0	+
		<i>Cirrhinus mrigala</i>	3	3	24	100	0	+
		<i>Clarias batrachus</i>	3	3	48	100	0	+
		<i>Mastacembalus armatus</i>	3	3	48	100	0	+
		<i>Mystus cavasius</i>	3	3	24	100	0	+
		<i>Mystus seenghala</i>	3	3	24	100	0	+
		<i>Notopterus notopterus</i>	3	3	72	100	0	+
		<i>Puntius ticto</i>	3	3	24	100	0	+
<i>Trichogaster fasciatus</i>	3	3	24	100	0	+		

nandus, the present findings are supported by Srivastava, 1980 d; who isolated it as a parasite of *Anabus testudinus*.

Achlya klebsiana was reported pathogen to fresh water fish *Channa punctatus*, the present observations are supported by Sati,²¹ who reported it as

pathogen of *Chela labuca*, *Closia fascitus*, *Cyprinus carpio var. communis* and *Puntius sophora*.

Achlya prolifera was found pathogenic to 10 freshwater fishes as *Catla catla*, *Channa striatus*, *Cirrhinus mrigala*, *Mystus cavasius*, *Mystus seenghala*, *Puntius ticto*



- Fig. 2: Showing *Achlya* spp. culture on Corn Meal Agar
Fig. 3: Showing *Achlya proliferata* infected *Mystus seenghala* with red patches on mouth and hyphae on body.
Fig. 4: Showing *Achlya* spp. colony on Soyabean seed (Glycine seeds).
Fig. 5: Showing *Achlya hypogyana* infected *Labeo rohita* with white cottony patches on head and caudal region.
Fig. 6: Showing *Achlya hypogyana* zoosporangia with encysted zoospores on its mouth.
Fig. 7: Showing gammae, oogonia and zoosporangia of *Achlya flagellatea*.
Fig. 8: Showing *Achlya Americana* Oogonia.

and *Trichogaster fasciatus*, the present findings are supported by findings of Srivastava and Srivastava²⁷, who also reported it as a pathogen of *Anabus testudineus*, *Colisa fasciatus*, *Colisa lalia*, *Puntius sophore* and *Notopterus notopterus*. Srivastava and Srivastava²⁶, reported it as a pathogen of *Cyprinus carpio var. communis*. Srivastava and Srivastava²⁴, reported it as a pathogen of eggs of fish *Channa punctatus*.

The present piece of work revealed that water bodies of Bhopal showed considerable intensity of fungal infection in fishes. Among the six species of *Achlya* studied, *A. prolifera* was found to be most pathogenic and showed maximum host range in *mystus* spp.

Conflict of Interest The authors declare that they have no conflict of interest.

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