

FUNGAL INFECTION IN SOME ECONOMICALLY IMPORTANT FRESH WATER FISHES IN GANDAK RIVER NEAR MUZAFFARPUR REGION OF BIHAR.

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ABSTRACT

Fungal diseases of fresh water fishes are known to be problematic disease. The aim of this study was to investigate fungal infections in five economically important fresh water fishes viz: *Catla catla*, *Notopterus chitala*, *Channa striatus*, *Labeo rohita*, *Channa marulius* were collected from Gandak river near Muzaffarpur region of Bihar during June 2013 to November 2013. The fishes were collected with the help of fisherman in sterile polythene bags in aerated water and transported immediately to plant pathology and microbiology laboratory, department of Botany, Patna Science College, Patna for mycological study. The specimens from infected organs of fish were inoculated on different culture media supplemented by suitable antibiotics. Among them nearly 25 fungi were identified. Identification and characterization of the fungi were made with the help of authentic manuals of fungi. The most common among them viz; *Pythium sp.*, *Thraustotheca sp.*, *Aphanomyces sp.*, *Dictyuchus sp.*, *Achlya sp.*, *Aspergillus sp.*, *Fusarium sp.*, and *Penicillium sp.* were isolated, identified and characterized. The seasonal variation and percentage of infection of fishes were analyzed.

Key words: Culture media, Diseases, Fish farming, Aquaculture, Fresh water fishes, Fungal infection.

INTRODUCTION

Fresh water fishes are an important protein source for people of many countries (Hussain *et al.*, 2011; Rubbani *et al.*, 2011). However, globally fish from freshwater and marine sources are in severe decline, driven in large part by economic and human population growth (Limburg *et al.*, 2011). Fish farming in various parts of the world has increased many folds in the last decade. As a result, fish culture has now become commercially an important industry worldwide. Diseases in fresh water fishes are a great threat to achieve optimum production and become a limiting factor to economic success of aquaculture. Fungi which are responsible for a number of diseases are present in fresh water. Fungal infections are mainly caused due to immune suppression. Fungi can attack fishes of all the ages and it can also prevent successful hatching when it invades fish eggs. Among numerous aquatic fungi, species of

Oomycetes have special importance because of their effect on fish health. (P. V. West, 2006). S. C. Sati (1991), R. Chauhan and T.A. Qureshi (1994) and T.A.Qureshi, *et al* (2002) also reported the different kinds of aquatic fungi belong to *Saprolegnia*, *Pythium*, *Thraustotheca*, *Aphanomyces*, *Dictyuchus*, *Protoachlya* and *Achlya* were the most virulent parasites in the comparison to others pathogenecity of *saprolegnia* on fresh water fishes. Several species like *Penecillium sp.*, *Alternaria sp.*, *Fusarium sp.*, and *Aspergillus sp.* were also reported by Firoz, Mehdi, Hamidreza (2011). Research on fungal diseases has been conducted in specific regions and due to increase in organic pollutants, occurrence and parasitic activity of aquatic fungi is also expected more. Therefore present study has been aimed to isolate, identify the fungi which cause disease in

fish and the seasonal variation and percentage of infection of fishes were analyzed.

MATERIALS AND METHODS

Five economically important fresh water fishes viz: *Catla catla*, *Notopterus chitala*, *Channa striatus*, *Labeo rohita*, *Channa marulius* were collected in Gandak river near Muzaffarpur region of Bihar during June 2013 to November 2013. The fishes were collected with the help of fisherman in sterile polythene bags in aerated water and transported immediately to plant pathology and microbiology laboratory, department of Botany, Patna Science college, Patna for mycological study. The fishes were kept separate in glass aquarium with continuous air supply at ambient temperature. Isolation of fungi from infected fishes was carried out by taking small pieces from muscles about 2 mm in diameter from different portions of body and washed thoroughly with distilled water. These tissues were then inoculated over plates containing on different culture media viz; Potato Dextrose Agar medium (Peeled potato- 250gm, Dextrose- 20gm, Agar- 15 gm, Distilled water- 1000ml), Richard's medium (Potassium nitrate- 10gm, Potassium dihydrogen phosphate-

5gm, Magnesium sulphate- 2.50gm, Ferric chloride- 0.02gm, Sucrose- 50gm, Distilled water- 1000ml), Czapek- Dox Agar medium (Agar- 15.0 gm Sodium nitrate- 2.0 gm, Potassium dihydrogen phosphate 1.0gm, Magnesium sulphate- 0.5gm, Ferrus sulphate- 0.01gm, Sucrose 30.0gm, distilled water 1000ml). Streptomycin (50mg/l) was added in the medium to avoid bacterial contamination. Among three media the Potato Dextrose Agar medium was found to be the best medium for invariably all the isolated fungi. An average three to seven days of incubation at a temperature ranging between 26°C to 32°C in the incubator was provided to the different isolated fungi. Identification and characterization, were made by observing the colony colour and texture and the prepared slides by comparing with the authentic manuals of fungi (Raper and Thom, 1949; Barnett and Hunter, 1972; Cooney and Emerson 1964; Samson and Tansey 1977; and Domsch *et al*; 1980, Bilgrami *et al*; 1991, Jamaluddin, 2004). Unidentified species, devoid of fruiting bodies were designated as sterile mycelia. The seasonal variation and percentage of infection of fishes were analyzed. The present frequency of fungal species occurrence was calculated as follows :-

$$\% \text{ of frequency of species} = \frac{\text{Average number of total colonies of species in one plate.}}{\text{Average number of total colonies of all the species in one plate}} \times 100$$

RESULTS AND DISCUSSION

In the present study, total 3073 fishes were examined out of which 416 were found to be infected with fungal diseases. Mostly five species of fishes viz: *Catla catla* (14.42%), *Notopterus chitala* (21.63%), *Channa striatus* (12%), *Labeo rohita* (32.69%), *Channa marulius* (19.23%) were found infected. Total 25 fungi were isolated viz: *Thraustotheca* sp., *Aphanomyces* sp., *Achlya americana*, *Achlya apiculata*, *Achlya conspicua*, *Achlya prolifera*, *Allomyces anomalous*, *Aspergillus niger*, *Aphanomyces laevis*, *Dyctiuchus achlyoides*, *Fusarium* sp., *Pythium aphanidermatum*, *Pythium undulatum*, *Saprolegnia ferax*, *Saprolegnia hypoglycana*, *Saprolegnia parasitica*, *Mucor* sp., *Rhizopus* sp., *Chaetomium globosum*, *Alternaria tenuis*, *Verticillium* sp., *Aspergillus fumigatus*, *Penicillium funiculosum*. Along this *Aspergillus*

niger and *Neurospora* sp. have been isolated first time from the fishes in India (Firoz *et al*, 2011). In the present study, *Achlya* and *Aspergillus* sp. were found most of the time during study period and showed maximum virulence (Table 1,2,3,4, and 5). Maximum infected fishes were observed during the month of October due to suitable temperature and minimum in June (Table 1,2,3,4,5). Some species like *Alternaria tenuis*, *Rhizopus* sp. and *Verticillium* sp. were obtained in low counts in infected fishes. Some other fungi like *Mucor*, *Penicillium*, and *Aspergillus* showed a progressive increase in the month of August and September (Table 1,2). Besides above mentioned fungal members some sterile hyphae without fruiting body were also observed.

TABLE -1
Name of fish - Catla catla

S.No.	Name of Fungi	% frequency of the isolated fungi					
		June	July	August	September	October	November
1.	<i>Achlya americana</i>	11.9	10.3	7.1	5.2	6.2	10.8
2.	<i>Achlya apiculata</i>	10.2	5.1	7.5	6.1	4.1	12.9
3.	<i>Achlya conspicua</i>	9.0	6.3	3.1	4.1	5.2	10.5
4.	<i>Achlya prolifera</i>	4.3	3.1	5.2	4.1	1.0	14.3
5.	<i>Allomyces anomalus</i>	3.0	--	2.2	5.1	1.0	--
6.	<i>Alternaria tenuis</i>	--	3.2	--	--	2.3	2.2
7.	<i>Aphanomyces laevis</i>	2.0	--	5.1	4.1	3.9	3.2
8.	<i>Aphanomyces sp.</i>	--	2.0	--	3.1	4.9	2.1
9.	<i>Aspergillus fumigates</i>	4.1	1.0	3.0	4.1	5.2	1.0
10.	<i>Aspergillus niger</i>	--	--	4.5	2.3	1.5	1.0
11.	<i>Aspergillus nidulans</i>	--	--	3.1	6.1	5.1	2.6
12.	<i>Chaetomium globosum</i>	--	3.1	3.1	--	4.1	--
13.	<i>Dyctiuchus achlyoides</i>	5.8	6.3	--	5.5	4.0	6.3
14.	<i>Fusarium sp.</i>	--	3.1	2.0	3.1	2.0	3.4
15.	<i>Neurospora sp.</i>	--	--	1.0	2.0	4.2	2.0
16.	<i>Mucor sp.</i>	6.1	9.0	4.1	6.1	2.3	4.2
17.	<i>Penicillium sp.</i>	7.3	8.2	7.5	6.5	6.2	4.1
18.	<i>Pythium aphanidermatum</i>	8.8	9.6	9.6	6.1	9.1	6.1
19.	<i>Pythium undulatum</i>	6.1	7.8	6.9	4.3	5.2	2.0
20.	<i>Rhizopus sp</i>	--	2.1	--	--	1.3	1.2
21.	<i>Saprolegnia ferax</i>	4.0	6.3	5.3	3.8	4.2	3.0
22.	<i>Saprolegnia hypogiana</i>	1.0	3.8	6.2	4.1	5.1	--
23.	<i>Saprolegnia parasitica</i>	5.9	6.1	5.5	5.3	6.2	--
24.	<i>Thraustotheca sp</i>	4.9	2.4	6.3	5.2	4.5	2.0
25.	<i>Verticillium sp.</i>	--	--	1.0	--	1.0	--

Note:- A standard deviation of ± 0.5 to 3 were observed of some of the fungi.

TABLE -2
Name of fish - *Notopterus chitala*

S.No.	Name of Fungi	% frequency of the isolated fungi					
		June	July	August	September	October	November
1.	<i>Achlya americana</i>	10.7	7.0	8.2	3.0	4.2	14.3
2.	<i>Achlya apiculata</i>	12.3	3.5	6.3	7.1	6.1	10.3
3.	<i>Achlya conspicua</i>	13.0	4.8	4.2	4.9	3.2	13.1
4.	<i>Achlya prolifera</i>	3.1	5.3	4.2	5.6	2.1	11.2
5.	<i>Allomyces anomalous</i>	2.0	--	3.1	7.3	2.1	5.2
6.	<i>Alternaria tenuis</i>	--	--	2.1	--	3.1	--
7.	<i>Aphanomyces laevis</i>	4.0	--	3.1	5.3	4.1	1.3
8.	<i>Aphanomyces sp.</i>	--	4.1	--	4.6	5.2	3.1
9.	<i>Aspergillus fumigatus</i>	1.3	3.0	2.2	5.8	4.1	2.0
10.	<i>Aspergillus niger</i>	--	2.1	3.7	1.3	2.5	2.3
11.	<i>Aspergillus nidulans</i>	--	1.7	4.3	3.4	3.1	2.6
12.	<i>Chaetomium globosum</i>	--	--	2.3	2.3	4.6	1.0
13.	<i>Dyctiuchus achlyoides</i>	8.2	4.8	3.1	6.3	4.3	2.1
14.	<i>Fusarium sp.</i>	--	4.1	3.5	2.1	1.3	3.8
15.	<i>Neurospora sp.</i>	--	1.5	2.5	1.2	5.3	3.2
16.	<i>Mucor sp</i>	4.7	4.0	8.2	5.3	4.0	3.1
17.	<i>Penicillium sp.</i>	8.8	6.5	2.7	8.2	5.2	3.5
18.	<i>Pythium aphanidermatum</i>	8.9	5.9	--	3.4	8.1	5.2
19.	<i>Pythium undulatum</i>	6.8	9.9	2.6	5.0	3.2	1.0
20.	<i>Rhizopus sp</i>	--	--	2.7	1.2	1.5	--
21.	<i>Saprolegnia ferax</i>	--	6.3	3.0	--	4.0	1.0
22.	<i>Saprolegnia hypogiana</i>	2.1	6.8	3.2	2.1	6.1	--
23.	<i>Saprolegnia parasitica</i>	4.7	5.6	--	6.1	7.0	--
24.	<i>Thraustotheca sp</i>	5.6	5.2	6.2	3.4	5.8	4.2
25.	<i>Verticillium sp.</i>	--	2.0	--	1.2	1.0	--

Note:- A standard deviation of ± 0.5 to 3 were observed of some of the fungi.

TABLE -3
Name of fish - *Channa striatus*

S.No.	Name of Fungi	% frequency of the isolated fungi					
		June	July	August	September	October	November
1.	<i>Achlya americana</i>	7.7	11.8	10.1	7.2	4.2	5.3
2.	<i>Achlya apiculata</i>	13.3	4.2	12.3	5.4	4.3	8.3
3.	<i>Achlya conspicua</i>	10.3	4.9	4.1	3.4	4.1	10.9
4.	<i>Achlya prolifera</i>	7.3	5.1	4.3	5.1	5.3	8.6
5.	<i>Allomyces anomalus</i>	--	3.1	--	2.3	2.1	4.3
6.	<i>Alternaria tenuis</i>	--	1.2	--	5.1	1.1	1.3
7.	<i>Aphanomyces laevis</i>	5.3	4.1	3.6	2.9	4.0	1.9
8.	<i>Aphanomyces sp.</i>	5.1	--	3.5	5.3	5.1	3.1
9.	<i>Aspergillus fumigates</i>	2.1	--	2.1	5.2	4.1	4.3
10.	<i>Aspergillus niger</i>	--	3.1	3.9	3.5	1.9	1.9
11.	<i>Aspergillus nidulans</i>	2.1	2.1	4.0	3.1	3.3	5.7
12.	<i>Chaetomium globosum</i>	--	--	4.2	2.3	2.1	--
13.	<i>Dyctiuchus achlyoides</i>	6.8	3.9	2.2	7.1	6.2	3.2
14.	<i>Fusarium sp.</i>	--	2.3	2.4	3.4	2.3	3.2
15.	<i>Neurospora sp.</i>	--	1.0	2.1	--	3.1	2.8
16.	<i>Mucor sp</i>	4.2	4.8	4.3	6.1	4.2	5.3
17.	<i>Penicillium sp.</i>	3.6	6.0	3.5	5.2	8.1	7.2
18.	<i>Pythium aphanidermatum</i>	5.4	7.1	4.9	3.1	10.9	3.3
19.	<i>Pythium undulatum</i>	4.6	5.5	2.8	--	7.1	2.3
20.	<i>Rhizopus sp</i>	--	3.5	--	--	1.9	--
21.	<i>Saprolegnia ferax</i>	9.1	3.7	8.1	4.6	5.6	3.2
22.	<i>Saprolegnia hypogyna</i>	--	3.9	4.3	5.2	7.1	1.1
23.	<i>Saprolegnia parasitica</i>	6.1	6.5	3.5	6.2	8.3	2.3
24.	<i>Thraustotheca sp</i>	4.3	--	5.2	4.1	3.0	--
25.	<i>Verticillium sp.</i>	--	--	1.5	--	1.3	--

Note:- A standard deviation of ± 0.5 to 3 were observed of some of the fungi.

TABLE -4
Name of fish - Labeo rohita

S.No.	Name of Fungi	% frequency of the isolated fungi					
		June	July	August	September	October	November
1.	<i>Achlya americana</i>	8.7	6.3	7.2	3.4	5.4	12.3
2.	<i>Achlya apiculata</i>	11.4	5.2	11.2	7.3	5.8	15.9
3.	<i>Achlya conspicua</i>	10.2	4.3	6.3	5.3	6.3	8.5
4.	<i>Achlya prolifera</i>	5.2	6.4	6.1	6.2	3.2	14.2
5.	<i>Allomyces anomalous</i>	4.1	4.8	1.0	--	1.3	3.5
6.	<i>Alternaria tenuis</i>	1.0	3.2	1.2	--	1.0	--
7.	<i>Aphanomyces laevis</i>	--	2.3	3.3	6.2	2.3	4.1
8.	<i>Aphanomyces sp.</i>	1.3	2.1	--	4.3	3.7	--
9.	<i>Aspergillus fumigatus</i>	5.3	--	4.2	5.9	3.7	2.5
10.	<i>Aspergillus niger</i>	2.1	2.3	3.6	3.2	2.1	--
11.	<i>Aspergillus nidulans</i>	--	3.2	5.2	3.5	3.8	2.6
12.	<i>Chaetomium globosum</i>	--	1.0	--	2.3	6.3	--
13.	<i>Dyctiuchus achlyoides</i>	3.1	5.2	2.1	7.9	4.2	5.2
14.	<i>Fusarium sp.</i>	--	4.3	4.1	6.3	3.3	--
15.	<i>Neurospora sp.</i>	1.6	2.1	--	1.3	4.6	3.2
16.	<i>Mucor sp.</i>	2.3	3.1	5.1	6.3	3.9	5.0
17.	<i>Penicillium sp.</i>	6.2	7.3	5.2	4.3	5.2	3.9
18.	<i>Pythium aphanidermatum</i>	7.5	5.6	4.1	4.6	4.1	4.9
19.	<i>Pythium undulatum</i>	5.3	2.9	5.2	6.5	8.2	2.4
20.	<i>Rhizopus sp</i>	--	1.2	1.2	--	1.2	1.6
21.	<i>Saprolegnia ferax</i>	2.2	8.9	6.3	5.6	6.2	3.1
22.	<i>Saprolegnia hypogiana</i>	2.5	6.2	3.1	--	3.1	1.5
23.	<i>Saprolegnia parasitica</i>	4.7	3.7	2.1	3.8	2.2	--
24.	<i>Thraustotheca sp</i>	8.6	3.4	4.1	2.2	3.5	2.0
25.	<i>Verticillium sp.</i>	--	--	--	--	1.0	--

Note:- A standard deviation of ± 0.5 to 3 were observed of some of the fungi.

TABLE -5
Name of fish - *Channa marulius*

S.No.	Name of Fungi	% frequency of the isolated fungi					
		June	July	August	September	October	November
1.	<i>Achlya americana</i>	13.5	8.5	9.5	5.9	7.1	10.7
2.	<i>Achlya apiculata</i>	8.4	7.3	6.8	5.3	3.2	13.0
3.	<i>Achlya conspicua</i>	11.0	5.2	5.3	6.3	3.9	8.9
4.	<i>Achlya prolifera</i>	5.2	4.6	7.3	2.0	1.2	10.2
5.	<i>Allomyces anomalous</i>	8.4	3.2	5.1	4.8	1.6	2.1
6.	<i>Alternaria tenuis</i>	--	1.3	1.6	--	2.9	--
7.	<i>Aphanomyces laevis</i>	1.0	2.1	7.8	3.2	3.0	--
8.	<i>Aphanomyces sp.</i>	--	3.1	3.5	--	4.0	--
9.	<i>Aspergillus fumigatus</i>	6.1	1.3	3.8	3.8	4.3	--
10.	<i>Aspergillus niger</i>	2.3	1.8	1.3	4.1	1.2	4.0
11.	<i>Aspergillus nidulans</i>	--	1.7	--	5.2	3.9	4.6
12.	<i>Chaetomium globosum</i>	--	--	5.3	--	3.2	--
13.	<i>Dyctiuchus achlyoides</i>	4.2	3.4	6.2	3.5	2.0	9.8
14.	<i>Fusarium sp.</i>	2.3	--	--	5.3	3.0	6.8
15.	<i>Neurospora sp.</i>	--	--	1.6	3.5	6.1	5.3
16.	<i>Mucor sp.</i>	--	5.3	6.2	2.5	6.3	8.1
17.	<i>Penicillium sp.</i>	8.1	6.1	8.5	7.8	1.2	6.3
18.	<i>Pythium aphanidermatum</i>	9.0	8.9	--	5.8	9.8	--
19.	<i>Pythium undulatum</i>	3.6	8.7	5.6	2.2	5.6	5.2
20.	<i>Rhizopus sp.</i>	--	3.2	--	1.5	2.0	--
21.	<i>Saprolegnia ferax</i>	4.9	--	2.3	3.9	3.1	--
22.	<i>Saprolegnia hypogiana</i>	3.6	8.6	--	6.2	2.9	--
23.	<i>Saprolegnia parasitica</i>	3.7	7.3	6.5	4.2	7.6	--
24.	<i>Thraustotheca sp</i>	1.9	4.7	--	4.1	6.3	--
25.	<i>Verticillium sp.</i>	--	--	2.0	--	1.2	--

Note:- A standard deviation of ± 0.5 to 3 were observed of some of the fungi.

Some of the fungal infection in fishes were observed during October month (M.J.Robert *et al*, 2003). It was also observed that fishes suffer from two types of fungal diseases *Saprolegniasis* and *Aphanomycosis*. (T.A.Qureshi *et al*, 2002,

R.J.Roberts *et al*, 1993 and K.Hatai *et al*, 1994). Also reported pathogenecity of same on fishes. The symptoms of the disease are characterized by brownish patches of cottony fungal growth on skin and gills. Initially the infection is in form of

small patches and in advanced cases big ulcerative lesions which penetrate up to muscles. Similar symptoms have been reported by (R.D.Khulbe and

S.C Sati, 1981, Hatai, K. and G.Hoshaiai 1992 and K.Hatai *et al*, 1994) . Present observation is in agreement with the above findings.

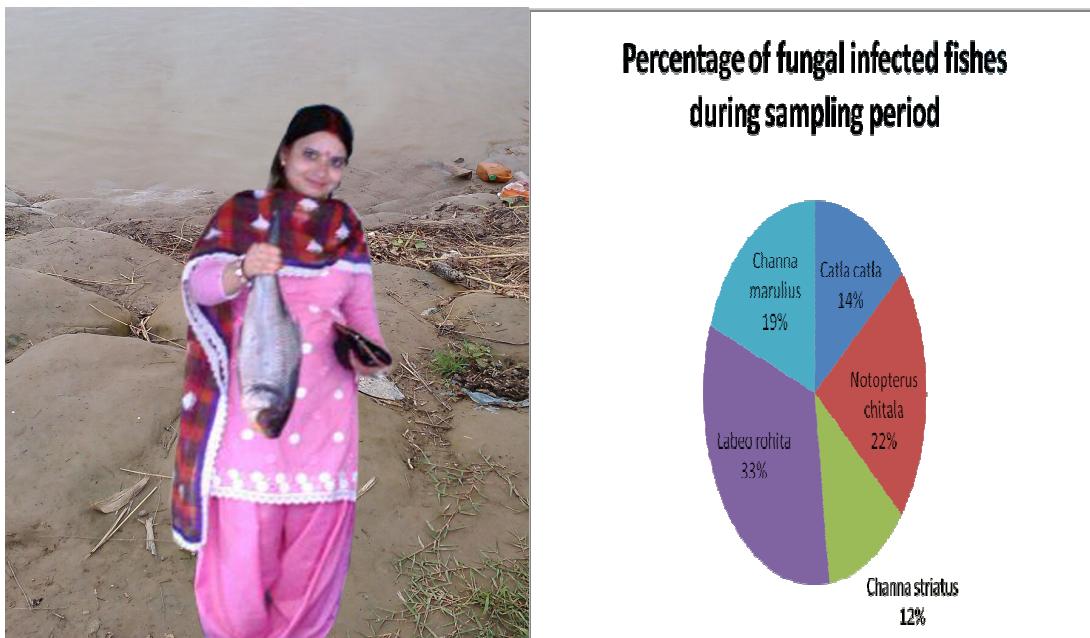
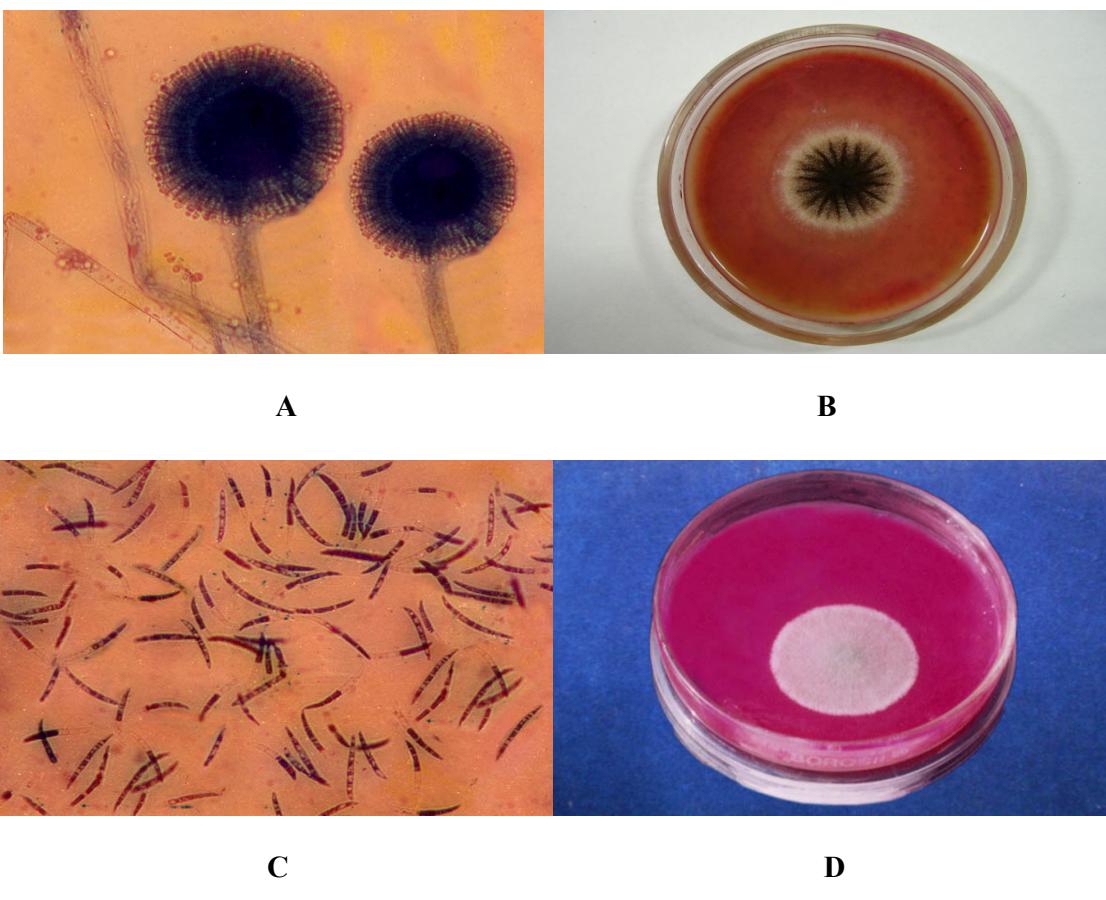


Figure - Sample collected by author.





E

F



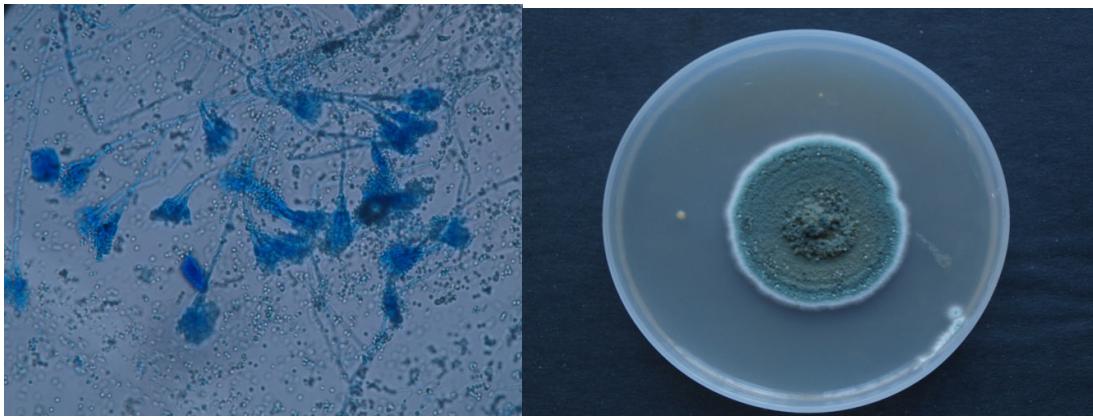
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K

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Figure showing –

A & B - Microscopic photograph and culture plate of *Aspergillus niger*.
 C & D - Microscopic photograph and culture plate of *Fusarium sp.*.
 E & F - Microscopic photograph and culture plate of *Aspergillus fumigates*.
 G & H - Microscopic photograph and culture plate of *Neurospora sp.*.
 I & J - Microscopic photograph and culture plate of *Verticillium sp.*.
 K & L - Microscopic photograph and culture plate of *Penicillium sp.*.

CONCLUSION

The present study is to investigate fungal infections in five economically important fresh water fishes viz: *Catla catla*, *Notopterus chitala*, *Channa striatus*, *Labeo rohita*, *Channa marulius* were collected from Gandak river near Muzaffarpur region of Bihar. Nearly 25 fungi were identified. Identification and characterization of the fungi were made with the help of authentic manuals of fungi. The most common among them viz; *Pythium sp.*, *Thraustotheca sp.*, *Aphanomyces sp.*, *Dictyuchus sp.*, *Achlya sp.*, *Aspergillus sp.*, *Fusarium sp.*, and *Penicillium sp.* were isolated, identified and characterized.

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