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PATHOGENECITY OF THREE SPECIES OF ASPERGILLUS (*A.FUMIGATUS , A.NIGER & A.SYDOWII*) ON SOME FRESH WATER FISHES

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ABSTRACT:

Role of *Aspergillus* in causing fish mycoses is of outmost importance because in recent years *Aspergillus* infections have increased in fresh water fishes. The study was carried out on randomly collected 102 infected fresh water fishes from different water bodies. Three different species of *Aspergillus viz. A.fumigatus, A.niger, A.sydowii* have been isolated from 9 different species of fishes *viz. Channa striatus, Cirrhinus mrigala, Clarias batrachus, Labeo rohita, Macrognathus aculeatus, Mystus seenghala Puntius sarana, Puntius ticto* and *Trichogaster fasciatus.* Total 38 isolates were recorded from infected fishes. Maximum isolates were of *A.fumigatus* (71%) followed by *A. niger*(18%) and minimum of A.*sydowii*(11%). Among the fishes, the maximum infected specimens were of *M.seenghala* and *P.sarana*. Organ wise, most infected area was caudal region (53%) and least was gills (8%). On Corn Meal Agar (CMA) fastest growth was observed in *A.fumigatus* (47mm) within 10 days duration. Pathogenecity tests showed all the isolated species of *Aspergillus* were pathogenic to fishes causing infection and mortality of most of the fishes.

KEY WORD: *Aspergillus, Pathogenicity, Ornamental fishes.*

INTRODUCTION:

The study of fish mycoses is of outmost importance both from point of view of fishery management and to check the spread of human and animal disease. There are so many reports on zoosporic fungi (Oomycetes) in fishes but work on Eurotiomycetes (*Aspergillus*) infection in fishes have been sporadic and role of *Aspergillus* as the cause of fish mycoses in fresh water fishes is

not been well documented. *Aspergillomycoses* in fishes have been reported by (Olufemi *et al*.,1983,1985 &1986). Bhattacharya,1988 and Bhattacharya *et al*.,1988 reported *A. niger* and *A.terreus* as fish pathogen. Shrivastava, 1996 reported *A.terreus* from fresh water fishes and tested their pathogenecity on same species of fishes. Some other workers isolated *Aspergillus* from fresh water fishes are (Refai *et al*.,1987&2010; Salem *et al.*,1989b; Shabazain *et al*.,2010 ; Junaid *et al*.,2010; Fadaifard *et al.*,2011; Iqbal *et al.*, 2012b; Iqbal & Mumtaz,2013 and Chauhan, 2013). Present study was aimed to investigate the *Aspergillus spp.* associated with fish mycoses by isolation and identification of species, along with that pathogenic nature have been tested by experimentally challenged the various species of fishes.

MATERIALS AND METHODS:

A total number of 102 fishes showing external symptoms were 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. The fishes were observed to note external symptoms. For the isolation of *Aspergillus spp*. from the body of fish, innocula were taken from all the fins , gills and skin with sterile needle and inoculated on agar plates. The media used in the present study were Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Sabourauds Dextrose Agar (SDA) and Corn Meal Agar (CMA). To avoid bacterial contamination all the glass wares, instruments and media were sterilized, along with all aseptic conditions, Teracycline 100mg/ml and Streptomycine sulphate 100mg/ml were used in media. Innoculation was done in Laminar flow in sterilized conditions. The agar plates were incubated at 28-30 ˚C for the growth of cultures. Growth of colony was observed in 3-4 days. For full growth of colony, plates were kept for 8-10 days for incubation. For identification, slides were prepared from each colony by taking small tuft of mycelium and stained with Lacto-phenol cotton blue . The slides were observed under microscope. Identification of fungi was carried out on the basis of keys of (Raper , 1965,Refai *et al*,1987,Willoughby 1994, Shrivastava ,2009). Fishes were identified by the keys of (Jhinghran, 1982and Qureshi and Qureshi ,1983).

Pathogenicity Challenge

To determine the pathogenecity of three species of *Aspergillus* identified as *A. fumigatus, A. niger* and *A.sydowii* same fish species were used from which the particular strain of fungi was isolated. After identification pure cultures were prepared and maintained on Corn Meal Agar at 28±2˚C and conidial spore suspension was prepared for innoculation. 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 conidial suspension was counted by haemocytometer and suspension was diluted to reach $8x10^5$ spores/ml for all the three species of *Aspergillus* used for pathogenecity test**.**

Experimental Set up

Healthy fishes with average weight of 35-40 gm were collected and kept in aquaria of 10L capacity under observation for three days with continuous aeration and fed with artificial feed. For experimental purpose fishes were injected intramuscularly with 0.2 ml conidial suspension. The experimental troughs were aerated continuously and temperature was maintained between 28-30˚C Injected fishes were observed for seven days. Infection and mortality was recorded. The dead fishes were sampled for observation and re-isolation.

RESULTS:

A total number of 102 randomly collected freshwater fishes were observed for isolation of *Aspergillus spp*. Based on morphological characterstics three different species of *Aspergillus* viz. *A.fumigatus, A.niger, A.sydowii* have been isolated from nine different species of fishes viz. *Channa striatus, Cirrhinus mrigala, Clarias batrachus, Labeo rohita, Macrognathus aculeatus, Mystus seenghala Puntius sarana, Puntius ticto* and *Trichogaster fasciatus*.(Table-1).

Among the 38 isolates obtained from infected fishes .Maximum (27) isolates were of *A.fumigatus* followed by (7) isolates of *A.niger* and minimum (4) isolates of *A.sydowii* (Fig-1). Among the fishes , maximum infection was found in *M.seenghala* and *P.sarana.* Organ wise study of fishes showed that in most of the fishes caudal region was most infected area (53%) then head region (29%),fins (10%) and minimum infection was found in gills (8%).(Fig-2).

All the three species of fungi showed growth on Corn Meal Agar ,*A. fumigatus* was found to be the fastest growing fungi shown growth up to 47 mm in ten days duration. Petriplate was full of conidia .*A.sydowii* showed 44mm growth and *A. niger* grown up to 39mm which was slowest among the three.(Fig-3).

Results of artificial infection showed that, all the three species of *Aspergillus* were pathogenic to challenged fish from which it has been isolated. Within the period of seven days infection was observed in almost all the inoculated fishes resulting in mortality.

A.fumigatus was inoculated on six different species of fishes, among them *C.striatus, L.rohita,M.seenghala* and *P.sarana* showed external symptoms of infection on second day of innoculation in all the three specimens of each species leading to 100% mortality. In *M. aculeatus* all the three inoculated fishes got infection on third day leading to death of two fishes. In *C mrigala* only one fish showed infection on third day and died in the period of seven days.

A.niger was inoculated on three species of fishes, *C.batrachus* showed external infection on fourth day of innoculation in two specimens among them one led to death in seven days time period. *M.seenghala* showed external infection in two fishes on third day of innoculation with 100% mortality. In *P.ticto* , on second day of experiment all the fishes got infection leading to 100% mortality.

A.sydowii was tested on two species of fishes, *P.ticto* and *T.fasciatus* . In both species of challenged fishes external infection was observed in two specimens from each on the second day but with in the period of seven days all the fishes died due to infection.

Among nine challenged species of fishes 100% mortality was observed in six species of fishes, viz. *C.striatus, L.rohit., M.seenghala* , *P.sarana* , *P.ticto* and *T.fasciatus* (Table-2)

Artificially challenged fishes showed similar symptoms as that of naturally infected fishes and re-isolation of fungi showed the confirmation of presence of inoculated fungi.

DISCUSSION:

This study reported three species of *Aspergilus,*viz. *A. fumigates ,A.niger, A.sydowii* isolated from various fresh water fishes. These findings are consistent with the findings of (Olufemi *et al.*,1983) who reported Aspergillomycoses in cultured Tilapia from Kenya and (Olufemi *et al.,* 1985) who reported *Aspergillus* as pathogen of cultured fishes. This work has been supported by some other workers (Refai,*et al.*,1987,2010 ; Iqbal *et al.,* 2012b ; Chauhan ,2012 & 2013 and Chauhan and Benkhede,2013).

While observing the fishes it was found that maximum infected fishes belong to ornamental group and most infected fish was *P.sarana.* These findings are supported by the work of (Iqbal & Mumtaz, 2013) who reported *Aspergillus* as pathogen of ornamental fish , Black moor . In infected fishes most affected region was caudal region which is in agreement with the findings of (Iqbal & Mumtaz,2013) who reported the tips and base of fins and body surface seems very easy and accessible site for fungal spores to attack, settle and to establish the virulence of infection. Growth of colony of isolated species of *Aspergillus* showed, maximum growth in *A.fumigatus* Within 10 days duration full colony growth was observed , Similar growth pattern was observed by (Alisa *et al.*,2001) in *A.sydowii*. During the study it was found that, among all the three isolated species *A. fumigatus* was fastest growing and virulent fungi . External lesions of this species affected fishes were more prominent.

Work on pathogenecity tests of *Aspergillus spp.* on fishes is very rare. During the present study 0.2 ml dose of 8X 10^5 concentration of spores suspension was found pathogenic to all the challenged fishes and death of most of the fishes within seven days .These findings are comparable with the reports of (Shrivastava,1996) who reported pathogenecity of *Aspergillus* on fresh water fishes and death within ten days.(Refai *et al*.,2010) also confirmed *Aspergillus* as pathogenic fungi for fish with their histopathological findings. Present study is in agreement with this view and pathogenic nature of *Aspergillus spp.* was confirmed by re-isolation of fungi from body of experimentally challenged fishes.

CONCLUSION:

From the above piece of work it has been found that *Aspergillus spp.* Were highly pathogenic to fish and conidia spreads at very fast rate leading to mortality of fish and even on dead fish these fungi grow and can spread disease in humans so control measures should be taken.

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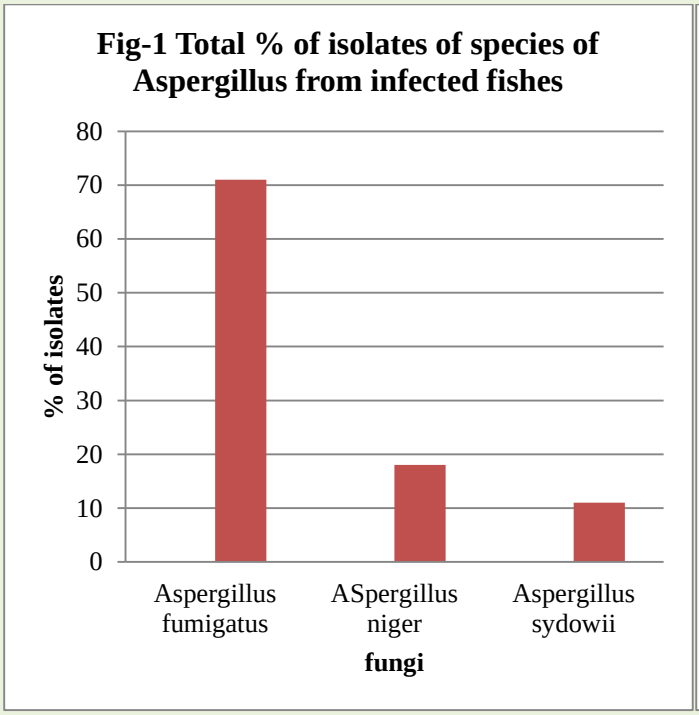
Table-1: Species of *Aspergillus* isolated from different regions of naturally infected fishes

S.No.	Fungi	Naturally infected fish	Caudal region	fins	gills	Head region
1.	*Aspergillus fumigatus*	*Channa striatus*	+	-	-	+
		Cirrhinus mrigala	-	-	+	-
		Labeo rohita	+	+	-	-
		Macrognathus aculeatus	-	-	-	+
		Mystus seenghala	+	-	-	-
		Puntius sarana	+	+	+	+
2.	*Aspergillus niger*	*Clarias batrachus*	+	-	-	-
		Mystus seenghala	-	+	-	+
		Puntius ticto	-	-	-	+
3.	*Aspergillus sydowii*	*Puntius ticto*	+	-	-	+
		Trichogaster fasciatus	+	+	-	-

Table-2 Pathogenecity tests of isolated species of *Aspergillus* on various species of fishes

S.No.	Injected fungi	Fish experimented	No. of fish	Conc. Of spores	Dose(ml)	No. of infected fishes	Infection within 7 days	Mortality%
1.	*A.fumigatus*	*Channa striatus*	3	8x 10^5	0.2	3	2nd day	100
		Cirrhinus mrigala	3	8x 10^5	0.2	1	3rd "	33
		Labeo rohita	3	8x 10^5	0.2	3	2nd "	100
		Macrognathus aculeatus	3	8x 10^5	0.2	3	3rd "	67
		Mystus seenghala	3	8x 10^5	0.2	3	2nd "	100
		Puntius sarana	3	8x 10^5	0.2	3	2nd "	100
2.	*A.niger*	*Clarias batrachus*	3	8x 10^5	0.2	2	4th day	33
		Mystus seenghala	3	8x 10^5	0.2	2	3rd "	100
		Puntius ticto	3	8x 10^5	0.2	3	2nd "	100
3.	*A.sydowii*	*Puntius ticto*	3	8x 10^5	0.2	2	2nd day	100
		Trichogaster fasciatus	3	8x 10^5	0.2	2	2nd "	100



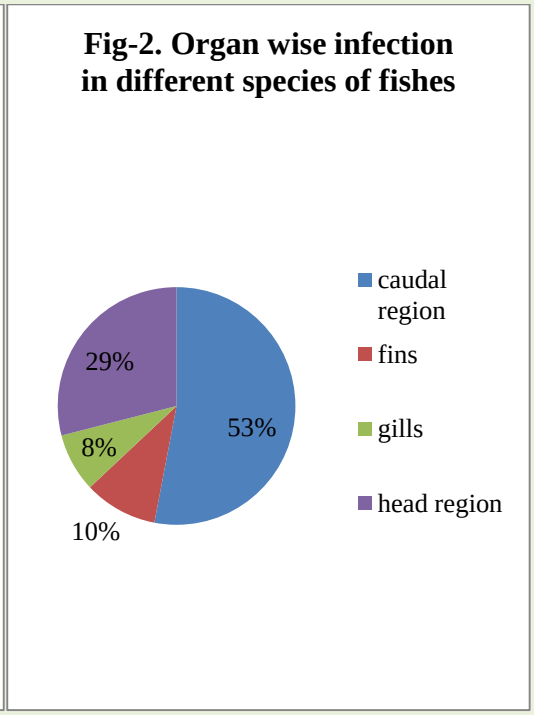


Fig-3.Growth of colony of Aspergillus spp.on CMA at 28-30"C for 10 days