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Pathogenicity of some species of *Achlya* and *Saprolegnia* on Indian Major carps viz. *Catla catla*, *Cirrihinus mrigala and Labeo rohita*

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Abstract: An investigation was conducted on fungal infected Indian Major Carps viz. *Catla catla, Cirrhinus mrigala* and *Labeo rohita* collected from Sarangpani Lake. During the study period from July 2011 to December 2011, total seven species of fungi have been isolated from the lesions and affected muscles of infected fishes. These fungal isolates were cultured under laboratory conditions and identified as Achlya *americana, A. klebsiana, A. orion, A. prolifera, Saprolegnia diclina, S. ferax and S.parasitica.* Pathogenicity of all the seven species of fungi was tested on different major carps. All the isolates were found pathogenic to fishes but *A. prolifera* and *S. parasitica* were found to be most virulent showed 100% mortality of the experimental fishes. Percentage prevelance of infection have also been found out. Maximum percentages of infections (47.4%) were recorded in Catla *catla* in the month of December and minimum (8.9%) in *Cirrhinus mrigala* in August.

Keyword: Oomycetes, fish mycoses, infection, pathogenic fungi, major carps.

INTRODUCTION

Phycomycete fungi are ubiquitous components of aquatic habitats. They are opportunistic facultative parasites. Saprolegnious fungi often act as wound parasites and handling fish often predisposes them to infection. Fish succumb to infection either under circumstances which damage their skin or when

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predisposed to environmental stressors. Often both conditions occur together^{1,3}. There is a good evidence to suggest that saprolegnian fungi can act as primary invaders in physiologically debilitated and immunologically compromised fish,⁴ and their eggs^{5,6}. Among all Oomycetes *Saprolegnia* and *Achlya* were more virulent parasites in comparison to others,^{7,8} reported fungal infection of common carps⁹ also tested the pathogenecity of *Saprolegnia* and *Aphanomyces* on major carps. The aim of present study was to report the incidences of dermal mycoses in Indian Major *Carps Catla, catla, Cirrhinus mrigala and Labeo rohita*, their percentage prevalence, isolation, culture and identification of fungi present on selected species of fishes and to test the pathogenecity of isolates.

EXPERIMENT DESIGN AND SETUP

Collection of Fishes: For the investigation of incidences of mycoses in major carps, Sarangpani lake have been selected which is used for culture of fishes. The infected fishes were collected on weekly basis for the period of six months from July 2011 to Dec. 2011. A total number of 1,765 fishes were examined. Sampling of infected fishes were carried out by collecting the fishes in polythene bags. These were bought to the laboratory in living condition. The lesions, ulcerations, white greyish patches and cottony tufts were examined grossly with the help of hand lens. Isolation of fungi was carried out by cutting a small piece of infected skin with the help of sterile scalpel and by plucking a small piece of mycelium with the help of sterile forceps or needle. After washing it with sterile distilled water they are baited with different baits like sesame and mustard seeds.

Purification: Purification of cultures have been done by preparing the cultures on Potato Dextrose Agar (PDA) and Glucose Yeast Agar (GYA). Techniques of ^{10,11} have been used for purification. To inhibit the bacterial growth 500ug/ml each of penicillin and streptomycin was added to PDA and GY agar plates. All the cultures were incubated at temperatures 18+2 °c. All the isolates were identified up to species level with the help of morphological and reproductive structures by using the monographs and hand book ^{12,13}.

Pathogenecity Test: To find out the pathogenecity of isolated species of fungi all the three Major Carps (*C.catla, C. mrigala and L. rohita*) were selected as experimental fishes having the average size of 3cm to 5cm and weight. The fishes were separately challenged against all the seven species of isolated fungi viz. *Achlya Americana, A. klebsiana, A. orion*, *A. prolifera*, *Saprolegnia diclina, S. ferax and S. parasitica*.

For this purpose nine fishes three from each species were placed in aquaria containing sterilized tap water. Before keeping the fishes in experimental troughs the initial pathogenecity is controlled by adding 2-3 drops of 0.05 ppm malachite green . Fishes are provided with aeration and readymade food. To remove the traces of malachite green experimental fishes have been transferred in sterile tap water. The pathogenecity tests were carried out by keeping nine fishes at a time for one isolate of fungi in a sterilized trough containing sterile tap water. The fishes were inoculated with small mycelial tuft subcutaneously , this have been done by small scalpel abrasion. To increase the concentration of zoospores in troughs small blocks of media containing fungi, approx 1.0cm2 were kept in the trough . Four blocks in each trough. For control , in separate trough nine fishes three from each species were kept without innoculation⁶ Continuous aeration was provided and temperature was 18 to 22 °c. The experiment were conducted for six days and infection was monitored regularly on the basis of behavioural changes, patches, lesions on skin , mycelial growth and mortality of fish.

RESULTS AND DISCUSSION

Present studies were conducted on three Indian Major Carps which were found infected during the study period from July 2011 to December 2011. All the three carps viz. *C. catla, C. mrigala* and *L.rohita* were found infected with fungi showing symptoms like de scaling, ulcerations and

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cottony tufts of mycelium. From naturally infected selected carps total fourteen isolates were recovered which belong to two genera, *Achlya* and *Saprolegnia* contributing seven species viz. *A.americana, A. klebsiana, A. orion, A. prolifera, S.diclina, S.ferax and S.parasitica*. During the whole study period *A.prolifera* and *S.parasitica* were more dominant by showing their presence on all the three selected species of fishes. (**Table-1**)

Table -1: Species of fungi isolated from Indian Major Carps, collected from Sarangpani lake --July2011 to Dec. 2011.

S.NO.	Sp. of fungi	Host Fish
1	Achlya americana	Cirrhinus mrigala, Labeo rohita
2	Achlya klebsiana	Cirrhinus mrigala
3	Achlya orion	Labeo rohita
4	Achlya prolifera	Catla catla, Cirrhinus mrigala, Labeo rohita
5	Saprolegnia diclina	Cirrhinus mrigala , Labeo rohita
6	Saprolegnia parasitica	Catla catla ,Cirrhinus mrigala , Labeo rohita
7	Saprolegnia ferax	Cirrhinus mrigala , Labeo rohita

Maximum percentage of infection was recorded in *C.catla* followed by *L.rohita* and minimum in *C. mrigala*. In C.catla it was (47.4%) and in C. *mrigala* (8.9%). Infection was maximum in the month of December when the temperature was low and minimum in August, during heavy rains.(**Fig.1**).





All the recovered isolates from naturally infected fishes were detected as pathogenic through experimental infection under laboratory conditions. All the seven species of fungi successfully produced abnormal protrusions, spots on the body, lesions, mycelial growth on skin and in most of the cases death of the fish.(**Table-2& Table-3**).

Table-2: Pathogenicity test of four species of Achlya on Indian Major Carps, C.catla, C. mrigala and Labeo rohita.															
_															
Fungal												% of		% of	
Isolate	Fish sp.	1		2		3		4		5	6		infection		mortality
		Ι	Μ	Ι	Μ	Ι	Μ	Ι	Μ	Ι	Μ	Ι	Μ		
Achlya															
americana	A-3,B-3,C-3	-	-	-	1	2	2	1	2	1	1	-	1	44.4	77.7
A.klebsiana	A-3,B-3,C-3	-	1	1	-	2	2	-	3	-	-	-	-	33.3	66.6
A.orion	A-3,B-3,C-3	-	-	-	1	1	1	1	2	1	-	-	2	33.3	66.6
A.prolifera	A-3,B-3,C-3	1	2	3	2	2	3	2	2	-	-	-	-	88.8	100
Control	A-3,B-3,C-3	-	-	-	-	-	-	-	-	-	-	-	-	0	0

Table-3: Pathogenicity test of city test of three species of Saprolegnia on Indian Major Carps,															
C.catla, C.mrigala and L.rohita.															
		DAYS													
													% of	% of	
Fungal Isolate	Fish sp.	1		2		3		4		5		6		infection	mortality
		Ι	Μ	Ι	Μ	Ι	Μ	Ι	Μ	Ι	Μ	Ι	Μ		
Saprolegnia diclina	A-3,B-3,C-3	2	1	3	2	1	2	1	-	1	1	-	-	88.8	66.6
S.ferax	A-3,B-3,C-3	1	-	2	2	1	-	-	2	2	3	-	1	66.6	88.8
S.parasitica	A-3,B-3,C-3	2	1	2	3	3	2	2	3	-	-	-	-	100	100
Control	A-3,B-3,C-3	-	-	-	-	-	-	-	-	-	-	-	-	0	0

I- Infection, M – Mortality, A -C. catla, B-C. mrigala, C-L.rohita.

Achlya Americana: In these isolate lesions start developing on third day of innoculation by showing 44.4% infection and 77.7% fishes died.

A.klebsiana : In this isolate lesion starts on second day of innoculation although one fish died on the first day by showing abnormality in behavior like unbalanced and slow swimming. 33.3% of fishes showed the development of infection and mortality was 66.66%.

A.orion: After inoculating this isolate on first day only behavioural changes were observed. On second day one fish died. Infection was visible on third day and 33.3% of fishes got visible infection. Mortality was 66.6%.

A.prolifera: Lesions developed on very first day of innoculation and 88.8% of fishes showed infection. Mycelium was clearly observed from lesion. All the fishes died on fourth day of experiment showing 100% mortality.

Saprolegnia diclina : On first day two fishes showed the development of infection and percentage of lesions was 88.8% while mortality was 66.6%.

S.ferax : Swelling and lesion starts developing within 24 hours of innoculation and on fourth day there was clearly visible mycelial growth. 66.6% of fishes got external infection and 88.8% fishes died.

S.parasitica : This isolate also found very quick in developing infection showing growth of hyphae on lesions within 24 hours. On fourth day all the fishes died. Percentage of infection was 100% and mortality was also 100%.

In control troughs all the fishes remain alive without any infection.

Experimental studies showed that among genus *Achlya*, *A. prolifera* was found to be most virulent by developing lesions in 88.8% of fishes and 100% mortality within four days of innoculation. In genus *Saprolegnia, S.parasitica* was found most vigorous showing infections and mortality in 100% fishes and complete mortality within four days of experiment.

Oomycetes which play an important role among the family Saprolegniaceae is responsible for causing fungal disease in fishes. 5 ^[32] Reported the saprolegnian fungi are one of the major causes of fungal disease in fresh water fishes and their eggs.

Some other workers also reported Saprolegnia and Achlya as pathogenic fungi from different species of fishes ¹⁴⁻²¹ also reported Saprolegnia parasitica from infected Salmonids in Japan ^{22,23}, reported Achlya and Saprolegnia as major fungal pathogens. In present study also from all the selected fishes Achlya sp. and Saprolegnia sp. have been isolated. A number of studies shown low temperature delay the immune response in fish which may be the cause of fungal infection²⁴ During this study also maximum infected fishes were recorded in the month of December when the temperature was low²⁵ reported that Indian Major Carps were much more susceptible to EUS²⁶ found Saprolegnia was the most common pathogen in L.rohita and C.catla²⁷ also stated that Saprolegnia sp. has great impact on aquaculture especially it can infect Carp and Tilapia. The above studies favours the present observations²⁸, was the first to demonstrate the ability of *S. parasitica* (Coker) to parasitize a wide range of fishes²⁹ demonstrated parasitic ability of sixteen species of aquatic fungi by conducting laboratory experiments.^[33] reported A. Americana as a parasite of C. mrigala which supports the present observation.⁵ reported A. orion as pathogen of L. rohita. In present work also this species have been isolated from L. rohita. A. prolifera was isolated from all three major carps and A. klebsiana from C. mrigala.^{5,30,31} reported S.diclina, S.ferax and S.parasitica as pathogen of major carps which supports the present findings. In genus Saprolegnia, S. parasitica is most virulent parasite of fish, 3,32,33, reported S. parasitica most virulent. The experimental studies of Saprolegnia on different species of fishes have also been reported by 34^[24]. Present study is supported by above findings by showing dominance of S. parasitica in major carps and it act as most virulent fungal parasite by showing 100% mortality of experimental fishes.

CONCLUSION

It is concluded that Major carps are in great threat to fungal infections and all the isolated species of *Saprolegnia* and *Achlya* were found pathogenic to fishes. During low temperature months incidences of disease were more. For prevention and treatment further study is required.

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