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Observations on saprolegnia infection in freshwater fishes of Lake Kolleru

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Abstract
The present study was design to investigate the Saprolegnia infection in fishes of Kolleru Lake, Andhra Pradesh and the percentage of infection of various species of fishes. A total number of 1400 fishes were screened and fungal infected fish samples were brought to the laboratory in sterilized plastic bags and kept in glass aquaria to observe the symptoms of infected fishes. Identification of various species of fungus was done on the basis of their vegetative and reproductive characters and standard keys described by various workers. Experimental infection trails were conducted with isolated fungal isolates to know the pathogenic nature of the fungi. Out of 1400 fishes examined, only 287 fishes were found to be infected with fungus. Eight species of fish viz. *Catla catla*, *Labeo rohita*, *Channa punctatus*, *Channa striatus*, *Clarias batrachus*, *Mastacembalus armatus*, *Mystus cavasius* and *M. seenghala* were found infected. Forty one isolates of fungi have been isolated from the diseased fishes, which belong to 8 genera and 14 species. Among all the eight genera, *Achlya* and *Saprolegnia* found to be highly virulent. In case monthly percentage of infection, the maximum percentage of infection was recorded in December, 2013 (23.2%) followed by January, 2014 (16.8%) and February, 2014 (15.3%) and minimum infection (9.2%) was reported in the month of October, 2013. In case fish species, the maximum infection was observed in *Clarias batrachus* (25%) while minimum was noticed in *Catla catla* (4%). Results of experimental infection trails indicates that *Saprolegnia parasitica* and Achlya sp are highly virulent in nature.

Keywords: Upputeru, fungus, infection, fishes

1. Introduction
Fungal infections of fish by oomycetes, commonly known as water moulds, are widespread in fresh water and represent the most important fungal group affecting wild and cultured fish. Most fungi are multicellular and assimilate nutrients by means of extracellular digestion. The *Saprolegniaceae*, in particular members of the genus *Saprolegnia*, are responsible for significant infections, involving both living and dead fish and eggs, particularly in aquaculture facilities. Oomycetes are classical saprophytic opportunists, multiplying on fish that are physically injured, stressed or infected [17]. Members of this group are generally considered agents of secondary infection arising from conditions such as bacterial infections, immunosuppression, poor husbandry, and infestation by parasites and social interaction. However, there are several reports of Oomycetes as primary infectious agents of fish [1, 20, 19] and their eggs [12, 19, 2]. These can readily become pathogens, resulting in epizootics among Salmonids and other teleosts [9, 1, 2, 5]. *Saprolegnia* may occur anywhere on the body of fish, but normally appears as a conspicuous, circular or crescent-shaped, white, cotton-like mycelium, particularly around the head and the caudal, adipose and anal fins [14, 21, 15, 10, 11]. It may spread over the body by radial extension until adjacent lesions merge. The present paper reports the isolation, purification and characterization fungal pathogens associated with diseased fishes of Kolleru Lake, Andhra Pradesh

2. Materials and Methods
For the purpose of this investigation, fungal infected fishes were collected from Upputeru creek, (Its centre lies at a Latitude: 16.348335, Longitude: 81.545723) near Akividu Mandal, West Godavari, Andhra Pradesh. This study was carried out from October, 2013 to March, 2014. The infected fishes were brought to the laboratory of PG Department of Biotechnology, DNR College, Bhimavaram.

By observing external symptoms like discoloration of skin, appearance of whitish brown patchy or extensive cotton wool cover of the fungal mycelia, hemorrhagic lesions on body and excess mucus secretion. Fungal tufts covered the skin, fins, gills, eyes of the fish and complete fish eggs. Infected fishes were found by naked eyes and by using hand lens.

2.1 Isolation of fungi from infected fishes

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 different agar media like PDA, GY agar and SPS agar. To inhibit bacterial growth, 500 mg/ml each of penicillin and streptomycin was added to the medium. The isolates were incubated at 18^0C – 22^0C for 3-4 days until fungal mat developed. Isolates were then used to make pure cultures

2.2 Purification of cultures

Pure cultures were prepared by taking small piece of media with fungal growth and transferred to the plates containing baits like hempseeds, sesame seeds, mustard seeds and broken pulses with sterile tap water. These cultures were incubated at 18^0C-22^0C for growth of colony on bait and development of sexual characters for identification. Isolated fungi were identified by morphological and sexual characters described in keys and monographs of Coker [4], Khulbe [12], Willoughby [21].

2.3 Experimental infection trails

To determine the pathogenicity of isolated species of fungi pure cultures were prepared on CMA and SDA and maintained at required temperature. Zoospores concentration was prepared as wet cultures by using baits, Glycine seeds for Saprolegnia and Sorghum seeds for Aphanomyces. Conidial suspensions were prepared on media. Concentrations were prepared by using haemocytometer.

Healthy fishes with average length of 10.5±2cm and average weight of 18±4gmwere collected and kept in aquaria of 10Lcapacity under observation for three days with continuous aeration and fed with artificially. For this purpose fishes were injected intramuscularly with 0.1 ml of concentration of Zoospores with $2x10^4$/ml of each species of fungi. Temperature was maintained as 18±2°C and 28±2°C for zoosporic and conidial fungi, respectively. Fishes were observed for one week and symptoms were recorded. The percentage of fish infection was calculated using following formula:

Formula: No. of fishes infected ÷ No. of fishes examined x100

3. Results & Discussion

In the present work, a total of 1,400 fishes were examined out of which 287 were found to be infected with fungal disease. Eight species of fishes viz. *Catla catla, Labeo rohita, Channa punctatus, Channa striatus Clarias batrachus, Mastacembalus armatus, Mystus cavasius* and *M. seenghala*, were found infected.

During study period, forty one isolates were collected from infected fishes which belonged to 8 genera viz. Achlya, Allomyces, Aphanomyces, Aspergillus, Dictyuchus, Fusarium, Pythium and Saprolegnia and these eight genera contributed 14 species such as Achlya americana, Achlya prolifera, Achlya conspicua, Allomyces anomalus, Aspergillus niger, Aphanomyces laevis, Dyctiuchus achlyoides, Fusarium sp., Pythium aphanidermatum, Pythium undulatum, Saprolegnia ferax, Saprolegnia hypogyana, Saprolegnia parasitica and S.diclina (Table.1).

Table 1: Isolation of fungi species from infected fishes

S. No	Host fish	Oct, 13	Nov	Dec	Jan, 14	Feb	Mar
1	*Clariuas batrachus*	S.pa, S.di	Ach.am	S.p, S.fe	Ach.pr	Fu.sp	Asp.ni
2	*Channa striatus*	S.pa,Aph.lev	S.hy	S.pa, Aph.lev	S.pa, Ach.co	Asp.ni	S.di
3	*Channa punctatus*	Aph.lev	S.pa	Aph.lev	S.p	Aph.lev	Ach.pr
4	*Catla catla*	S.pa	-	Ach.pr	Aph.lev	-	-
5	*Labeo rohita*	S.pa	Py.un	S.pa	Ach. am	S.pa	-
6	*Mastacembalus armatus*	S.p	S.di	S.pa	All.an	-	S.p
7	*Mystus cavasius*	S.hy	Aph.lev	-	-	-	Aph.lev
8	*Mystus seenghala*	S.pa	S.pa	Dy.ach	S. di	Asp.ni	-

Association of saprolegnia and Aphanomyces with fish disease has been reported by various workers [8, 21, 3]. A. niger and F. solani from infected fishes and eggs of West Iran and found these species responsible for internal and external infections of fishes [11, 3]. In the present study, Achlya and Saprolegnia were found most of the time during study period and showed maximum virulence. Achlya was the leading pathogen which contributed twelve isolates and broadest spectrum of species. Maximum species of fungi were isolated from C. batrachus [3]. In this study, maximum infected fishes were observed during the month of December, 2013 (23.2%) and minimum in October, 2013 (9.2%) (Fig.2).

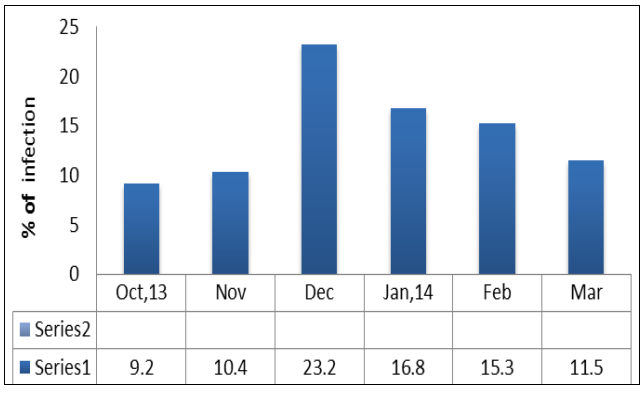

Fig 1: Monthly percentage of fungal infection in fish

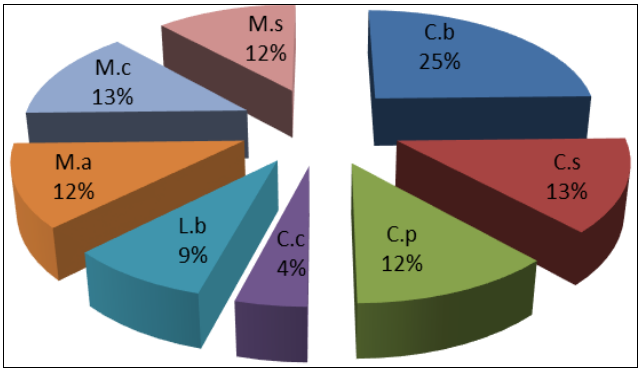

Fig 2: Fungal infection in various species of fishes

(C.b: Clariuas batrachus, C.s: Channa striatus, C.p:Channa punctatus, C.c: Catla catla, L.b: Labeo rohita, M.a: Mastacembelus armatus, M.c :Mystus cavasius, M.s: Mystus seenghala).

In case fishes, maximum infection was observed in Clarias batrachus (25%) while minimum was observed in Catla catla (4%). Severities of fungal infections in fishes during cooler months have also been reported by Mastan *et al.*, [13, 18].It was also observed that fishes suffer from two types of fungal diseases Saprolegniasis and Aphanomycosis. Hatai and Hoshiai [8] and Hussain *et al.*, [11] also reported pathogenecity of same species 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 upto muscles. Similar symptoms have been reported by Chauhan *et al.*, [3]. Present observation is in agreement with the findings of workers. It was observed that fish of all ages got infected with fungal disease irrespective of their age and size [18. 3, 13]. In the present study maximum infection was found in catfishes which get the support from the findings of Chauhan *et al.*, [3] and Khulbe, [12]. The relation of some physicochemical parameters of lake with disease incidences is also depicted. Maximum incidences were reported when the temperature was low and the retardedness was observed in the diseased fishes as the temperature increased. Low values of pH and high amount of DO also favours fungal growth. These findings were confirmed by the reports of Chauhan [3].

4. Conclusion

The incidences of fungal diseases varied with fungal species and the season of the year. The highest prevalence of infection was observed in colder months from November to January and the lowest during February to July. Low temperature is conducive for pathogenic potentiality of the water molds, retardation of pathogenic potentiality of the water molds at higher temperature above 28ºC, which cannot infect and require a certain period for multiplication. In the present study, it was observed that Saprolegnia parasitica and Achlya sp. are highly pathogenic in nature and causes huge economic losses to fishes.

Table 2: Experimental infection trails with various species of fungi

S. No	Fishes used	Fungi injected	No. of fish used	Con. of Fungal spores/ml	Mortality in days					Total Mortality in %	Re-isolation Fungi
					2	4	6	8	10		
1	*Clariaus batrachus*	*S. parasitica*	8	$2x10^3$	1	2	4	1		100	+
2	*C. batrachus*	*S. diclina*	8	$2x10^3$	-	1	2	2	-	62.5	+
3	*C. batrachus*	*A. americana*	8	$2x10^4$	1	-	3	2	-	75	+
4	*C. batrachus*	*S. ferax*	8	$2x10^3$	-	3	2	2		90	+
5	*C. batrachus*	*A. prolifera*	8	$2x10^4$	1	1	3	-	-	62.5	+
6	*C. batrachus*	*Fusurium sp*	8	$2x10^4$	-	2	1	-	1	50	+
7	*C. batrachus*	*Aspergillus sp.*	8	$2x10^4$	-	3	1	-	-	50	+
8	*Channa straitus*	*S. parasitica*	8	$2x10^3$	1	2	3	2	-	100	+
9	*C. straitus*	*S. hypogyna*	8	$2x10^4$	-	1	1	2		50	+
10	*C. straitus*	*Aphanmyces laevis*	8	$2x10^3$	-	2	2	3	-	87.5	+
11	*C. straitus*	*Achlya conspicua*	8	$2x10^4$	2	2	3	-	-	87.5	+
12	*C. straitus*	*Aspergillus niger*	8	$2x10^4$	-	2	2	2	-	62.5	+
13	*C. straitus*	*S. diclina*	8	$2x10^4$	1	2	-	3	-	62.5	+
14	*C. punctatus*	*Aphanomyces laevis*	8	$2x10^4$	1	-	3	1	-	62.5	+
15	*C. punctatus*	*S. parasitica*	8	$2x10^3$	2	3	3	-	-	100	+
16	*C. punctatus*	*A. prolifera*	8	$2x10^3$	1	-	3	-	1	62.5	+
17	*Cat catla*	*S. parasitica*	8	$2x10^3$	3	3	2	-	-	100	+
18	*Catla catla*	*S. diclina*	8	$2x10^3$	1	-	2	3	1	87.5	+
19	*Catla catla*	*Aphanomyces laevis*	8	$2x10^3$	2	2	3	-	-	87.5	+
20	*Labeo rohita*	*S. parasitica*	8	$2x10^3$	1	2	4	-	-	87.5	+
21	*Labeo rohita*	*Pythium undulatam*	8	$2x10^3$	-	2	2	2	-	62.5	+
22	*Labeo rohita*	*Achlya americana*	8	$2x10^4$	2	2	3	1	-	100	+
23	*Mastacembalus armatus*	*S. parasitica*	8	$2x10^3$	2	3	3	-	-	100	+
24	*M. armatus*	*S. diclina*	8	$2x10^3$	-	2	2	2	-	62.5	+
25	*M. armatus*	*Allomyces anomalus*	8	$2x10^5$	-	-	2	2	-	50	+
26	*Mystus cavasius*	*S. hypogyna*	8	$2x10^3$	1	2	2	-	-	62.5	+
27	*M. cavasius*	*Aphanmyces laevis*	8	$2x10^4$	1	3	2	-	-	75	+
28	*M. seenghala*	*S. parasitica*	8	$2x10^3$	2	3	3	-	-	100	+
29	*M. seenghala*	*S. diclina*	8	$2x10^3$	-	2	2	2	-	75	+
30	*M. seenghal*	*Dyctiuchus achlyoides*	8	$2x10^5$	2	3	1	-	-	75	+
31	*M. seenghala*	*Aspergillus niger*	8	$2x10^5$	-	2	2	1	-	62.5	+

5. Conflicts of interest

Author has none to declare.

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