



RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF FUNGI FROM INFECTED FRESHWATER FISH *CATLA CATLA*

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ABSTRACT

Wheat The present observation infected fresh waterfish *Catla catla* were collected from Chidambaram area, Tamil Nadu and fungal species were isolated. Totally five different fungi were isolated from the infected carp gill, intestine and muscles samples, namely species of *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus flavus*, *Cunninghamella* sp., and *Penicillium* sp. These fungal strains were caused by diseases in the fishes.

Key words:

Catla catla,
Freshwater fish,
Fungi.

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INTRODUCTION

The fungal pathogens first attack the body surface of the carps and progressively grows deeper into the tissues. Histo-pathological studies indicate the growth of mycelia over epidermis, destroying the body muscles and gradually passing through dermis causing necrosis of muscle layers. The fungal pathogens are caused severe problems in carps and complete loss of fish production. The fungi are two types namely saprophytes and parasites. Saprophytes depend on dead on dead organic matter whereas parasites live on or in the living bodies of other organisms. In fungi reproduction is of three kinds vegetative, bisexual and sexual.

However, of late the species has been observed to be fungal pathogens that have resulted in the recurrence of India from Andhra Pradesh, Tamil Nadu and Kerala by devastating the carp culture industry in India and inflicting losses amounting to approximately US\$ 10 million. Fungi are known to attack fish eggs, fry, fingerlings and adult fish. Water molds infections cause losses of freshwater fishes and their eggs in both natural and commercial fish farms (Bangyeekhun and Sylvie, 2001). The fungal diseases occur in brood stock and all life stages of fish and eggs. Fungal infection cause low productivity of fry and low production in fish culture (Kwanprasert *et al.*, 2007). The mortality rate due to fungal infection may reach some time up to 80-100% in incubated eggs (Chukanhom and Hatai, 2004). According to post-harvest handling of fishes may also result in

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infection with microorganisms such as bacteria and fungi (Akande and Tobor, 1992). Interaction of physiochemical factors generally has influence on the diversity of water molds (Paliwal and Sati, 2009). After that numerous water mould species parasiting in different species of fishes and their eggs have been described by different researchers. The fungal pathogens first attacks the body surface of the fishes and progressively grow deeper into the tissues have been reported by Phyllis (2007). However, Refai *et al.* (2010) has characterized *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp., as normal mycoflora and these spp. may be regarded as opportunistic pathogens (Refai *et al.*, 2004) as many of them possess virulence factors which enable them to cause disease (Refai *et al.*, 2010), especially under favorable predisposing conditions. Ecological differences play an important role in species diversity of fungi that develop on both fish and eggs (Hussein *et al.*, 2001). Rekha Chauhan *et al.*, (2012) have been reported the seven species of fungi from the lesions and affected muscles of infected fishes Indian Major Carps viz. *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita*. Rajakumar *et al.*, (2011) reported that the fungal pathogens like *Aspergillus flavus*, *Aspergillus tamari*, *Aspergillus Nidulans*, *Aspergillus fumigatus*, *Mucor* and *Penicillium* were isolated from fresh water carp culture pond. In recent years, the carp culture industry has been facing serious problems due to microbial diseases in the coastal belts of Tamil Nadu, India. In view of this, the present investigation has been carried out to study the outbreak of fungal diseases in the carp *Catla catla* cultured in semi-intensive ponds in the Chidambaram area of Cuddalore District.

MATERIALS AND METHODS

Collection of Fish samples: Infected carp *Catla catla* were collected from carp culture pond at Chidambaram area Cuddalore district, Tamil Nadu.

Isolation of fungi: From serial dilution fungal species were isolated and 0.1 ml of the sample taken from 10^{-2} , 10^{-3} , and 10^{-4} tube and spread aseptically over on the Rose Bengal agar medium. Over plate maintain as a control without sample. All the Rose Bengal agar plates were incubated

37°C for 72 h. After incubation the plates were observed the fungal growth and the different fungal pathogen were isolated (Gillman, 1957).

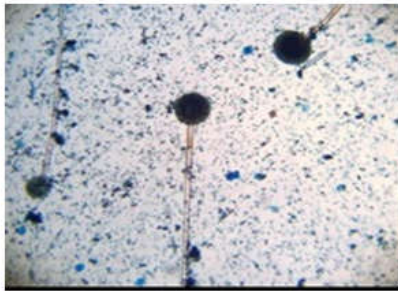
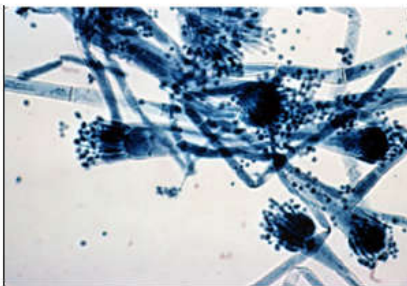
Identification of fungi: The pure culture of the pathogens was maintained in rose Bengal agar medium by streaking method and stored further use. The organism was observed under microscope by using lacto phenol cotton blue staining technique (Gillman, 1957). Lacto phenol cotton blue is a stain commonly used for making semipermanent microscopic preparations of fungi. It stains the fungal cytoplasm and provides a light blue background, against which the walls of hyphae can readily be seen. It contains four constituents phenol, which serves a fungicide lactic acid, which acts as a cleaning agent of cotton blue, which stains the cytoplasm of the fungus and glycerine, which gives a semipermanent preparation. Photographs were taken by using Nikon microscope (Nikon, Japan).

RESULTS

Isolation and identification of fungi: The fungal strains were isolated from infected freshwater carp *Catla catla* Gill region 4 fungal species, muscles region 2 fungal species and Intestinal region 2 fungal species. Totally 5 different fungal strains were observed from infected carp on the culture characteristics (Table 1 and Fig. 1). The fungal strains belonging to a genera were isolated, among them *Aspergillus* sp., was the dominant genus (3 species). All the fungal strains were identified by Lactophenol cotton blue method. The staining results of each fungus were compared with standard fungal identification manual. Totally five pathogenic fungi were isolated by dilution plating method technique, the isolated fungi is harmful in *Catla catla*. The fungal species were isolated such as *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus flavus*, *Cunninghamella* sp., and *Penicillium* sp. Among them *Aspergillus* was the dominant genus (3 species). All the isolated fungi were identified by Lactophenol cotton blue staining. The staining results of each fungus were compared with standard fungal identification manual, the maximum number of fungi was belonged to Deutromycetes of them a great majority was contributed by species of *Aspergillus*.

Table 1. Isolation of fungi from infected carp *Catla catla*

S.No.	Name of the bacteria	Gills	Muscles	Intestine
1.	<i>Aspergillus niger</i>	+	-	-
2.	<i>Aspergillus nidulans</i> ,	+	-	+
3.	<i>Aspergillus flavus</i>	-	+	-
4.	<i>Cunninghamella</i> sp.,	+	-	-
5.	<i>Penicillium</i> sp.,	+	+	+

*Aspergillus niger**Aspergillus nidulans**Aspergillus flavus**Cunninghamella Sp.,**Penicillium Sp.,***Figure 3. Isolated fungi from infected carp *Catla catla***

DISCUSSION

In the present study bacterial species were isolated from the infected carp gills, intestine and muscles. Totally 5 fungal species were isolated by diluting plating technique. The infected carp *Catla catla* were collected from Chidambaram area of Cuddalore District. Dominance of *Aspergillus* a universal features of various fresh water habitats studies of also confirmed. The isolated fungi were identified and characterized according to their asexual characteristics described by Khulbe (1993). The clinical signs found with the infected fishes were similar to the signs of fungal infected fishes described by Hatai and Wada (1994). The clinical signs due to *Saprolegnia* and *Achlya* infection were characterized here by the presence of a cotton wool like growth and small white patches on the heads and fins of the affected fish. These signs were similar to the signs described by Stuart and Fuller (1968) and Srivastava (1980b).

According to Siddique *et al.*, (2009) were examined 44 fungal infected fresh water carp *Cirrhinus mrigala* specimens from October to December and January to February where symptoms were recorded as ulcer, EUS and gill rot. Jalilpoor *et al.*, (2006) was observed the fungal genera like, *Penicillium* spp., *Fusarium* spp., *Mucor* spp. and *Saprolegnia* sp. were isolated from *Aspenser percicus* eggs, where they caused 22% mortality of these eggs. The common fungi invading smoke dried fish species were isolation and pathogenicity of cultured fish was reported by the earlier workers (Doe, 1983; Fafioye *et al.*, 2002). The infected fishes showed fungal growth on gills, skin and fins. Infected fishes had eroded scales, fins and hemorrhages over body surface and tips of caudal fin were eroded. Fin infection is considered less pathogenic as such fishes survive but this infection may lead to complete damage of the fins. The single most affected site was gills. The infection on sensitive areas like gills and eyes of fish may lead to serious disease conditions, as the growth of fungal hyphae in eyes may cause partial or complete blindness and in such condition the treatment or cure is impossible and ultimately the fishes die were recorded (Srivastava, 2009).

Conclusion

The fungi are perhaps the most important pathogens in fish culture ponds causing severe mortalities and financial losses. In the present investigation, the fungal species showed harmful effect on the culture organisms which needs to be addressed and create awareness among fish eating people.

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