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RESEARCH ARTICLE

IDENTIFICATION OF *SAPROLEGNIA* IN SELECTED INDIAN MAJOR CARPS CULTURED IN AN EUTROPHIC POND

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ABSTRACT

Aquaculture plays a vital role in many countries by offering better nutrition, higher income, foreign exchange and better employment opportunities. Aquatic ecosystems are affected by several health stressors that significantly deplete biodiversity. Fungal infection is an important economic and limiting factor in intensive fish production. Microbial quality of farmed fish is largely determined by the quality of water in which they are cultivated. Aquatic fungal diseases are more acute in cold water than in warm water culture and may be aggravated by the unfavourable conditions, i.e., over-crowding, malnutrition and unstable temperature. Therefore, a study was attempted to estimate the physio-chemical parameters of pond water and pathogenic fungi in different tissues of the cultured freshwater carps.

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INTRODUCTION

Water is a renewable resource; reckless usage and improper management of water systems may cause serious problems in availability. The quality of water is usually determined by its physiochemical characteristics. It is a well established fact that domestic sewage and industrial effluent discharged into natural water result in deterioration of water quality and cultural eutropication (Shaw *et al*., 1991). The other important sources of water pollution include mass bathing, disposal of dead bodies, rural and urban waste matters, agricultural run-off and solid waste disposal (Tiwana, 1992). From a fish pathologist's view, there are mycoses that hinder the function of organs and kill the fish on mass scale and there are mycoses depriving fish body of its natural strength. Moulds, which cause mycoses, are microscopic organisms producing filamentous coating on various substrates. It is still widely believed that mould infestation of fishes is largely a secondary phenomenon. Therefore, mycological examination ought to become an integral part of monitoring the health of the fish. Indeed, every freshwater fish is exposed to at least one species of fungus during its life time (Neish and Hughes, 1980; Noga, 1993, 1996), starting from the embryonic stage through adulthood (Bruno and Wood, 1994). Yet, studies or surveys on pathogenic or parasitic fungi from Indian waters are very scanty (Ramaiah, 2006) and hence the present study was attempted to identify *Saprolegnia* in selected freshwater fishes of a pond in Tamil Nadu, India.

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MATERIALS AND METHODS

Fish Maintenance and Water Quality Management

Four to five month old fingerings of Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinnus mrigala*) (mean body weight: 6.1 g and mean body length: 7.2 cm) produced in local hatchery were stocked in ponds at the rate of 10 sq m^{-1}. They were regularly fed with supplemented feeds (rice bran and mustard oil cake + coconut oil cake) at 5% body weight. The important water quality parameters were analysed weekly using standard methods (APHA, 2005). Ten live infected and non-infected fish each were randomly collected and maintained in aseptic conditions from each pond after 2, 6, 10 and 20 weeks from the beginning of infection.

The fishes were sacrificed and body weight recorded. The patch that appeared on the body surface of fishes were removed by a sterile inoculating loop and incubated in Sabouraud dextrose agar (HiMedia, Mumbai, India) plates (pH 5.6) and stored at 22 - 30°C for 5-10 days. The plates were observed everyday for growth. For identification of fungus, the cultures were subjected to lactophenol cotton blue (LCB) stain (HiMedia, Mumbai, India) following procedures of Chakraborti (2003), Thomas *et al.* (1991) and Pelczar *et al.* (2008). A drop of LCB stain was placed on the centre of slide and using a sterilized mounting needle, a small portion of culture was mixed gently with the LCB stain. A cover slip was placed gently to avoid air bubble and examined under a light microscope at magnifications between 10 - 100X and photos captured.

RESULTS AND DISCUSSION

The physio-chemical condition of the aquatic system during the period of study – summer (March-May) and rainy season (September-November) are recorded in Table 1. Results of the present study indicate that *Saprolegnia* does cause significant fungal infection in all the three species of carps.

The first sign of infection appears to be the presence of reddish to greyey patches and after the onset of infection, mortality appeard after 35-43 days (summer) and 21-26 days (winter) of infection. In general, they appeared to invade tissues starting either from the head or tail fin region and then spread over the entire surface of the body.

Table 1. Physico-chemical conditions of the eutrophic pond

Parameters	Unit	Summer Season (March-May)	Rainy/Winter Season (March-May)
Water Temperature	°C	30	28
pH		7.8	7.4
Alkalinity	mg/l	120	128
Phosphate	mg/l	0.004	0.005
Nitrate-N	mg/l	0.33	0.38
Ammonia-N	mg/l	0.04	0.03
Calcium	mg/l	48	50
Magnesium	mg/l	10	12
Chloride	mg/l	20	22

Table 2. Seasonal collection of infected fishes

	Summer season		Winter season	
	No. of fishes	Infected fish	No. of fishes	Infected fish
Cirrhinus mrigala	50	18	50	31
Labeo rohita	50	10	50	19
Catla catla	50	15	50	24

Table 3. Cirrhinus mrigala mortality and recovery from disease condition recorded between 0-6 weeks of treatment

Treatments	Number of fish treated (n)	Mortality (n; %)	Disease recovery(n; %)
Control	40	2 (5)*	NA
A	40	16 (40)**	3 (19)*
B	40	21 (22.5)**	2 (10)*
C	40	14 (12.5)**	3 (21)**

NA:Not applicable, means without common asterisks along common significantly differences ($P < 0.05$)
values presented are mean □ SD
A:5 g NaCl / l for 3 minutes
B :5 ppm $KMnO_4$ / l for 3 minutes
C:5 g NaCl / l for 3 minutes followed by 5 ppm $KMnO_4$ for 3 minutes

Disease treatments

The fishes at the initial stage of infection were caught alive and grouped into three (n = 40). The fishes in each group were subjected to different treatments (A: dip treatment with 4 g salt per litre of water for 2 min.; B: Dip treatment with 5 ppm $KMnO_4$/lit for 3 min.; C: dip treatment with 5 g salt l^{-1} of water for 3 min followed by 5 ppm $KMnO_4$/lit. for 3 min). The treatments were given thrice every week in a plastic tub (capacity: 50 l) upto 6 weeks from the beginning. The observations were recorded everyday for recovery for improvement in condition, growth attainment and mortality, if any, in each treatment. The data obtained were then statistically analysed.

This observation is similar to the findings of other workers (Zaki *et al.*, 2008; Willoughby and Roberts, 1992). However, Das *et al*. (2012) reported that red patches first appear in the mid part of the body and then spreads to other parts. This suggests that the site of infection and the patterns that arise from infection can vary between farm raised fishes as suggested by Beakes (1982), Pickering and Willoughby (1982). In the present study, among the three carps, *Catla catla* appeared to be the most common fish that recorded the highest rate of infection followed by *C. mrigala* while *Labco rohita* recorded the least. According to Neish (1997), the physiological state of the fish and environmental conditions determine the successful establishment of fungal infection.

Table 4. Labeo rohita mortality and recovery from disease condition recorded between 0-6 weeks of treatment

Treatments	Number of fish treated (n)	Mortality (n; %)	Disease recovery (n; %)
Control	40	3 (7.5)*	1 (12)*
A	40	18 (45)*	7 (11)*
B	40	10 (25)**	14 (10)*
C	40	8 (20)	16 (25)**

NA:Not applicable, means without common asterisks along common significantly differences
(P < 0.05) values presented are mean □ SD
A:5 g NaCl / l for 3 minutes
B :5 ppm $KMnO_4$ / l for 3 minutes
C:5 g NaCl / l for 3 minutes followed by 5 ppm $KMnO_4$ for 3 minutes

Table 5. *Catla catla* mortality and recovery from disease condition recorded between 0-6 weeks of treatment

Treatments	Number of fish treated (n)	Mortality (n; %)	Disease recovery (n; %)
Control	40	6 (15)*	Nil
A	40	19 (48)*	1 (5.2)*
B	40	15 (37)**	2 (13)*
C	40	11 (28)*	4 (36)**

NA:Not applicable, means without common asterisks along common significantly differences
(P < 0.05) values presented are mean □ SD
A:5 g NaCl / l for 3 minutes
B :5 ppm $KMnO_4$ / l for 3 minutes
C:5 g NaCl / l for 3 minutes followed by 5 ppm $KMnO_4$ for 3 minutes

Bruno and Wood (1994) reported that sudden changes in temperature can make fish vulnerable to saprolegniasis due to the increased physiological stress. This appears to be true in the present study as the rate of infection was less during the summer season when compared to the rainy season as temperatures were on the favourable side for carp culture (26-33° C) as suggested by Das *et al.,* 2004). According to Willoughby and Roberts (1992) and Aly and El-Ashram (2000) *Saprolegnia* has a wide range of tolerance (3-33°C) but they will attack fishes only when they became stressed or have weak immune system (Bruno and Wood, 1994; Pickering, 1994).

This is probably the reason for heavy mortality rates during the winter season than the summer season as the swimming, feeding, oxygen consumption and thermal regulation rates are favourable during the summer season. Generally, fungal infections are difficult to treat especially during acute conditions eventhough few chemicals are in use for aquaculture (Fitzpatrick *et al*., 1995; Meyer, 1991). Some of them are malachite green (Willoughby and Roberts, 1992), formaline (Mitchell and Collins, 1997) and hydrogen peroxide (Marking *et al*., 1994). However, there are concerns about their potential mutagenic/teratogenic properties. Das *et al*. (2012) used a combination treatment of NaCl and $KMnO_4$ which is also experimented in the present study but with different concentrations (Tables 2-4). Among the various treatments, the combination treatment of NaCl and $KMnO_4$ appeared to be the best as mortality rates were lowest. As far as disease recovery was concerned, here also, the combination treatment recorded the highest recovery rates with values ranging from 21% (*C. mrigala*) to 36% (*C. catla*). However, eventhough the results are not promising, this method can be used as an emergency measure to kill the growth of Saprolegniasis temporarily.

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