

Original Research Article

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## Isolation, Detection of Saprolegniasis: A Fungal Disease and its Management at Meghalaya

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### ABSTRACT

#### Keywords

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The occurrence of saprolegniasis in freshwater carp nursery at Ri-bhoi district, Meghalaya was investigated. The farmers encountered a severe outbreak of saprolegniasis in *Labeo gonius* fingerlings in the month of August after heavy rainfall which drops the temperature suddenly. The fishes were collected, examined, isolated and culture in selective media. The gross observation shows cotton like growth of fungus on abdomen and tails of the fish. After examination of moribund fishes, the farmers are recommended for treatment with 10 ppm common salt and 1 ppm potassium permanganate once daily for three consecutive days for three weeks. The treatment was eco-friendly management with locally easily available and non hazardous to animal and as well as environment. After the treatment the fishes recovered from infection of saprolegniasis.

### Introduction

*Saprolegnia* are ubiquitous in freshwater ecosystems and affects wild and cultured fishes, including living and dead eggs (Hussein *et al.*, 2001). The major fungal diseases which infect fishes belongs to the order Saprolegniales and falls within the genera *Saprolegnia*, *Aphanomyces* and *Achlya*. Saprolegniasis often contributes a heavy mortality of fishes. It invades epidermal tissues, generally beginning on the head or fins and can spread over the entire surface of the body. Visible signs are white or grey patches of filamentous mycelium. The spores most easily penetrate into the fish body

when surface of the skin or gills is damaged mechanically and/or by parasitic or bacterial infections and when the fish is weak. The Oomycetes are parasitic to a large diversity of host species (Beakes *et al.*, 2012). *Saprolegnia* generally considered as agent of secondary infection arising from conditions as bacterial infections, poor husbandry including poor water quality, adverse water temperature, poor nutrient, overpopulation etc (Bailey, 1984).

The seasonal variation play an important role in spreading of the *Saprolegnia* infections in freshwater fish especially during late autumn, common in winter months and early spring

where the temperature is low (Hughes, 1962). For the treatment of fungal disease, many works has been devoted to the examination of various types of fish fungicides and their effects either on aquatic animals or on the environment. Malachite green and formalin are the most potent fish fungicides although they have an acute impact on the aquatic ecosystems (Schreier *et al.*, 1996). Other problems with these substances include an immune suppressive effect on repeatedly treated fish (Prost and Spinska, 1989). Fungicides reported as terratogenic and mutagenic effect due to hazardous residues in fish tissues (Bruono and Wood, 1999). Formalin, solution 37% formaldehyde is effective in treating *Saprolegnia* but has effect on both environments personal who handle it (Fitzpatrick *et al.*, 1995). For these reasons many researchers have been investigating the use of safer compounds that have no harmful effect on fish and their eggs or on ecosystem (Willoughby and Roberts, 1992).

In the present study, farmers encountered a severe outbreak of Saprolegniasis in *Labeo gonius* fingerlings in the month of August under mid-hill altitude condition. The disease outbreak was occurred in an earthen nursery pond (400 m<sup>2</sup> area) where *Labeo gonius* spawn was stocked for rearing up to fingerling stage. The average size of the infected fingerlings was 4-6 cm TL.

## **Materials and Methods**

### **Sample collection**

Moribund fish were collected from the pond by examining individually for the presence of external lesions and brought to the laboratory. One fish with visible lesions was sampled for histopathology. Fish with lesion was killed and two pieces of muscle of size 5 mm<sup>3</sup> each taken from the lesion and surrounding muscle

for histology study. Sample was fixed immediately in 10% NBF

### **Clinical examination**

The main clinical signs appeared on the infected fish were lethargy, loss of equilibrium, unable to feed, hemorrhage at the base of the fins, in some fishes, the hemorrhages extended to cover all the body surface, as seen in fig [1]. The main characteristic lesions of saprolegniasis was appearance of cotton wool like tufts on the tail (caudal) of fish as seen in fig [2 & 3].

### **Post-mortem examination**

The main postmortem lesions were appearance of cotton wool tufts on caudal fin (tail), pale to grayish gills, serious fluid or exudates in the abdominal cavity, intestine free from any food particles, dark enlarged liver, distended gall bladder with bile, splenomegaly.

### **Mycological examination**

The wet preparation of skin lesions showed masses of mature and immature sporangia filled with large number of sporangiospores, the hyphae appeared profusely branched and were non septate, these morphological findings were characteristic of the saprolegnia species as seen in fig [4]. Slides were prepared from each colony and stained with lacto phenol cotton blue. The slide was observed under microscope and photographed.

### **Isolation of Fungi**

For the isolation of fungi associated with fish, swab of skin was cultured in Potato Dextrose Agar (PDA). To inhibit the bacterial growth, 40ug/ml of Ampicillin was added to the medium. The plates were incubated at room

temperature (24-28°C) and fungal growth was observed after 3-7 days (Fig 5). For purification, grown fungi were transferred to fresh medium.

### **Treatment and control**

Fishes were given permanent bath with 10 ppm common salt and 1 ppm potassium permanganate once daily for three consecutive days and then once in a week for three weeks. The fishes responded to the treatment rapidly and showed significant improvement after one week. The most

effective method for controlling and preventing saprolegniosis in fish ecosystem is a combination of good fish management as good water quality and avoidance of adverse water temperature.

Intensified aquaculture with high stocking density and feeding rate often causes disease outbreak in fish industry causes huge losses of farmers. *Saprolegnia* infection occurs mainly nursery phase with adverse environment event, mainly due to low temperature, which plays a critical role in developing saprolegniasis.

**Fig.1 Hemorrhage over the abdomen**



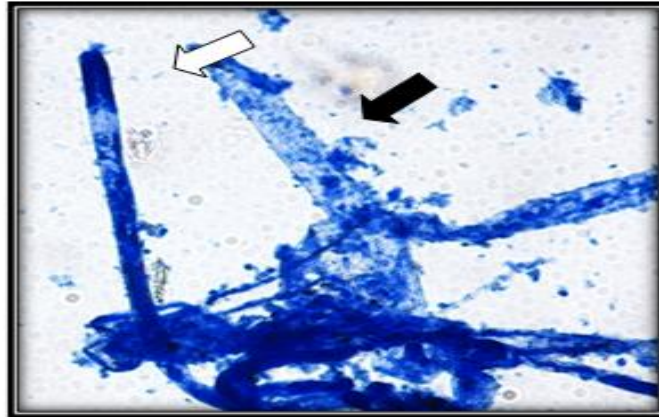
**Fig.2 Cotton wool like tufts on the tail**



**Fig.3 Cotton wool like tufts on the tail**



**Fig.4** Sporlagnia filled with sporangio spores (white arrow), non-septated hyphae (black arrow)



**Fig.5** Fungal growth on PDA (cotton wool like structure)



As fungicides are carcinogenic reported by different authors, therefore it is better to adopt semi-intensive culture system with medium stocking density, in mid-hill condition at Meghalaya where installation of water temperature regulating system is difficult for poor fish farmers. Treatment with common salt and potassium permanganate also give good response in treating saprolegniasis.

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