



Watermoulds Parasitizing Eggs and Neonates of Certain Fresh-water Scaly Fishes and Prawns in Water-bodies of Gorakhpur and Maharajganj, U.P., India

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ABSTRACT

Watermoulds are known to attack eggs and fries of fishes and as a general rule the infection starts when the host gets injured either mechanically or as a result of infections other than fungal. The present investigations have shown infection on eggs and fries of *Labeo rohita* L. and fries of *Macrobrachium lamarrei* Milne Edw. (prawn) by *Saprolegnia diclina* Harvey, *Achlya diffusa* Nees von Esenbeck and *Pythium oligandrum* Dreschler. The pathogenicity of the fungal isolates has been tested using healthy and disease-free eggs and fries of *Labeo rohita* and fries of *Macrobrachium lamarrei*. In the *in vitro* test experiments, the pathogenic nature of all the fungal isolates has been confirmed.

Key words: Watermoulds, Eggs and fries, *Labeo rohita*, *Macrobrachium lamarrei*, *Saprolegnia diclina*, *Achlya diffusa*, *Pythium oligandrum*, Pathogenicity tests

Only after the Indian Independence, fisheries together with agriculture have been recognized as an important sector. The vibrancy of the sector can be visualized by the 11-fold increase that India achieved in fish production in just six decades, i.e. from 0.75 million tonnes in 1950-51 to 9.6 million tonnes during 2012-13. This resulted in an unparalleled average annual growth rate of over 4.5 percent over the years which have placed the country on the forefront of global fish production, only after China. Besides meeting the domestic needs, the dependence of over 14.5 million people on fisheries activities for their livelihood and foreign exchange earnings to the tune of US\$ 3.51 billion (2012-13) from fish and fisheries products, amply justifies the importance of the sector on the country's economy and in livelihood security. India is also an important country that produces fish through aquaculture in the world. India is home to more than 10 percent of the global fish diversity.

The most important task is that, the production of healthy fish fries and fingerlings are procured. The incidence of diseases and infections in fishes, their eggs, fries and fingerlings is a very big challenge for fish farmers

and fish industries in the world. The occurrence and magnitude of infections are closely related to the sanitary conditions prevalent in the water and also the general health of the fishes themselves. The death of fishes caused by diseases are of highest significance in fish culture, hence to achieve healthy fish stock one should implement programmes like fish parasitological research, control of diseases and maintenance of health relationship between fishes and their environment (Eli *et al.* 2011).

The first significant work on fish mycoses was conducted by Tiffney (1939) who demonstrated the ability of *Saprolegnia parasitica* Coker to parasitize a wide range of fish and amphibians. Vishniac and Nigrelli (1957) demonstrated the ability of fourteen saprophytic species of Saprolegniaceae to infect Mexican Platyfish, *Xiphophorus maculatus*. Other noteworthy works are that of Scott and O'Bier (1962), Scott and O'Warren (1964), Sprague (1965, 1966), Wolke (1975). The latest researches in this field includes those of Czczuga and Muszynska (1997, 2001), Czczuga *et al.* (2002), Kiziewicz (2004), Al-Rekabi *et al.* (2005), Udomkunsri *et al.* (2007), Refai (2010), Eli *et al.* (2011), Hussian (2013). The latest Indian contribution in this field include the research contributions of Prabhuji (2010, 2011), Mastan *et al.* (2011), Prabhuji *et al.* (2012, 2016), Prabhuji and Srivastava (2013, 2014), Chauhan (2014), Pachade *et al.* (2014 a,b).

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Fungi are known to attack eggs, fry, fingerlings and adults of fishes and as a general rule the fungal infection starts when the host gets injured either mechanically or as a result of infections other than fungal. In India, the incidence of disease has been observed in major carps cultured in different parts of the country (Gopalakrishnan 1963, 1964). The fries and fingerlings, when transported over long distances, get bruised on the body and, unless properly disinfected, these become sites of fungal infection, resulting sometimes in large scale mortality. The present communication deals with the eggs and fries of certain fresh-water scaly fishes and prawns parasitized by watermoulds collected from the water-bodies in Gorakhpur and Maharajganj districts (India).

MATERIALS AND METHODS

Infections were observed on the eggs, fries and fingerlings of some of the common edible scaly fishes and prawns of eastern U.P. The infected specimens have been collected from the mentioned sites (Table 1) during November, 2013 to December 2014. The fish individuals were identified using the keys provided by Srivastava (2010). The infections were observed on the eggs and fries of *Labeo rohita* and *Macrobrachium lamarrei* (Prawn).

Table 1 Collection of infected eggs and fries specimens and the watermoulds isolated

Name of fish	Symptoms	Watermoulds isolated
<i>Labeo rohita</i> (G/M)	On Eggs	<i>Pythium oligandrum</i> <i>Achlya diffusa</i> <i>Saprolegnia diclina</i>
	On Fries	<i>Pythium oligandrum</i>
<i>Macrobrachium lamarrei</i> (G)	On Fries	<i>Achlya diffusa</i>

G: Ramgarh Lake, Gorakhpur

M: Private Fish Farm, Maharajganj

The fish eggs and fries samples, having cottony outgrowth on the surface, were brought safely in oxygenated poly-bags to the laboratory. The cottony outgrowths were aseptically removed from the fish eggs and fries and placed on 4-5 boiled hempseed cotyledons floated over 10-15 ml sterile distilled water in sterilized Corning Petri-dish and incubated at 22-25°C.

Purification and culture of watermoulds:

Unifungal, bacteria free cultures of the fungal isolates obtained from the fish eggs and fries were purified by the method suggested by Sinha (1985) from a mixed colony of mycelia growing on hemp seed cotyledons, a spore mass was drawn out with the help of capillary tube. This mass was, then, separated into individual units by means of narrow jet of sterile water from a wash bottle. Some of the individual spores were also removed from the water by means of platinum wire loop and were streak inoculated on the surface of semi-solid Potato Dextrose Agar. After 24-48 hours incubation at 20–25°C temperature, germinating spores / hyphae were cut off along with a bit of medium and transferred, inoculated surface downwards, on the second

plate of agar. With the appearance of definite mycelial colony around the inoculum, a second block of agar, about 1 cm² was cut from the edge of the colony and was placed inoculated surface downwards, on a third plate of agar. Repetition of this process resulted in bacteria-free isolates (Prabhujii et al. 2016). The growing hyphae was transferred to sterile hemp-seed cotyledons in sterile distilled water and incubated for 24-48 hours at 22-25°C temperature for its life cycle study.

Identification of the isolates

Identification of the parasitic watermoulds has been done with help of the keys provided by Prabhujii (1979), Khulbe (1983), Sinha (1985), Johnson (2002).

Pathogenicity test studies on fish eggs

In order to test the pathogenic nature of the isolates obtained from diseased fish eggs and fries-controlled infection test was conducted in the laboratory for each of the isolates on the lines described below:

Three glass troughs (24 cm in diameter and 12 cm in height) were sterilized in hot air oven at 175°C for three hours; sterilized pond water was poured in the troughs. Freshly laid healthy eggs were brought to the laboratory in 100 ppm solution of chloramphenicol (Scott and O'Bier 1962). Hundred healthy eggs were placed in each trough. In one of the troughs few infected eggs bearing fungal mycelia were introduced in the second trough hemp seed cotyledons having fungal mycelia were introduced. The third trough containing healthy eggs only in sterilized water was used as control. The experiments were repeated thrice for each of the isolates.

Disease control studies on fish eggs and fries

In order to perform disease control studies, the biocontrol method using *Trichoderma viride*, suggested by Srivastava et al. (2017) has been used and found to be highly effective.

RESULTS AND DISCUSSION

Several fungal infections of fish eggs and fries have also been collected. The infected eggs and fries were partially or completely covered with fungal hyphae. The transparency of the infected eggs was lost and they never hatched.

- Labeo rohita*: The infected eggs collected showed the presence of hyphae, later identified to be *Saprolegnia diclina*, *Achlya diffusa* and *Pythium oligandrum* (Fig 1A, 2A–C). The infected fries of the same fish were collected with hyphal tufts of *Pythium oligandrum* (Fig 1B).
- Macrobrachium lamarrei*: The infected fries collected showed the presence of hyphal tufts on the body surface and the ramified hyphal net inside the body (Fig 2A–C). Later, the fungus has been identified to be *Achlya diffusa*.

c) The controlled re-infection studies made on the eggs and fries of *Labeo rohita* and the fries of *Macrobrachium lamarrei* using pathogenic watermoulds, viz. *Saprolegnia diclina*, *Achlya diffusa* and *Pythium oligandrum* have shown positive results and their pathogenic nature has been confirmed (Table 2-3).

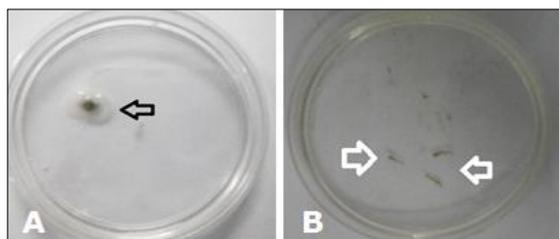


Fig 1A Infected egg of *Labeo rohita*.
Fig 1B Infected fish fries of *Labeo rohita*

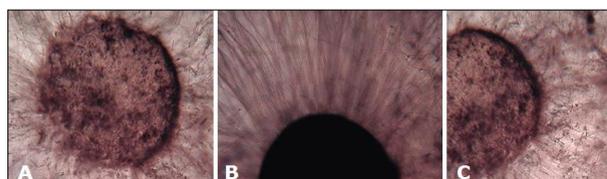


Fig 2A-C Infected eggs of *Labeo rohita*. X 400
A) Egg showing infection of *Saprolegnia diclina*
B) Egg showing infection of *Achlya diffusa*
C) Egg showing infection of *Pythium oligandrum*

Table 2 Controlled re-infection studies demonstrating parasitic ability of watermoulds on eggs/fries of *Labeo rohita*

Pathogen	Eggs / Fries	Mycosis evident (hours)
<i>Saprolegnia diclina</i>	Eggs	15 – 20
<i>Achlya diffusa</i>	Eggs	15 – 20
<i>Pythium oligandrum</i>	Eggs	12 – 15
<i>Pythium oligandrum</i>	Fries	15 - 24

In earlier reports of fungi associated with fish diseases, many workers have made little effort to differentiate between saprophytic invaders and parasitic watermould

species. Therefore, following the isolation and identification of the naturally occurring fungal pathogens, it was considered necessary to verify the pathogenicity of the isolates under controlled laboratory conditions. Moreover, except Sinha (1985), care has not been taken by various fish mycologists to test the pathogenic nature of the isolates on the same fish species or eggs or fries from which it was originally isolated. It is also important because of the fact that fishes of different species may differ in their metabolic activities, resistance and their response to foreign organisms. Therefore, the pathogenicity of all the isolates has been experimentally verified on the original host species of the fish eggs and fries. During disease control experiments using *Trichoderma viride*, 99–100% eggs remained disease-free and normally hatched whereas 100% fries, too, remained disease-free and developed into fingerlings.

Table 3 Controlled re-infection studies demonstrating parasitic ability of watermoulds on fries of *Macrobrachium lamarrei*

Pathogen	Eggs / Fries	Mycosis evident (hours)
<i>Achlya diffusa</i>	Fries	20 – 24

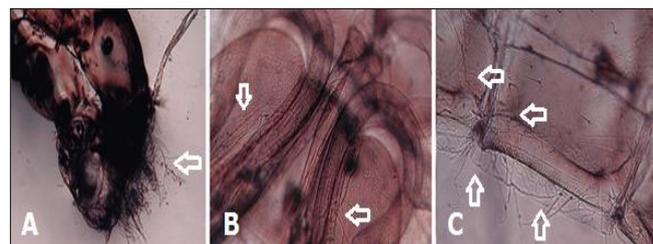


Fig 3A-C Infected fries of *Macrobrachium lamarrei* (Prawn).
Arrow shows the fungal hyphae of *Achlya diffusa* X 400

The pathogenic watermoulds responsible for severe infections in the eggs and fries of common edible scaly fish (*Labeo rohita*) and fresh water prawns (*Macrobrachium lamarrei*) cause heavy loss to pisciculture. During the present investigations the watermoulds have been isolated from the eggs and fries of *Labeo rohita* and *Macrobrachium lamarrei* and their pathogenicity has been confirmed. The disease control in eggs and fries, using *Trichoderma viride*, has been found to be highly effective.

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