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***In vitro* study on dermal lesion disease of *Puntius* spp. of Tarang River: A tributary of Champabati**

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Abstract

Indigenous fish varieties found in rivers and beels of Kokrajhar district provides livelihood support of the people of the region. Most of the local fishes are brought from different Beels, Rivers, Ponds and Agricultural crop land to the daily market. But due to some unplanned fishing activity and anthropogenic pressure on the aquatic ecosystem the major fish varieties are dwindling day by day and sometimes severely get infected with certain diseases. The present investigation revealed the potential cause of the dermal lesion of *Puntius sp.* of Tarang River which causes tremendous loss annually. Clinically, cotton wool type lesions on the fish body surface, ulceration and erosion of skin and muscles were observed. The agricultural runoff during monsoon and dumping of household's garbage are the some major source of the pollution of the Tarang River. Diseased *Puntius sp.* were tested in the laboratory with an aim to identify the possible cause of the disease and exploring the ecological status of the said river canal.

Keywords: *Puntius spp.*, *Tarang River*, *Dermal lesion*, *Actinomyces spp.*, *Penicillium spp.* and *Fungal infection*.

1. Introduction

Rivers plays important role as a major fresh water resource supporting wide range of flora and fauna of the world^[24]. In recent past, expanding human population, industrialization, intensive agricultural practices and discharge of large amount of waste water into the rivers have resulted in the deterioration of the water quality and environment which acts as a potential source of stress to biotic community^[2, 4]. The polluted water body always provides the favorable condition for the growth of various pathogenic bacteria that cause microbial infection on fish species that can result the spread of water borne diseases in human. Ahmed *et al.*^[3] found that seasonal variations in pH, temperature and dissolved oxygen play important roles in the multiplication of pathogens thus leading to diseases in fish. The disposal and release of waste can also affects the physicochemical properties and microbiological quality of the river ecosystem^[8]. Therefore, water quality assessment and effective water management is very important factor to determine why such contamination is present; how serious is it and what step could be taken to eliminate such type of incidence of fish disease^[6].

Tarang River is flowing from north to south (Fig. 1) covering 45- 50 kms; a tributary of River Champabati that supports more than 1000 farmers for their agriculture activities and various poor families who are associated with fishing activity (Fig. 2) depends on this River. The average temperature of the water body is 20- 25 °C and the pH ranges from 6.6- 7.1 which shows neutral condition of the water. The increase in pH in water body could be due to either increased of carbonates or increased photosynthetic activities of producers^[15]. The average depth of the water during winter is 0.68 meter and during summer the depth raises up to 2.5 meter when sluice gate is closed. The dumping of the household waste, domestic discharge into the River the continued surface runoff of agricultural area and construction of dam are the some major source of water pollution of this river. Presently the water body is slightly more or less in a good condition, still sometimes the major non culturable wild fishes such as *Puntius spp.*, *Mystus spp.*, *Channa punctatus*, *Clarius spp.*, *Heteropneustes fossilis* etc. severely gets infected with certain diseases.

In most cases, fungi and bacteria serve a valuable ecological function by processing dead organic debris. But they can become a problem if fish are stressed by poor nutrition, pressure of population and over exploitation of fishes^[21]. Fungal infections of freshwater fish are common and distributed worldwide and associated with immune suppression^[21]. Bacterial diseases of fish are also considered to be a major problem for both fisheries and aquaculture^[20]. Majority of water borne pathogenic microorganisms enter water courses as a result of faecal and waste water contamination^[1]. Emphasis has been given on the diseases incidence on *Puntius spp.* causing cotton wool like dermal lesion on the dorsal fin of the said fish varieties. The bacterial infections mostly occur on fishes that are physically injured, stressed or infected. Many bacteria are suspected to parasitize the fish and their eggs causing great losses of fish population. Therefore, a study has been undertaken to assess the water quality of the said River and *in vitro* investigation on causal organism along with histopathology to understand such disease appearance in epidemic form.

2. Materials and methods

Puntius sp. (Puthi) showing pathological lesions were collected from the Tarang River during winter for isolation of the pathogens. Materials were collected in a sterile container with liquid medium for primary culture of possible microbial presence. Later, culture and isolation of the pathogen was done by inoculating small pieces of muscle about 2mm in diameter from infected portion of the body in a PDA medium and incubated at $27 \pm 0.5^\circ \text{C}$ for 24- 48 hours. Growth of colony was observed in 2-3 days. For full growth of colony plates were kept for 5- 8 days for incubation and later used for subculture of the organism. Pure cultures were prepared by using the methods of Scott^[16].

For histological observation infected tissue portion were taken with the help of pointed forceps and properly stained sample was taken on slide and observed under LABOMED Lx 300 binocular microscope. Microphotograph of *Actinomyces sp.* and *Penicillium sp.* were taken in LABOMED Lx 400 photomicrographic digital camera. And the seasonal variation of disease incidence on *Puntius spp.* was calculated by the following formula:

Disease incidence (%) = (No. of fish infected/ Total no. of fishes examined) \times 100

3. Results

3.1 Identification

Preliminary identification was made on the basis of the colony characters such as colour, shape, structure. In addition to this, gram staining was done to identify the bacteria by following the standard protocol and observed under microscope. Identification was confirmed with the help of available literature and by comparing photograph. For identification of secondary fungal growth, slides were prepared from the colony by taking small tuft of mycelium and stained with lacto-phenol cotton blue and observed under microscope^[19]. Fish species were identified using the keys^[7, 22]. The common fish species found in different season in Tarang River are *Clarius batrachus*, *Channa punctatus*, *Xenentodon cancila*, *Chanda nama*, *Heteropneustes fossilis*, *Clarius batrachus*, *Glossogobius giuris*, *Mystus spp.*, *Nandus nandus*, *Puntius spp.*, *Rasbora elenga*, *Wallago attu*, *Rasbora daniconius*, *Anguilla bengalensis*, *Devario assamensis*, *Conger cinerius*, *Panaeus indicus* and

Amphipnous cuchia. However, during the study period it has been observed that only 9 varieties were found available in different ratio (Fig. 7). And conspicuously the disease incidence on *Puntius spp.* was observed to be higher in the month of February (Table: 1).

Table 1: Month wise distribution of disease incidence on *Puntius spp.* observed during the study period.

Month	No. of Fish sampled		Disease Incidence (%)
	Observed	Infected	
February	200	13	6.5 %
March	250	12	4.8 %
April	300	14	4.67 %
May	274	09	3.28 %
Total:	1024	48	Average: 4.68 %

3.2 Morphological observation of causal organism

The colonies of *Actinomyces sp.* which was isolated in PDA medium appeared golden yellow, round, convex, flattened, semitranslucent in nature loosely attached with the media and slimy shining surface with mycelium like appearance on the periphery which resembles a mass of branched mycelium of fungi that breaks up into poorly branching forms (Fig. 4). Later, the organism was observed to be filamentous and Gram positive when observed under microscope and was identified as *Actinomyces sp.* (Fig. 5).

4. Discussion

It is beyond doubt that the isolated pathogen is *Actinomyces sp.* associated with *Penicillium sp.* (Fig. 5 & Fig. 6) responsible for causing cotton wool like growth in skin and dorsal fin of the *Puntius spp.* The association growth of the *Actinomyces* and *Penicillium* suggest that natural control or ecological balance of the pathogenic microbial growth. The causal organism of dermal lesion disease in *Puntius spp.* (Fig. 3) is *Actinomyces sp.* (Fig. 5) which belongs to the class Bacteria and the order Actinomycetales. The actinomycetes multiply by means of germinating spores attached to sporophores and by means of fragmentation where they break up into hyphae^[5, 9]. The present study revealed the pathogenic nature of the Actinomycetes which cause dermal lesion disease in local fish varieties.

Even though clear record has not been found on *Actinomycetes* associated with fish diseases but the present study indicates the role of this organism to cause cotton wool like fin rot. The histopathological observation made by different workers showed that the infection can also cause ulcerative disease of fish; the organisms may play an important role not only in necrotizing the muscle tissue but also damaging the internal organs like kidney, liver and spleen besides the peritoneum.

Penicillium spp. was observed to be as secondary pathogen that growth after the 5- 6 days of incubation and it was confirmed by the secondary surface growth colony in a small portion. They act as opportunistic parasites which take advantage on stressed or weakened fish and already infected fish. Therefore, some fungal diseases are considered to be secondary infection^[17]. They can not harm healthy fish due to presence of the mucus layer on the skin of a fish that prevents the spores from infecting its living tissues.

Mentioned is to be made that poor water conditions always enable diseases to break out fast. Low temperatures are too primary causes of such infections^[10]. Fungal infection is also associated with environmental stress, presumably because

fish exhibit a weaker immune response when they are not properly taken care of; chilling, poor water quality and inappropriate water chemistry are all common reasons why aquarium fish develop fungus^[11]. Similar physico-chemical properties has been found in Tarang River water as the present sluice gate blocks the running water which enables to grow different infectious microorganisms during late winter as well as pre monsoon.

From fish pathologist point of view, pathogenic fungi destroy the functions of the organs and kill the fishes on mass scale and also deprive the natural strength of the fishes^[12]. The initial sign of microbial infection on fishes are distinct dermal lesion including ulcers. The family Saprolegniaceae

contains the majority of fungi that have been associated with disease in fish^[23, 25]. Saprolegniasis constitute one of the most severe diseases causing problems in fish culture. The studies made by^[13, 17, 18, 26] and various other workers have shown that not only saprolegnia but many other fungi are also associated with infection of fish and fish eggs. It has also been reported that mycological infestations on fishes are largely a secondary phenomenon and this type of infection occurs when the host resistance is lowered by environmental stress factors such as high organic load, overcrowding and sub lethal oxygen levels in water. In addition to water stagnancy the higher temperature also favours rapid proliferation of the bacteria and subsequent host mortality.

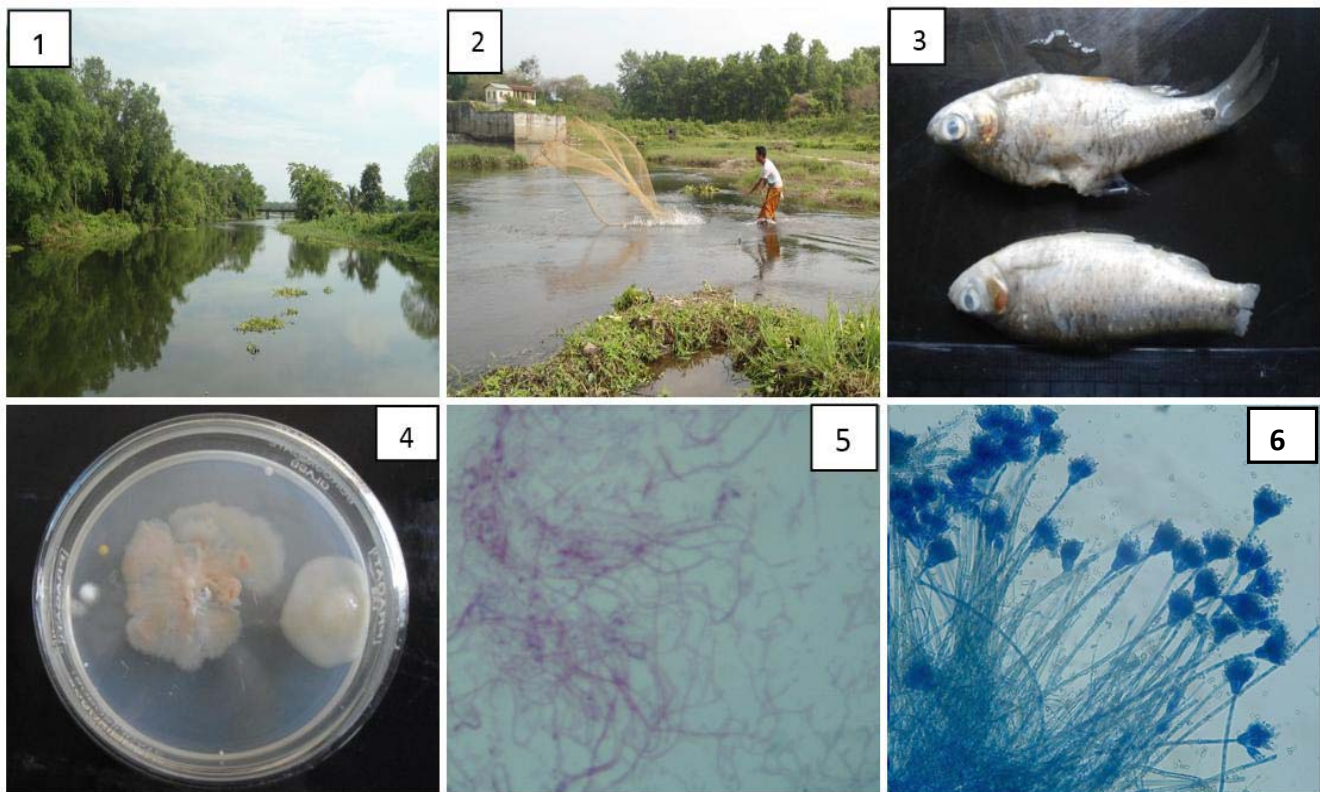
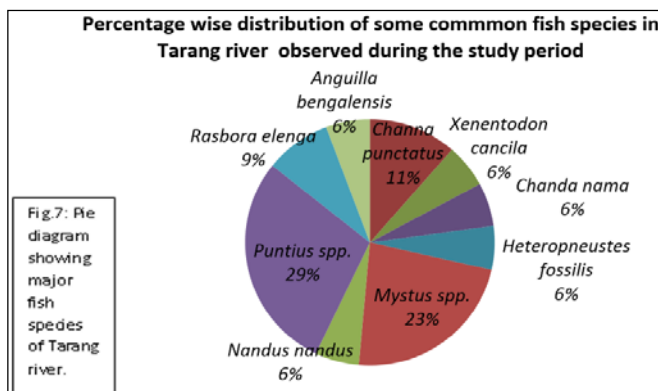


Fig 1: A view of Tarang River, Fig 2: Fishing activity in Tarang River, Fig 3: Infected *Puntius sp.*, Fig 4: *In vitro* culture of the causal organism, Fig 5: Microphotograph of *Actinomyces sp.*, Fig 6: Microphotograph of *Penicillium sp.*



5. Conclusion

The present finding showed the ability of *Actinomyces sp.* and *Penicillium sp.* to cause cotton wool like dorsal fin rot disease on *Puntius spp.* The disease was observed to be higher mainly during late winter and pre monsoon which cause great loss of fish productivity. Regular water quality assessment is very important to reduce such incidence of

microbial infection on fish. Therefore, cleanliness and removal of organic matter from the River bed by allowing the River to flow, and reducing the dumping of garbage and dead animals is very important which causes rapid proliferation of pathogenic microbiota causing various diseases and infections on indigenous fishes. *Actinomyces sp.* causing disease can be partially control by maintaining water current through sluice gate keeping on regularly and further study is recommended for complete eradication of such disease incidence from Tarang River.

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