

Introduction

From the inception of human civilization people used and exploit the water bodies in different ways. Water was being used not for drinking purpose and water ways but became a spot of hunting as there lived consumable aquatic animals. Man learned that the edible lives in water bodies are very cheap and started to exploit the same.

Fish have long been regarded as an important source of protein for human and consumed all over the world. It is consumed for its wide variety of tastes and nutritive qualities. There is ever increasing demand for more fish protein. Recently efforts have been made to increase fish production by adopting intensive and semi-intensive method of production to meet the ever increasing demand for fish. Intensive rearing through high stocking densities, artificial manuring and feed use has created the condition leading to physiological stress and increased the risk of disease out break (Pillay, 1996; McLean 1996).

Vast and varied water bodies of India are potentially one of the richest in the world. There are big and famous rivers, their tributaries, large creeks, canals and a long coastal line. The dug up impounded water bodies like ponds, tanks and lakes are also potential areas for pisciculture. Oxbow lakes locally known as beels and baors are also highly productive.

Recently, inland fish production of India has gone up through adoption of improved technology and culture method. India produced 6.3 million tones of fish during 2004-05 out of which 3.2 million tones of fish and other aquatic products came from the inland sector (Vass, 2006). Fish culture is also important from ecological point of view because fish is the most efficient among farm animals in converting feed into nutritious food.

The state West Bengal has rich fishery resources and inland fish production is quite substantial. The state has largest brackish water fishery resources in the India. The river of the state harbour twenty four species of economically important fishes (Jain, 1990). The state of West Bengal has more than 150 beels with an estimated water spread area of 42,000 hectares

and twenty four endemic fish species have so far been recorded from beels of West Bengal (Chauhan, 2006). The large agricultural field also harbour a large number of edible fishes which are not only rich in protein percentage but also cheap.

The outbreak of fish disease in India till 1988 had not been alarming. The most common diseases of fresh water fish in India were haemorrhagic septicemia, dropsy, ulcerative disease, columnaries disease, microsporidiasis, dactylogyrosis, gyrodactylosis, ligulosis, argulosis and saprolegniosis (Kumar and Dey, 1992; Das and Das 1995). The epizootic ulcerative syndrome the most destructive of all fish diseases ever witnessed by India broke out for the first time in May, 1988 in various states of north-eastern India such as Tripura, Magalaya, and Assam (Das, 1988). In October 1988 the disease spread to northern districts of West Bengal such as Jalpaiguri, Cooch Behar, West Dinajpur, Maldah and the plains of Darjeeling district. (Pal and Prodhan, 1990). The incidence of the disease was also reported from southern part of West Bengal such as Murshidabad, Nadia, North and South 24-Parganas and Midnapore (Pradhan and Pal, 1990). The disease affected almost all the district of West Bengal except Purulia by 1989 and spread to other states of India like Orissa, Bihar, Uttar Pradesh by 1990 (Jhigram and Das, 1990). Almost all the states of India except Gujarat, Punjab, Himachal Pradesh and Jammu & Kashmir were affected by 1993 (Das and Das, 1993).

The disease caused panic among the common people as a result the rate of fish consumption declined sharply. The fish farmers, fish traders and fishermen suffered heavy economic losses. The situation became so alarming that the Government of West Bengal and Ministry of Agriculture, Government of India jointly organized a "National Workshop on ulcerative disease syndrome in fish" 1990 at Calcutta, West Bengal.

The name epizootic ulcerative syndrome (EUS) was adopted in 1988 at the consultation of Experts meeting on ulcerative fish disease in Bangkok (FAO, 1986). It was accepted that the condition was primarily an infectious

disease and a complex condition involving more than one etiological factors like bacteria, fungus and viruses.

Various pathogenic organisms e.g. viruses (Frerichs *et al.* 1986; Ahne *et al.*, 1988; Lio-Po *et al.*, 2001) bacteria (Llobrera and Gacutan, 1987; Boonyratpallin, 1989; Callinan and keep, 1987; Pal and Pradhan, 1990; Subassinghe *et al.* 1990; Jhingran, 1990; Chakraborty and Dastidar, 1991; Lio po *et al.* 1992; 1998; Yadav *et al.* 1992; Saha and Pal, 2000) and fugus (Frazer *et al.* 1992; Roberts *et al.* 1993; Chinabut *et al.* 1995; Willoughby *et al.* 1999) have been isolated from affected fishes. Opinion also prevailed that some environmental factors such as lowering of water temperature, low alkalinity and pH fluctuation might act as potential predisposing factrors (Bondad-Reantoso, 1992, Das and Das 1993; Callinan *et al.* 1995; Lumanlan-Mayo *et al.*, 1997; Roy and Pal 2005).

Epizootic ulcerative syndrome (EUS) was redefined at a DFID Regional Seminar in Bangkok in 1994 as "a seasonal epizootic conditions of fresh water and estuarine warm water fish of complex infectious etiology characterized by the presence of invasive *Aphanomyces* infection and narcotizing ulcerative lesion typically leading to a granulomatous response" (Robersts *et al.*, 1994a).

Heterogenicity of viral isolation e.g. snake head virus (Saitanu *et al.*, 1986), Rhabdoviruses (Frerichs *et al.* 1986, 1989; Roberts *et al.* 1993, Lio-po *et al.*, 2001) a retrovirus (Freirichs *et al.* 1991) and reovirus (Frerichs 1995) with low recovery rate led same worker to conclude that viruses are adventitious agent (Frerichs, 1995).

But bacteria have been claimed to play a key role in causing EUS (Llobrera and Gocutan, 1987; Boonyaratpalin, 1989, Costa and Wijeyaratne, 1989; Subasinghe *et al.* 1990; Jhingran and Das, 1990; Pal and Pradhan, 1990; Chakraborty and Dastidar, 1991, Lio-Po *et.al.*, 1992; Saha and Pal, 2000).

Pal and Pradhan (1990) isolated two fluorescent Pseudomonads (R_1 and R_2) and one Aeromonad (R_3) and one coccus (c) from the ulcer of air breathing fishes during the initial phase of outbreak of the disease in North Bengal. R_1 , R_2 and R_3 were found to be pathogenic in healthy fishes. Mohan and Sankar (1995) reported detection of aseptate highly invasive fungus in the histological section of EUS affected fish. Only a few Indian workers reported presence of fungus in EUS affected fish (Karunasagar *et al.* 1994; Quereshi *et al.*, 1995; Pal, 1996; Viswanath *et al.* 1997, 1998; Saha, 1998; Mohan *et al.* 1999). Very little work has been carried out on the experimental induction of EUS with fungal isolates in India.

At present there is a declining trend of EUS in India. Outbreak of EUS is reported every year from various regions of North Bengal. So it appears that the primary source of the pathogen or the transmission factors are not yet controlled.

Under this circumstance it was considered worthwhile to study about the role of fungus and bacteria as causative agents of epizootic ulcerative syndrome.

The main objectives of this study are: (1) Isolation and characterization of fungus from EUS affected fishes (2) Isolation and characterization of bacteria from EUS affected fishes. (3) Induction of ulcer by fungal isolates (4) Induction of ulcer by bacterial isolates. (5) Induction of ulcer by fungus and bacteria in mixed condition (6) Histopathological studies of ulcer tissue, liver, kidney and spleen of naturally infected fishes and experimentally infected fishes by fungus in pure condition and bacteria in pure and mixed condition.