Summary

The present study dealt with "Evaluation of the role of fungi and bacteria in causing epizootic ulcerative syndrome in fishes", consisting of (1) Observation on the epizootic ulcerative syndrome (EUS) affected fishes, (2) Histopathological observations of some EUS affected fishes, (3) Isolation of fungus, *Aphanomyces* sp. from some EUS affected fishes, (4) Isolation of bacteria from naturally infected fishes, (5) Studies on the pathogenicity of R1, R2 (fluorescent pseudomonads) and R3 (*Aeromonas caviae*), isolated from EUS affected fishes on *Channa punctatus* and *Cyprinus carpio*, (6) Studies on the pathogencity of fungus (F_{cs1}) isolated from EUS positive *Channa striata,* (7) Studies on the pathogenicity of the bacteria isolated from EUS positive fishes, (8) Studies on the pathogenicity of zoospore of fungus (F_{cs1}) and bacteria (R1, R2 and R3), (9) Histopathology of experimentally infected fishes, *Channa punctatus* and *Cyprinus carpio* after intramuscular injection with R1, R2 and R3 singly and in mixed condition, (10) Histopathology of experimentally infected fishes, *Channa punctatus* after intramuscular injection of zoospore suspension of fungus, *Aphanomyces* sp. isolated from *C. striata*. (F_{cs1}).

In May 1988, EUS first occurred in India. From the beginning it affected a wide variety of fish species in both wild and cultured water. The disease spreaded all over India barring a few states by the year 1993. Some areas of North Bengal witnessed the disease recurringly every year.

Altogether 300 infected fish such as, *Channa punctatus, C. striata, C. gachua, Mystus* sp. *Clarias batrachus, Labeo rohita, Labeo bata, Catla catla, Cirrhinus mrigala, Puntius* sp. and *Macrognathus aculeatus* where collected. The ulcers were categorized into superficial, moderate and severe.

Histopathological studies were conducted on naturally EUS affected fishes, *Labeo rohita, Labeo bata* and *Channa* striata. The Grocott methenamine siliver stain and Periodic acid-schiff stained section of ulcer showed the presence of fungal hyphae. H-E stained sections showed that there was loss of epidermis. Necrosis of muscle, granulomatous changes and

blood capillary infiltration in the dermal and subdermal layers at the site of ulcers were the prominent changes. Liver showed vacuolation of hepatocytes, necrosis and infiltration of blood capillaries. In the kidney, vacuolation, tubular degeneration and haemorrhage were the major changes. Spleen also showed degenerative changes.

Fungus was isolated from the ulcers of naturally infected *C. striata, C. punctatus, L. rohita* and *L. bata*. The isolated aseptate fungus was identified as *Aphanomyces* sp. by its characteristic zoosporangia which was not wider than the hyphae A ball of spore at the tip of sporangium was observed. A single row of primary zoospores was found within the zoosporangia. In culture media the fungus became slender. The isolated fungus did not grow at 37^0C. All the four isolates (F_{cs1}, F_{cp1}, F_{lr1} andF_{lb1}) showed similar characteristics.

Bacteria were isolated from the ulcers of naturally infected *Labeo rohita* and *C. striata*. Among the isolates of *L. rohita* one belonged to the genus *Pseudomonas,* three belonged to the genus *Aeromonas,* one belonged to the genus *Micrococcus*. Among the isolates of *C. striata* one belonged to the genus *Aeromonas*, and four belonged to the genus *Pseudomonas*.

Pathogenicity of R1, R2 and R3 were tested on *Channa punctatus* and *Cyprinus carpio*. The experimental fishes were injected intramuscularly pure and mixed culture of three bacteria (R1, R2 and R3) @0.05 mL/100 g body weight (6-8 x 10^9 cells / mL) and @ 1 mL/100 g body weight (6-8 x 10^9 cells / mL) respectively. The result showed that all the three bacteria were virulent. The virulence of R3 higher than R2. The virulence of R1 was slightly low which caused superficial ulcer at the site of injection with slight redness on the surrounding. Results also showed that the mixed bacterial suspension was more pathogenic than the pure bacterial suspensions. Severe ulcers were induced at the injection site in fishes treated with a mixed and R3 suspension while superficial and moderate ulcers were induced in fishes injected with pure bacterial suspensions of R1 and R2 respectively. Both the fish species were equally susceptible to R1, R2 and R3 when injected intramuscularly either in pure or in mixed form.

The pathgenicity studies with the zoospores of isolated fungus (F_{cs1}) *Aphanomyces* sp. showed that the isolated fungus was pathogenic and it induced ulcer at the site of injection and caused 44% mortality in experientally infected fishes, *Channa punctatus*.

Pathogenicity studies with the bacterial isolates showed that nine strains of bacteria belonging to the genus *Aeronomonas* and *Pseudomonas* were virulent. *Micrococcus varians* was non pathogenic. Virulent L_{r1}, L_{r2}, L_{r4}, and C_{s5} belonging to *Aeromonas* and C_{s1}, C_{s2}, C_{s3} C_{s4} and L_{r5} belonging to *Pseudomonas* were found to be pathogenic.

Pathogenicity studies with mixed suspension of fungal zoospore (F_{cs1}) and bacteria (R1, R2 and R3) in pure condition showed that zoospore and R3 suspension is more virulent than that of the suspension of zoospore and R1. *Zoospore and R2 suspension is less virulent than zoospore and R1* suspension. The pathogenicity studies showed mixed suspension of fungal zoospore (F_{cs1}) and R3 is most virulent.

Histopathological studies were conducted on *Cyprinus carpio* after intramuscular injection given to healthy fishes with pure and mixed bacterial suspension of R1, R2 and R3. Results showed that, there was loss of epidermis, muscle necrosis, degenerative changes and blood capillary infiltration in the dermal and subdermal layers at the site of injection. Liver showed vacuolation of hepatocyes, necrosis and infiltration of blood capillaries. In the kidney, vacuolation, tubular degeneration and haemorrhage were major changes. Haemosiderin laden macrophages were detected in the kidney of all infected fishes. The degree of pathological changes were comparatively less in the section from fishes injected with pure bacterial suspension than the sections from fishes injected with mixed bacteria.

Histopathological studies were conducted on *C. punctatus* after intramuscular injection with the zoospores of isolated *Aphanomyces* sp. The loss of epidermis and dermis of the skin of ulcerative area were noticed but the dermis showed severe changes wherever it was present. Severe

myonecrosis and typical granuloma formation were found. Aseptate fungal hypae were found in the dermis and underlying musculature. In the liver vacuolation and haemorrhages were observed. Necrotic and degenerative changes were observed in the kidney and spleen. No evidence of fungal hyphae were observed in the liver, kidney and spleen.