General Discussion
5.0. GENERAL DISCUSSION

Many tropical countries in Asia have a long tradition of aquaculture, over 80% of fish produced by aquaculture comes from Asia. Aquatic animal disease and environmental related problems may cause annual losses of more than US$ 3 billion dollars among Asian countries (FAO, 1996). Fish in fresh water environments are susceptible to a number of bacterial, fungal, viral and parasitic diseases. A survey on the diseases in ornamental fishes *Cyprinus carpio* (Koi carp), *Xiphophorus maculatus*, *Xiphophorus hellerii*, *Poecilia sphenops*, *Poecilia reticulata* and *Serrasalmus elongatus* in Kanakumari District, TamilNadu, India have undertaken by the present study. Clinical signs in the chosen infected fish species reported were reddening of the abdomen, ulceration on the head, loss of scales, erosion of the tail fin, grey coloured lesions, eroded pectoral fin, reddening of the vent region, ulceration near the eyes, black coloured swelling on caudal fin, raised scales, swelling of the abdominal region, etc. Clinical symptoms of the experimental fish induced by bacterial infections were similar to those of naturally infected fish (Shih-Ling *et al.*, 1999).
Disease causing pathogens were also reported in the chosen ornamental fish species. Seventeen bacterial species were identified, of these *Shigella flexneri*, *Bacillus cereus* and *Vibrio sps* were reported to occur in all the fish species. Ibiwoye *et al.*, (1990) reported that, *Citrobacter freundii*, *Escherichia coli*, *Staphylococcus sps* and *Streptococcus sps* are the dominant forms of fish pathogens in the skin. Besides that, other bacterial pathogens *viz.*, *Escherichia coli*, *Klebsiella pneumoniae*, *Yersinia enterocolitica*, *Streptococcus pyogens*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Staphylococcus saprophyticus*, *Citrobacter freundii*, *Proteus myxofaciens*, *Proteus penneri*, *Clostridium sps* were also reported in the present study. It is important to notice that, single infected fish is reported to have cross infections with bacterial and fungal pathogenic forms.

Aydin *et al.* (1997) isolated *Citrobacter freundii* from naturally infected aquarium fish species namely *Poecilia sp*, *Xiphophorus demenciare*, *Xiphophorus pygmaeus*, *Carassius auratus* and *Astronotus ocellatus* with gross clinical symptoms. Several authors (Pradhan and Pal, 1990; Okpokwasili and Okpokwasili, 1994; Pal and Srivatsava, 1996) have reported that mixed cultures of two or more bacterial
strains are more virulent than individual pathogens. In this study since more than one pathogenic strains were isolated from each fish species, and it is likely that by nature, these strains are acting *en masse* to produce deep severe ulcers.

*Streptococcus* species is known to cause serious deseases in a number of host in the present study. Streptococcal disease of fish are not common; however, when they do occur, significant losses can result. *Streptococcus sps* have been isolated from a variety of ornamental fish including rainbow sharks, red- tailed black sharks, rosey barbs, danios and some cichilds which cause high mortality rate, over a period of 3 – 7 days. Ferguson *et al.* (1994) reported that, *Streptococcus* on the other hand does not seem to be a truly opportunistic pathogen as it can be more aggressive than many other environmental bacteria. Populations of Zebra danios and White cloud mountain minnows expose to high concentrations of *Streptococcus* in the water experienced 100% mortality with in 2 – 4 days of exposure. Austin and Austin, (1999) isolated *Streptococcus difficilis* from Israel; *Streptococcus milleri* from the kidney sample of Koi; *Streptococcus parauberries* from farmed turbot in Northern Spain.
Keun-Kwang et al. (1997) reported *Yersinia sps* from Olive Flounder *Paralichthys olivaceous*. Sung-Woo et al. (2001) reported the Staphylococcal infection in Mud Loach (*Misgurnus mizolepis*) cultured in recirculating aquaria. Chanratchakool (1996) also observed massive bacterial infection with *Vibrio sps*, in white spot diseased shrimp with reddish exoskeleton and he considered red discolouration and Vibriosis as stress induced diseases. *Staphylococcus epidermis* as a fish pathogen was first reported by Kusuda and Sugiyama (1981) which caused mass mortality in farmed yellow tail (*Serriola quinquiradiata*) and red sea bream (*Chrysophrys major*) in Japan. Trust and Bartlett (1974) reported that, *Aeromonas, Citrobacter, Enterobacter, Enterococcus* and *Pseudomonas* were often present, where as *Alkaligenesis, Flavobacterium* and *Vibrio* frequently occur in the ornamental fish culture water. Most of the bacterial diseases in silver carp grown in tropical ponds are caused by gram negative bacteria. The gram positive bacteria have rarely being found to be the aetiological agent in warm water fish (Griffin and Snieszko, 1951). Katoch et al., (2001) reported different kinds of pathogenic forms viz., *Vibrio sps, Aeromonas sps* and *Morexella sps* in diseased trout in Himachal pradesh. Allan
(1985) isolated *Aeromonas hydrophilla*, *Aeromonas salmonicida*, *Citrobacter sps.*, *Vibrio sps* and *Yersinia sps* in bacteriological investigations of a fish farm.

It is observed in the present study that, the predominance of bacterial pathogens depends upon the host organisms. For instance, *E.coli, Klebsiella pneumoniae, Proteus myxofaciens, Yersenia enterocolitica, Vibrio sps, Bacillus cereus, Staphylococcus aureus, Clostridium sps, Shigella flexneri* were reported at a maximum of 14.7% in *Xiphophorus hellerii* whereas, the bacterial species such as *E.coli, Klebsiella pneumoniae, Vibrio sps, Citrobacter freundii, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus pyogens, Clostridium sps* and *Shigella flexneri* were reported to present at a maximum of 15.4% in *Xiphophorus maculatus*. Qureshi et al., (1995) reported that, *Aeromonas* and *Pseudomonas spp.* are the dominant bacterial strains found to induce ulcers in healthy fishes from fresh and brackish water habitat.

Min-Soon et al. (2000) reported nine genera of bacteria namely *Vibrio sps* (35.7%), *Pseudomonas sps, Bordetella sps, Alkaligenesis sps, Flavobacterium sps* and *Staphylococcus sps* respectively, in which *Vibrio sps* are the predominant one. Singh (1996) isolated *E.coli, Salmonella*
typosa, *Shigella* sps and *Klebsiella aerogenes* from 15 marine fish samples collected from Satpati fishing center, Palghar, India of which *E.coli* was found to be the predominant one.

The incidence of mycotic infections in chosen fishes has also been reported in the present study. Fungi are known to attack eggs, fry, fingerlings and adult fishes. Generally most of the fishery biologists have wrongly assumed that almost all the fungal infections of fish and fish eggs are caused by the members of the genus *Saprolegnia*. But other fungal species have also been reported in variety of fishes (Srivastava and Srivastava, 1977; 1978). In the present study four fungal species reported *viz.*, *Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium restrictum*, *Pencilliurn chrysogenum* in the chosen fish species. These fungal infected fish species showed variety of clinical signs *viz.*, white patches on the ventral sides of the abdomen or opercular region and near the pectoral fin in *Xiphophorus inaculatus*, excessive fungal out growth from the dorsal fin and a cream patch near the dorsal fin. Of these, *Penicillium chrysogenum*, *Rhizopus stolonifer* and *Aspergillus niger* were found to be the predominant forms in *Poecilia sphenops* and *Serrasalmus elongatus*.
Allan (1985) reported that, *Fusarium sps, Penicillium sps*, and *Aspergillus sps* were the predominant fungal pathogens in fish. Richards *et al.* (1978) reported that, *Fusarium sps* was the predominant pathogen in salmonids. Hendricks and Bailey (1989) and Tacon (1992) reported that, *Aspergillus sps, Fusarium sps* and *Penicillium sps* were the predominant forms. Singh (1996) reported that *Aspergillus flavus* and *A. carbolarius* were the predominant pathogens in Satpati fishing centre, Palghar, India. Branislav Rankovie (2005) reported that *Penicillium, Aspergillus, Cladosporium, Fusarium, Rhizopus, Mucor, Phoma* and *Verticillium* were the predominant forms. In Egypt, *Aspergillus, Penicillium* and *Trichoderma* were found to be the most common genera among Nile fishes (Bagy *et al.*, 1993).

Opportunistic species may be expected to vary from one geographic area to another and one pond to another with in a farm depending upon the availability of these bacterial pathogens in the external medium (Jayasree *et al.*, 2000). To overcome these problems, various chemotherapeutants have been used for treatment or prevention of diseases. However, the use of antimicrobial agents have resulted in more resistant pathogenic microorganisms. These resistant
microbial pathogens could have a negative impacts on the therapy of fish diseases and the environment of the fish farms (Smith et al., 1994).

Herbs have been widely used in veterinary and human medicine. They are natural products that are not only safe for consumers but also widely available throughout Asia. Now a days herbs or herbal products also have a significant role in fish culture. Many kinds of herbal medicine have been used in China to control fish disease and have produced satisfactory results (Rajandra, 1990). Shangliang et al. (1990) reported the antimicrobial activity of five Chinese herb extracts against thirteen bacterial and two viral fish pathogens. In Vietnam, the Institute of Ecology and Bioresources has undertaken applied research on some medicinal herbs for prophylaxis and treatment of fish and shrimp diseases such as ulcer, intestinal diseases, white mouth, white head, red skin and red spot in fish, and luminescence and brown spot disease in shrimp (Dung, 1990). In Thailand, during the outbreak of Epizootic ulcerative syndrome in 1983, the snake-head fish farmers in Uthaitanee used the bark of cork wood tree Sesbania grandiflora for the treatment of haemorrhage lesions. Since 1990, many kinds of herbs have been introduced in shrimp farms
suffering from infectious diseases. For example, garlic and onion has been mixed into pellets for daily feeding to shrimp to prevent bacterial infections.

In the present study, an attempt has been made to find the herbal based disease treatment on ornamental fishes in South India. About 75 plant species belonging to 39 families have subjected for the extraction of bioactive compounds by using Chloroform. All the extracts were tested against 17 bacterial and 4 fungal pathogens isolated from the infected ornamental fish species. Out of which, 18 plant species showed sensitivity against one or more bacterial pathogens and 17 plant species showed sensitivity against one or more fungal pathogens.

Shu-tong et al. (2001) have screened 88 species of plant extracts against Phytophthora infestans and found that, 31 species were completely inhibitory; 41 species were strongly or moderately inhibitory and 11 were little inhibitory against Phytophthora infestans. Moreover 16 kinds of Thai traditional herbs were tested for their antibacterial efficacy against 10 strains of Vibrio species, 11 species showed antibacterial sensitivity, out of which 2 of them (P.guajava and
M. charantina) showed the highest effective against Vibrio species. In the present study, Cinnamomum verum showed maximum sensitivity (20 mm dia) against Klebsiella pneumoniae; Eupatorium odoratum showed maximum sensitivity (15 mm dia) against Vibrio species. The Cinnamomum which is native of India and Sri Lanka, being cultivated in many tropical countries including Mexico. This plant has been used in ayurvedic and other traditional medicines in Asia. In the American continent, most of the original uses are still prevalent; mainly as a treatment for diarrhea, stomach upset, against respiratory ailments (Gonzalez, 1998; Linares et al., 1994; Aguilar, 1999)

It is also used externally as a poultice to treat minor bacterial and fungal infections of the skin. Some of the plant constituents have proven value against bacteria and fungi, including molds that produce carcinogenic aflatoxins (McCann, 2003; Juglal et al., 2002). Cinnamomum constituents possess antioxidant action and may prove beneficial against free radical damage to cell membrane (Dragland et al., 2003; Jayaprakasha et al., 2003; Lee and Shibamoto, 2002). Its essential oil contains both antifungal and antibacterial principles that can be used to prevent food spoilage due to bacterial contamination
(Fabio et al., 2003 Guynot et al., 2003; Kalemba and Kunicka., 2003; Shur
and Nielson, 2003; Valero and Salmeron, 2003; Friedman et al., 2002;
Mejlholm and Dalgaard, 2002; Ranasinghe et al., 2002; Smith - Palmer
et al., 2002; Yuste and Fung, 2002; Mau et al., 2001).

*Cinnamomum* oil have also proven to be particularly effective
against some species of taxicogenic fungi (Juglal et al., 2002; Soliman
and Badeaa, 2002; Montes-Belmont and Carvajal, 1998) as well as
against respiratory tract pathogens, including species belonging to the
genera *Aspergillus, Candida, Cryptococcus,* and *Histoplasma* (Viollon and
Chaumont, 1994; Inouye et al., 2001) Likewise, the *Eupatorium* belongs
to the family Asteraceae also shown maximum antifungal sensitivity
against the tested pathogens. Rai et al., (2002) reported that, the
essential oil from *Eupatorium triplinerve* showed sensitivity against
*Sparoleguria* species of fungal pathogens causing diseases on fish. Many
investigators have worked out antimycotic potential of essesntial oils
(Barnes, 1963; Korta and Starzyk, 1963; Maruzzella, 1963; Hiller, 1964;
Birch, 1966; Korbely and Florian, 1971; Garg, 1994; Zutschi et al., 1975;
Overeem, 1976; Goutam et al., 1980; Jain et al., 1980; Ikram and Hag
1980, 1984; Deshmukh et al., 1986; Singh et al., 1986; Yadav and Saini,
1990; Kishore and Dwivedi, 1991; Jain and Agarwal, 1992; Perrucci
et al., 1994, 1995; Rai et al., 1999). Generally, the members of Asteraceae are known to contain essential oil which usually possesses antimicrobial sesquiterpenes.

However, the most effective antimicrobial agents from *Cinnamomum* have not been attempted to test any toxic effect in animal system and hence the present study has also conducted challenge trials on the effect of the *Cinnamomum* extract on the percentage survival of fishes. The interesting observation form the present study is that the supplementation of these plant extracts along with the feed increased the survival of the fishes *viz.*, *Cyprinus carpio*, *Xiphophorus maculatus* and *Poecilia sphenops* in the culture water inoculated with *Shigella flexneri* and *Rhizopus stolonifer*. Contrastingly, the extract from *Cinnamomum* with antibacterial effect showed negative results against the species *Helicobacter pylori* associated with gastric ulcers (Martin and Ernst, 2003). Direkbusarakom *et al.* (1997) reported that, eight species of Thai traditional herbs tested against Systemic Ectodermal and Mesodermal Baculo Virus (SEMBV) showed inactivation and further reduced the mortality rate of shrimp. However, the *Phyllanthus urinaria* showed 100% survival rate while 58 – 85% of these in other plant species tested.