**RESEARCH PROGRAMME**

**Project title:** Immunological, hematological and biochemical responses in common carp (*Cyprinus carpio* L.) following *Aeromonas hydrophila* infection and herbals treatment

(a) **Project Summary**

Fishery has been recognized as a powerful income and employment generation source as it stimulates growth of a number of subsidiary industries and is a source of cheap animal protein. Fish diseases, however, are one of the major limiting factors in aquaculture production, especially with the recent increase of aquaculture practices in rural areas. Rural small-scale aquaculture is the extensive or semi-intensive low cost farming.

The diseases caused by bacteria particularly genus *Aeromonas* and some other bacteria like *Pseudomonas, Staphylococcus* etc. are epizootic ulcerative syndrome (EUS) and hemorrhagic septicemia. Motile aeromonads were reported as most important bacteria among the etiological agents of EUS and hemorrhagic septicemia in a variety of fishes throughout world. *Aeromonas hydrophila, A. bestiarum, A. salmonicida, A. veronii, A. cavine* and *A. jandaei* have been reported as causative pathogens in various fish species like common carp (*Cyprinus carpio* L.), rainbow trout (*Oncorhynchus mykiss,* catla (*Catla-catla*), grasscarp (*Ctenopharygodon idella*), silver carp, bighead, golden carp, Chinese' bream and other fish species.

Antibiotics have made a tremendous contribution to the profitability of the fish industry. However, as a consequence of the increasing concern about the potential public health problems due to antibiotic resistant strains of bacteria, fish nutritionists are being challenged to develop an alternative for antibiotics. The herbal drugs have been in common use as a cure of many diseases and there is sufficient mention about their use in our ancient Ayurvedic literature. Charka Samhita (1000 B.C.) the earliest treatises of Indian medicine mention the use of over 2000 herbs for medicinal purpose. If herbal alternative to antibiotics can be found, fish nutritionists could formulate a diet that would meet the needs of the commercial fish industry without using antibiotics.

Keeping these points in view this research programme will be able to make alternates to antibiotics to cure fish bacterial diseases by using immune enhancers. I will select traditional Indian herbs (viz. neem, tulsi, turmeric, garlic etc.) and one bacterial pathogen *Aeromonas hydrophila* for this investigation. These herbs are incorporated in fish diet and then infected with same pathogen. The economics of herbal vis- a-vis antibiotics was also studied and found that use of herbs instead of antibiotics is more economical as compare to antibiotics. It was observed and found that herbs are immuno enhancers, which make the fish more resistant against the infection of *A. hydrophila*. There are thousands of herbs present in our environment which are easily available and more economical as compare to antibiotics. There is no residual effect of herbs in food chain also. If these types of practices are applied in fields regularly then we hope this environment will free from antibiotics residual effect. Considering the above facts in view the present investigation will be proposed.
Objectives of Research

1. To determine the ID$_{50}$ value of *A. hydrophila* in *C. carpio*.
2. To study the hematological and biochemical changes in blood of *C. carpio* following *A. hydrophila* infection and herbal treatment.
3. To study the immunological response in *C. carpio* following *A. hydrophila* infection and herbal treatment.
4. To study the economics of herbal *vis-a-vis* antibiotic treatment.

(b) Scientific Contribution and its Importance:

The herbal derivatives are the new generation of alternative measures for the prevention of bacterial diseases in fish which should be used for maintaining the fish health. The overall results of my study showed that the addition of these four herbals positively influenced a more rapid restoration of fish diseases associated with pathogenic bacteria. **Herbal derivative of garlic should be preferred over tulsi, neem and turmeric for preventing fish diseases and maintenance of fish health.** This practice will reduce the side effects of applying the synthetic compounds and the cost and also make it eco-friendly. Hence the alternative herbal biomedicines prove to be very effective in aqua-cultural operations. The use of herbs as immunostimulants prior to infection also merit further investigation.

(c) Origin of Proposal/Project

Fish is the primary source of high quality protein providing approximately 16 per cent of the animal protein consumed by the world's population; 26.2 per cent in Asia, 17.4 per cent in Africa, 9.2 per cent in Europe, 7.4 per cent in North and Central America and 7.2 per cent in South America, Fisheries and Food Security, 2006. Fisheries have been recognized as a powerful income and employment generator as it stimulates growth of a number of subsidiary industries and is a source of cheap animal protein. It is an instrument of livelihood for a large section of economically backward population of the country. Out of total world fish production, India shares 4.3 per cent (National Fisheries Development Board, 2009). The share of fisheries in the national GDP is 1.4 per cent, and in the agricultural GDP its share is 4.5 per cent (Ayyappan, 2006). Aquaculture continues to be the fastest growing animal food-producing sector to outpace population growth, with per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.8 kg in 2006, an average annual growth rate of 6.9 percent. World aquaculture is heavily dominated by the Asia–Pacific region, which accounts for 89 percent of production in terms of quantity and 77 percent in terms of value (FAO, 2009).

In Haryana, the area under fish culture in 2008-09 was 12,885 hectares and total fish production was 67,236 metric tonnes, and out of 30748 peoples engaged in this venture, 9466 were fish farmers and 21282 performed other related activities thus generating total income of 1862.85 million rupees. In district Hisar in 2007-08, the area under aquaculture was 1158 hectares and the state marketed 5710 tonnes of fishes, fetching an income of 171.3 million rupees (Statistical Abstract of Haryana, 2009).
The world’s total annual potential for fish production is estimated to be 8.9 million metric tonnes but total annual actual fish production is 7.4 million metric tonnes there is a loss of 1.5 million metric tonnes. This loss occurs due to mismanagement, unhygienic conditions and diseases outbreaks. The available figures on direct economic losses due to fish diseases indicate the significance of this problem. Estimates of economic losses due to fish diseases are available since 1990s which suggest that developing countries in Asia lost at least Rs. 7000 crores due to diseases in 1990 alone. Since then, losses have increased. A 1995 estimate suggests that aquatic animal disease and environment-related problems may cause annual losses to aquaculture production in Asian countries more than Rs. 13,500 crores per year. According to recent reports, global losses due to fish disease are more than Rs. 18,000 crores and the World Bank is investing Rs. 1240 crores in fish disease research. The above figures provide an indication of the overall economic significance of aquatic animal diseases. So, Fish diseases are now considered to be the most limiting factors in the fisheries production sector.

The most important species used for fish culture in India are the Indian major carps (Catla catla, Labeo rohita and Cirrhinus mrigala), common carp (Cyprinus carpio), grass carp (Ctenopharyngodon idella), silver carp (Hypophthalmichthys molitrix) and giant freshwater prawn (Macrobrachium rosenbergii). Fish species such as snakehead murrel (Channa punctatus), magur (Clarius batrachus), North African catfish (C. gariepinus), stinging cat fish (Heteropneustes fossilis) and sutchi cat fish (Pangasius hypophthalmus) are also farmed, but to a lesser extent. Cold-water fish such as rainbow trout (Oncorhynchus mykiss), brown trout (Salmo trutta fario), snow trout (Schizothorax richardsonii) and golden mahseer (Tor putitora) are cultured in the uplands of northern India, mainly for sport fisheries (Cerro et al., 2002), out of these Indian major carps (C. catla, L. rohita and C. mrigala), common carp (C. carpio), grass carp (C. idella), silver carp (H. molitrix), magur (C. batrachus), North African catfish (C. gariepinus), stinging (H. fossilis) and giant freshwater prawn (M. rosenbergii) are cultured in Haryana (Raj et al., 2008).

With the increasing fish culture activities, several bacterial diseases, causing morbidity and mortality in fish have been reported world over in fresh water aquaculture. Bacterial diseases and infections are very common in fishes. Disease has become one of the major limiting factors in aquaculture production of any country, especially with the recent increase of aquaculture practices in order to fulfill the protein deficiency of the rural people.

The diseases are like epizootic ulcerative syndrome (EUS) and hemorrhagic septicemia caused by bacteria particularly of the genus Aeromonas and some other bacteria like Pseudomonas, Staphylococcus etc. Motile aeromonads were reported as most important bacteria among the etiological agents of EUS and hemorrhagic septicemia in a variety of fishes throughout the world. Aeromonas hydrophila, A. bestiarum, A. salmonicida, A. veronii, A. cavine and A. jandaei have been reported as causative pathogens in various fish species like common carp (Cyprinus carpio L.), rainbow trout (Oncorhynchus mykiss, catla (Catla-catla), grass carp (Ctenopharyngodon idella), silver carp, bighead, golden carp, Chinese bream and other fish species (Kozinska, 2007; Sahoo et al., 2007; Sugita et al., 2008).
Earlier the control of fish diseases was focused on chemical compounds like formalin, organophosphates, malachite green, copper sulphate and various antibiotics (Ghosh et al., 2003). The use of antibiotics and chemotherapeutics for prophylaxis and treatment in intensive aquaculture has been widely criticized for their negative impact (FAO 2002). Therefore, avenues for the indiscriminate worldwide use of antibiotics in aquaculture has led to the development of drug-resistant in pathogenic bacteria which are becoming increasingly difficult to control and eradicate diseases. Besides development of drug resistant bacteria and pathogens, the antibiotics also exert adverse effect on the beneficial aquatic micro flora. The retention of harmful residues in aquatic animals finds their entry in to the human food chain causing health problem in human beings. The excess use of antibiotics is hazardous in human being, which may create antibiotic resistance in microorganisms. It is necessary to estimate residuals of antibiotics in fish. The retention of harmful residues in aquatic animals finds their entry in to the human food chain causing health problem in human beings (Esiobu et al., 2002; Sarter et al., 2007). Consequently, research on interactions between immunity, growth and development of eco-friendly alternatives to antibiotics that may keep fish healthy, such as impact of use of probiotic and plant based immunostimulants, has increased. Enhancement of the immune system seems to be the most promising method of preventing fish diseases.

Common carp (C. carpio) is an important fish used for pond culture in Haryana. This fish has been reported to be infected with many bacterial diseases including EUS and hemorrhagic septicemia. For the control of this disease, eco-friendly methods have not yet been discovered or evolved. Enhancement of immune response is one way to help the fishes to overcome the disease. This would be done through a study evaluating the dietary dosages of herbals on the immune response and disease resistance against infections due to pathogens. Aeromonads are notorious pathogens in fishes responsible for the cause of EUS and hemorrhagic septicemia in fishes, there is scanty information available about the herbal control of these pathogens.

Objectives:

1. To study the immunological responses, haematological and biochemical, changes in C. carpio following Aeromonas hydrophila infection and herbal treatment.
2. To study the residual effects of antibiotics in fish body.
3. To study the economics of herbal vis-a-vis antibiotic treatment.

Methodology:

1. To study the immunological responses, haematological and biochemical, changes in C. carpio following Aeromonas hydrophila infection and herbal treatment.
   These all parameter will be studied by following different standard protocols.

1.1 Bacterial agglutination test was performed for qualitative estimations.
The blood will be collected and used for bacterial agglutination test on slides for the qualitative estimation of Aeromonads in fish blood following standard protocol as mentioned by NHS (2004).

1.2 **Superoxide anion production / NBT assay for quantitative estimations.**
For the quantitative estimation of pathogen, estimation of superoxide anion production (which is produced due to the killing of Aeromonads by macrophages in blood) will be studied by NBT assay using protocol of Rajesh Kumar *et al.*, 2008 with some modifications.

1.3 **Expression kinetics of Interleukin 1β gene**
The fish will be taken out from experimental lot and sacrifice after dipping in MS-222 solution. The kidney head will be collected for this objective and mRNA will be extracted by following standard protocols. The expression of interleukin 1β gene will be observed in different herbal treatment against Aeromonads infection in Common carps (*C. carpio*).

2. **To study the residual effects of antibiotics in fish body.**
It is hazardous to have low amount of residual antibiotics in fish meat for human being, which brings unnecessary resistance to the pathogenic microbes. Keeping in view this objective of the project is to estimate the residuals of antibiotics in fish body by using standard methods is need of the time.

3. **To study the economics of herbal vis-a-vis antibiotic treatment.**
An experiment setup will be established to study this objective.

**Problem and Significance**

*Common carp (Cyprinus carpio)* is the major fish among Indian major carps in composite fish farming which provide a good animal protein to consumer and economic value for fish farmer. This fish is very susceptible to Aeromonads *i.e.* *Aeromans hydrophila* is most common aeromonad. Aeromonads are opportunistic Gram-negative pathogens causes an ulcerative condition. There has been various symptoms such as hemorrhagic septicemia, infectious abdominal dropsy, fin and tail rot in freshwater fish. Vaccines are being developed against Aeromonads but these are not yet commercially available. It is a heterogeneous species, having variable antigens, that vaccine development is extremely complex. In large aquaculture systems, traditional methods are ineffective against controlling new diseases. Therefore, alternative methods need to be developed to maintain a healthy microbial environment in the aquaculture systems and the health of the cultured organisms. There is reduced consumer preference for aquacultural products treated with antibiotics. Therefore, use of herbs is one of such methods that are gaining importance in controlling potential pathogens.
Bibliography


