Summary
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Aquaculture gains momentum at the advent of modern technologies in farming of aquatic organisms. The intensity of fish farming invades the gush of disease occurrences as in all other farming. The high sensitivity of fish to stress and the accelerated spread of disease in water, have put the squeeze an aquaculturists to ponder their venture on maintaining their fish in sound health, in order to obtain sustainable economic gains.

To nurture healthy fish, they require refining potent defence mechanisms against pathogen invasion. These are the non-specific and specific immune responses. The non-specific immune response is more important in fish than it is in mammals. These non-specific immune system in fish can be enhanced through feed additives. The main objective of the study was to improve the immune system fish by using plant leaf extracts as immunostimulants.

The first chapter includes the isolation and identification of bacterial pathogens associated with bacterial haemorrhagic septicaemia in Cyprinus carpio and its pathogenicity were studied. Biochemical characterization of the bacterial culture study indicates that the fish was infected with two types of bacteria namely, Aeromonas hydrophila and Pseudomonas fluorescens. The lethal dose for the two pathogens namely, Aeromonas hydrophila and Pseudomonas fluorescens were studied and the LD₅₀ values were found to be 3.65 x 10⁴ cells/ml and 4.33 x 10⁴ cells/ml respectively. The virulence study elucidated that among the two pathogens, Aeromonas hydrophila was found to be more pathogenic than that of Pseudomonas fluorescens.

The growth performance of freshwater fish, Cyprinus carpio fed with feeds incorporated with three different leaf extracts of Aegle marmelos, Andrographis paniculata and Euphorbia hirta were carried out. The experimental fish, Cyprinus
carpio of uniform size and were stocked in six troughs (60 liter capacity) with six fish each (including control). The complete artificial feed was incorporated with different concentrations of leaf extracts viz., 5, 10, 20, 25 and 50g / kg feed and fed for an epoch of about 50 days, to investigate the effective concentrations for growth of common carp, Cyprinus carpio. At the same time a control group was also maintained for the three plant leaf extracts in order to compare the growth stimulatory effect of leaf extracts.

During 50 days of feeding trials, experimental fish were fed with feeds daily at the rate of 2% of body weight. After feeding, the fish were allowed to feed two hours continuously and unconsumed feed was collected for drying and weighing. After 24 hours, faecal matter was collected by filtering the water through a fine sieve and dried. The weight gain or loss in fish were observed at required intervals of 10 days. The results of this study are presented and discussed in Chapter II.

It is clear that all the medicated feeds were readily accepted and no difference in feeding rate was observed. Difference in fish growth was noticed in fish which consumed medicated feed with plant leaf extract than the control fish. The maximum feeding rate was observed in fish fed with feeds incorporated with Euphorbia hirta leaf extract followed by Aegle marmelos. The maximum value of feeding rate of 1.96 ± 0.063% body weight / day was observed in fish fed with feed incorporated with 20g leaf extract/ kg feed of Euphorbia hirta, while the least feeding rate of 1.26 ± 0.075% body weight / day was found in fish fed with feed having 25g leaf extract of Andrographis paniculata / kg feed (fig 2.1). The maximum feed assimilation rate was observed in fish consumed feed incorporated with 20g leaf extract of Euphorbia hirta/ kg feed. Among the three plant leaf extracts tried, Euphorbia hirta showed the best assimilation and metabolic rate
The results of the feed conversion ratio indicates that feed conversion ratio were low in fish fed with lower concentrations of leaf extracts of Aegle marmelos and Andrographis paniculata. In the case of the fish fed feed with Euphorbia hirta, showed decrease in feed conversion ratio as the concentrations of leaf extract in the feed increased, whereas a reverse trend was observed in feed conversion efficiency. The better protein efficiency ratio was found in fish, which consumed feed incorporated with 5g leaf extract of Aegle marmelos/kg feed, followed by the fish fed with Euphorbia hirta and Andrographis paniculata. Among the three plant leaf extracts tried, the maximum specific growth rate of $0.253 \pm 0.010 \text{ g \% / day}$ was found in fish fed with feed 5g leaf extract of Aegle marmelos/kg feed, followed by the fish fed with feeds incorporated with 25 and 50g leaf extracts of Euphorbia hirta/kg feed and the values were found to be $0.228 \pm 0.009 \text{ g \%}$ and $0.234 \pm 0.014 \text{ g \% / day}$. The results also indicates that the growth performance irrespective of specific growth rate, protein efficiency ratio and feed conversion efficiency was found to be better in fish, which consumed 5 g leaf extract of Aegle marmelos, 25 and 50g leaf extracts of Euphorbia hirta, followed by fish fed with feed having 10g leaf extract of Andrographis paniculata incorporated feed.

The Chapter III includes the immunostimulatory effect of leaf extracts of Aegle marmelos, Andrographis paniculata and Euphorbia hirta in Cyprinus carpio. After 50 days of immunomodulation, the fish were infected with bacterial pathogen, Aeromonas hydrophila and Pseudomonas fluorescens through intraperitoneal injection at a dose of $1.5 \times 10^4 \text{ cells/ml}$, and after 5 days of infection immunostimulatory effect of leaf extracts of Aegle marmelos Andrographis paniculata and Euphorbia hirta were assessed through the assessment of haematological, specific, non-specific and enzymatic changes.
These studies were carried out once in every day up to 20th day. These responses were also studied prior to the challenge of pathogen.

It is clear from the haematological assay that the leaf extracts of *Aegle marmelos, Andrographis paniculata* and *Euphorbia hirta* were able to increase the RBC, Hb content and WBC count after feeding with leaf extract incorporated feeds and after challenging with the pathogen, the RBC count and Hb content decreased for about 10 days and thereafter showed an increasing pattern, whereas the WBC count increased after infection with the pathogen.

The medicated feeds with leaf extracts were able to stimulate the specific immune response by increasing the titre value of antibody. The fish which consumed feeds with *Andrographis paniculata* leaf extracts showed an increase in antibody titre value for about 15 days after infection and subsequently the titre value decreased. It is also observed that an increase in antibody concentration was found in fish fed with feed having 5g leaf extract. In the case of *Aegle marmelos*, the peak antibody production was high on 10th day in fish which consumed feed with 20g leaf extract. The leaf extract of *Euphorbia hirta* was also able to stimulate the antibody production only up to 5th day, when fed with higher concentrations of 25 and 50g plant leaf extract/kg feed.

The fish fed with feeds incorporated with leaf extracts of *Aegle marmelos, Andrographis paniculata* and *Euphorbia hirta* were able to stimulate the phagocytic ratio significantly. The fish fed with feed having 10g leaf extracts of *Aegle marmelos* and *Andrographis paniculata* were able to stimulate the maximum phagocytic ratio up to day 10 whereas feeds with *Euphorbia hirta* at 50g leaf extract/kg feed was able to stimulate
the phagocytic ratio up to 15th day and subsequently by the values decreased as noticed in other two cases.

The fish fed with feeds having 5g leaf extract of *Aegle marmelos* and 10g leaf extract of *Andrographis paniculata* were able to increase the NBT positive cells upto 15 days after infection. The fish fed with feed having 25 and 50g leaf extracts of *Euphorbia hirta* were able to enhance the NBT positive cells upto 20 days after infection.

The fish fed diets with three different plant leaf extracts were able to enhance the serum lysozyme activity and the maximum serum lysozyme activity was noticed on the 10th day in fish fed with feeds incorporated with 10g leaf extract of *Aegle marmelos*, 5 and 10g leaf extracts of *Andrographis paniculata* and 50g leaf extract of *Euphorbia hirta*.

The fish fed with feeds having leaf extracts of *Aegle marmelos* and *Andrograhis paniculata* and *Euphorbia hirta* were able to enhance the phosphatase enzyme activity significantly. The fish fed with 10g leaf extract of *Aegle marmelos*, showed high phosphatase activity and the peak response was noticed on the 10th day, whereas in the case of *Andrographis paniculata* and *Euphorbia hirta* incorporated feed enhanced the phosphatase activity significantly on 10th and 20th after infection and the respective effective concentrations were found to be 20 and 25g leaf extracts / kg feed.

The feeds with three different leaf extracts at different concentrations were able to enhance the serum peroxidase activity maximally up to 10 days and effective
concentration was found to be 10g leaf extract of *Aegle marmelos*, 5g leaf extract of *Andrographis paniculata* and 25g leaf extract of *Euphorbia hirta* / kg feed.

Pathogen clearance study elicits that the feeds incorporated with leaf extracts were able to eliminate the pathogens from its circulatory system in *Cyprinus carpio*. Among the three leaf extracts, the fish fed with 10g leaf extract of *Aegle marmelos*, 10g leaf extract of *Andrographis paniculata* and 50g leaf extract of *Euphorbia hirta* performed better in eliminating the pathogen.

Disease resistance study indicates that the fish fed with feeds having leaf extracts of three plants tried were able to increase their survival percentage significantly when compared with the control fish. The feeds with 5g leaf extract of *Aegle marmelos*, 10g leaf extract of *Andrographis paniculata* and 50g leaf extract of *Euphorbia hirta* / kg feed enhanced the survival percentage, as observed in other parameters of immune response.