SUMMARY

Aquaculture is the world’s fastest growing food-production sector, providing an acceptable, protein rich supplement to, and substitute for, wild aquatic animals and plants. Shrimp farming takes the lion share of total aquaculture production and has continued to expand and become a major source of income in many countries. Disease outbreaks are significant constraint to the development of the shrimp culture sector, with a global estimate of disease losses in the range of 3 billion dollar per year. In shrimp, viral diseases are the most devastating followed by bacterial outbreaks.

The attempts made for controlling or preventing such devastating outbreaks using conventional antimicrobials and other chemotherapeutants were mostly expensive and unsuccessful. Moreover the uncontrolled and repeated use of antibiotics causes a major setback in the successful treatment of bacterial infections due to the development of antibiotic resistant pathogens. Developing specific bacterins/vaccines are also impractical in crustaceans because of their poorly developed specific immune system.

The non-specific defense mechanisms for controlling diseases have considerable potential for health management in shrimp aquaculture. The immune level responses during the administration of chitosan and levamisole and their potential in disease resistance in tiger shrimp *Penaeus monodon* were characterized in the present study. For this forty days old shrimp, *P monodon* were collected from extensive farm near Rajakamagalm area, Kanyakumari district, Tamil nadu. For immunostimulation the two immunostimulants were used at different doses ranging from 50-300 mg/kg body wt of shrimp and the administration prolonged for 30 days.
The entire immunostimulated group showed the higher absolute growth rate (AGR) over the control. However the group treated with 50 mg levamisole/kg body wt of shrimp (group C) showed high AGR value of 0.106 g/body wt/day. The THC of the group C increased to 24.64% over the control group on the day 1 with a drastic increase in the day30 to the extent of 50.13%. More or less same trend was noted in all other experimental groups. The applied immunostimulants also influence the survival rate of *P. monodon* after challenging with *V. fischeri*. The group C shows survival rate of 60% was recorded as maximum. Based on the above investigation it is evident that levamisole (50mg/kg body wt of shrimp) and chitosan (50mg/kg body wt of shrimp) certainly enhances the nonspecific immunity of *P. monodon*. Thus chitosan and levamisole act as immunostimulants which appear to improve the immune status and growth of *P. monodon* in aquaculture.

The protein characterization was carried out using haemolymph collected from the immunostimulant administered treatment groups. The collected haemoph were used for poly-arylamide gel electophoresis. Then the noval 8.1 kDa molecular weighted protein band was excised using in-gel digestion method and it was submitted for MALDI-TOF analysis for peptide sequence determination. Finally the amounts of protein expression in different treatment groups were identified using Western Blotting method.

The protein characterization using SDS PAGE analysis revealed the presence of a unique peptide of 8.1 kDa in all treatment groups and it was subjected for further analysis. The penaeidin peptide (8.1 kDa) developed during the administration of immunostimulants had 75 amino acid length. The peptide consist of 16 different amino acids, among which valine contribute the major share of 12% followed by 10.6% of
leucine. Among the five different treatment groups the group treated with 30mg levamisole and 150mg chitosan/kg body wt of shrimp expressed the maximum amount of 8.1 kDa peptide. The amounts of peptide produced with in the control and different experimental groups were 3.21ng, 6.38ng, 11.40ng, 16.32ng and 18.17ng respectively. These significant variations revealed the impact of levamisole and chitosan on 8.1 kDa peptide production.

The 75 amino acid sequence of penaeidin was searched using BLAST-P algorithm using nonredundant database search. The sequence was assigned to penaeidin superfamily of peptides. The penaeidin peptide from the present experiment shows the maximum alignment with two penaeidin proteins isolated from *P monodon* (AF475082 and ACQ66007). Phylogenetic analysis depicts that the query peptide was evolutionarily segregated from the penaeidin counterparts. The structure prediction using 3D GIXA protein modeling server shows that the peptide consist one α helix unit and two coils. The \( \phi \) and \( \psi \) angles plotted for each amino acid shows that a major fall in right handed coils. The structural accuracy was evaluated using Z scores, a total of -1.32 shows that the model has an accurate 3D structure. Further the energy distribution of each amino acid read in terms of entropy shows that the model has minimum internal energy distribution, it shows maximum stability. Moreover, from their unusual features both at the structural level and in terms of biological activity, penaeidins could represent, along with other antimicrobial peptides, a new generation of therapeutic agents and find potential applications in aquaculture or agronomy.