VI. SUMMARY

White Spot Syndrome Virus first appeared in Taiwan in the early 1990’s and has spread throughout the world. In India it first appeared in 1994. WSSV is one of the first viruses infecting aquatic invertebrates that has been fully sequences and characterized in detail. The virus which infects shrimps and number of other crustacean species causes 100% mortality in shrimp within one week post infection. Several measures have been adopted to enhance immune system and improve disease resistance of shrimps which include use of immnostimulants, probiotics, better water quality management, stocking virus free larvae, etc. But none of these methods alone or in combination have offered complete protection against WSSV. Discovery of quasi-immune response in invertebrate including shrimps surviving WSSV infection has opened the way for development of vaccine. The whole inactivated viral particles are known to enhance disease resistance and envelop proteins of the virus are considered as potential vaccine candidate because these proteins will first come in contact with the host immune system. Recently RNAi mechanism has also been found in shrimps and this mechanism is as an antiviral agent that protect shrimps from viruses infection. In the present study the ability of four envelope proteins to induce protection and serve as potential vaccine candidate was studied. The protection induced by dsRNA and possibility of using this to protect shrimp against WSSV infection was also studied. The outcome of the investigations are summarized below.
The virus was purified from the WSSV infected shrimp.

Four coat proteins, VP28, VP281, VP39 and VP466 were amplified by PCR, cloned in *Escherichia coli* and expressed in this bacterial expression system.

Bacterially expressed recombinant proteins were purified by affinity chromatography.

Shrimps *P. monodon* were vaccinated with purified recombinant proteins by intramuscular injection. The relative percentage survival of the shrimps vaccinated with recombinant proteins is in the order VP28>VP39>VP466>VP281.

Shrimps vaccinated orally with bacteria expressing recombinant proteins and the extent of protection provided by the recombinant protein was in the order VP28>VP39>VP466>VP281.

The combined result of injection and oral vaccination with recombinant proteins suggested that injection method will offer better protection when compared with oral vaccination and also VP28 could be used as candidate for vaccination against WSSV.

dsRNA specific for the *vp28* gene of WSSV was prepared, also dsRNA of part of β-lactamase gene of *Edwardsiella tarda* which served as non specific negative control. dsRNA was delivered to shrimp by injection. Shrimp injected with vp28 dsRNA showed
100% survival following challenge while shrimp injected with $\beta$-lactamase dsRNA showed 100% mortality.

- RT-PCR was done to confirm the silencing of $vp28$ gene of WSSV in experimental shrimp and $\beta$-actin gene expression was used to check the quality of extracted RNA.

- Antisera raised against $vp28$ gene was used for western blotting to check the suppression of protein expression in the shrimp injected with VP28dsRNA.

- The results of the study suggest that recombinant protein vaccine and RNAi technology has potential to protect shrimp against WSSV infection.