9.0 SUMMARY AND CONCLUSION

The present study was made an attempt to identify the incidence of WSSV and MBV in different locations of India and to find out the susceptibility to infection and pathogenicity of white spot disease in *P. monodon* and *P. indicus* and to delineate the antiviral activity of unique chemical classes in both halophytic and non-halophytic medicinal plants responsible for the treatment of WSSV disease. The results of the present study are as follows:

- The results suggested that, Tamilnadu occupies the maximum percentage (39.5%) of WSSV infection particularly at Chidambaram but the maximum (8.6%) percentage of MBV infection was identified at Sirkali. Nagapattinam and Ramnad did not showed any sign of WSSV infections during the first year assessment.

- During the second year, the maximum (39.2%) percentage of WSSV infection was identified at Ponneri. Similarly, the maximum (15.5%) percentage of MBV infection was identified at Kalpakkam.

- During the third year, the maximum percentage of WSSV (46.6%) and MBV (13.3%) infections was identified at Chidambaram.
• In Andrapradesh, the maximum percentage of WSSV infection was identified by 41.1%, 46.6% and 61.9% at Ongole, Gudur and Kavali respectively. But, the maximum percentage of MBV infection was identified by 50% and 100% at Nellore and Ongole throughout the three years of assessment.

• In other states of India, the maximum percentage of WSSV infection was identified by 25 and 25% at Gujarat and Mumbai during first and second year assessment. But, none of the samples showed any sign of WSSV and MBV infections during the third year assessment.

• The present study was also made an attempt to identify the incidence of WSSV infections in broodstock and post larvae. The results reveals that, the maximum percentage of WSSV infection was identified by 85.7% and 85% with brood stock and post larvae’s during the month of October. But, the maximum (66.6%) percentage of MBV samples were identified during the month of December.

• In the present study, the results of incidence of WSSV infections suggested that, the maximum percentage (84.2%) of WSSV larval infection was identified during the month of October. Further, the maximum (84.2%) percentage of WSSV infection was also identified with post larval during the month of October. Similarly, the maximum
percentage (50%) of MBV infections were also identified with post larvae’s during the month of December.

- Shrimps inoculated with the 3 doses of WSSV by i.m route first displayed empty guts and reduced response to mechanical stimulus between 24 and 36hpi. The proportion of shrimp from each of the 3 doses that displayed these clinical signs. Shrimp used as controls did not display any of these clinical signs instead they remained healthy and survived throughout the experiments.

- The survival experiment reveals that, each of the 3 doses of WSSV inoculated by i.m. induced 100% mortalities. The mortality was recorded at 24hpi with each of the 3 doses tested. The cumulative mortality reached 100% at 84hpi in shrimps inoculated with $5.9 \times 10^6$ SID$_{50}$, $4.4 \times 10^5$ SID$_{50}$ doses while shrimps inoculated with $4.1 \times 10^4$ were all dead of 108hpi. Control animals survived entire experiment period of 96 hours. However, $5.9 \times 10^6$ concentration of viral cells have shown high virulence which have been died within 36hrs and in after concentration animals were died within 60hrs by injecting $4.4 \times 10^5$, But, $4.1 \times 10^4$ concentration injected animals have died within 84hrs. Besides that, all the animals has been observed sign of empty gut within 72hrs, but control animals did not showed any signs. However,
lowest concentration of $4.1 \times 10^4$ viral cells injected animals showed signs of empty gut after 36hrs. Control animals injected with saline solution did not showed any sign in animals and become active.

• It is important to noticed that, all WSSV-inoculated shrimps became infected. Control shrimp were WSSV-negative. Comparative study of different concentration of viral cells have been used to evaluate the stress and mortality. The concentration of viral cells or copies density is $5.9 \times 10^6 \mu L$, which is responsible for SID$_{50}$ (pathogenicity) to WSD and mortality in shrimps.

• The antiviral property of the crude extracts from chosen halophytes and non halophytes in *P.monodon* and *P.indicus* infected with the WSSV were carried out by the present study. The viability of the WSSV virus in the infected wild shrimps were checked with the control primer and confirmed the inoculated viral load of $5.9 \times 10^6$. Infected animals treated with non halophytic plant crude extracts reveals that, the *S.indicus* leaves decreased the viral load in *P. indicus* and *P. monodon* when compared with the control and the viral load was recorded as $5.14 \times 10^{11}$ and $4.18 \times 10^9$ with the thermal cycle value of 14.93 and 14.43 respectively.
• It is interesting to notice that, the Camphora drastically decreased the viral load up to 0.04 with the thermal cycle value of 31.81 in white shrimp *P.indicus* and tiger shrimp *P.monodon* with the viral load of $1.44 \times 10^{12}$ with the thermal cycle value of 14.36.

• In *Ocimum sanctum* treated animals, the viral load was recorded by $5.23 \times 10^9$ and $3.12 \times 10^7$ in *P.indicus* and *P.monodon*. The corresponding thermal cycle values were recorded as 18.42 and 20.12 respectively.

• In the case of halophytic plant *Avicennia marina*, the viral load was decreased by $8.85 \times 10^{11}$ in *P.monodon* and the thermal cycle value is 14.63. Controversely, the viral load was increased with $1.14 \times 10^{13}$ in *P.indicus* when compared with control with the thermal cycle value 20.12.

• Various plant parts (leaf, root, flower and stem) of the non halophytic plant *S.indicus* treated WSSV infected shrimps reveals that, the root extract decreased maximum $(2.12 \times 10^8)$ viral load with the thermal cycle value of 15.25 in *P.indicus* and decreased $3.14 \times 10^7$ in *P.monodon* with the thermal cycle value of 21.68. Moreover, the flower extract drastically decreased the viral load in *P.monodon* (1.34) with the thermal cycle value of 29.88. But in the case of *P.indicus*, the viral load was decreased with $1.34 \times 10^7$ and the thermal cycle value of 20.85. The
results of the *S.indicus* stem extract reveals that, the maximum reduction of viral load was recorded by 0.02 with the thermal cycle of 32.25 and minimum 0.05 with the thermal cycle of 31.77 in *P. monodon* and *P. indicus* respectively.

- The synergetic activity of the crude extracts from non halophytes in WSSV infected shrimps were also carried out by the present study. It reveals that, the complete removal of viral load was recorded in *P.monodon* and $8.25 \times 10^{11}$ level of viral load was recorded with the thermal cycle value 14.64 in *P.indicus*. Further, the synergetic activity of the crude extracts from halophytes and non halophytes reveals that, negligible amount (0.01) of viral load was recorded in *P.indicus* with the thermal cycle value 32.64 and the viral load was slightly decreased ($2.65 \times 10^{13}$) when compared with the control and the thermal cycle value was recorded as 12.73 in *P.monodon*.

- In addition to that, the animals were also treated with the positive control and negative control. It reveals that, the animals treated with the DMSO and PBS did not have viral load in negative control. But in the case of positive control, the viral load was recorded by $1.72 \times 10^{12}$ in *P.indicus* (T11W) and $2.12 \times 10^{13}$ in *P.monodon* with the thermal cycle values of 13.14 and 12.18 respectively.
• The present study was also made an attempt to identify the major chemical constituents of the most potent *S. indicus* stem extract by GC-MS analysis suggested that, of the selected 16 different chemical constituents four chemical constituents such as (E)-3,3-diphenyl-4-hexenoic acid, methyl (Z)-3,3-diphenyl-4-hexenoate, trans-5,5-diphenyl-3-hexenoic acid, cis-5,5-diphenyl-3-hexenoic acid were identified as major chemical constituents. Of the four compounds, the (E)-3,3-diphenyl-4-hexenoic acid is already reported to posses antimicrobial property which is commonly designated as Honokiol or Magnolol. This compound is recommended by the joint FAO/WHO expert committee as food additives. The Molecular formula is C\textsubscript{18}H\textsubscript{18}O\textsubscript{2} and the Molar mass is 266.334 g.mol\textsuperscript{-1}. The appearance is white solid, solubility in water sparingly (25 °C).

• It is concluded from the present study that, among the halophytic and non-halophytic medicinal plants, the *S. indicus* a non halophytic medicinal plants possess potential antiviral compounds for the treatment of white spot diseases in peneaid prawns so as to enable to increase the market potential of fishery resources as well as to enhance the economic return through the fishing sector.