<table>
<thead>
<tr>
<th>SL. NO.</th>
<th>CHAPTERS AND SIDE HEADINGS</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>REVIEW OF LITERATURE</td>
<td>4</td>
</tr>
<tr>
<td>2.1</td>
<td>Shrimp farming in India</td>
<td>4</td>
</tr>
<tr>
<td>2.2</td>
<td>Shrimp diseases</td>
<td>5</td>
</tr>
<tr>
<td>2.3</td>
<td>White spot syndrome virus</td>
<td>6</td>
</tr>
<tr>
<td>2.4</td>
<td>Host range of WSSV</td>
<td>7</td>
</tr>
<tr>
<td>2.5</td>
<td>WSSV histopathology</td>
<td>10</td>
</tr>
<tr>
<td>2.6</td>
<td>WSSV cytopathology</td>
<td>11</td>
</tr>
<tr>
<td>2.7</td>
<td>Morphology of WSSV</td>
<td>11</td>
</tr>
<tr>
<td>2.8</td>
<td>Techniques for WSSV detection</td>
<td>12</td>
</tr>
<tr>
<td>2.9</td>
<td>WSSV Genome</td>
<td>13</td>
</tr>
<tr>
<td>2.9.1</td>
<td>Major differences among the WSSV genomes</td>
<td>17</td>
</tr>
<tr>
<td>2.9.1.1</td>
<td>Occurrence of a large deletion</td>
<td>17</td>
</tr>
<tr>
<td>2.9.1.2</td>
<td>A variable region prone to recombination</td>
<td>19</td>
</tr>
<tr>
<td>2.9.1.3</td>
<td>Occurrence of a transposase sequence</td>
<td>19</td>
</tr>
<tr>
<td>2.9.1.4</td>
<td>Single nucleotide mutations</td>
<td>20</td>
</tr>
<tr>
<td>2.9.1.5</td>
<td>Variation in number of repeat units within hrs and direct repeats</td>
<td>21</td>
</tr>
<tr>
<td>2.10</td>
<td>Repeat patterns in genome and its relevance</td>
<td>26</td>
</tr>
<tr>
<td>2.11</td>
<td>Effect of differences on WSSV genes</td>
<td>27</td>
</tr>
<tr>
<td>2.12</td>
<td>Genomic studies in WSSV based on VNTRs</td>
<td>27</td>
</tr>
<tr>
<td>2.13</td>
<td>Evolutionary studies in WSSV</td>
<td>28</td>
</tr>
<tr>
<td>2.14</td>
<td>Fitness and Virulence differences in WSSV</td>
<td>29</td>
</tr>
<tr>
<td>3.</td>
<td>MATERIALS AND METHODS</td>
<td>30</td>
</tr>
<tr>
<td>3.1</td>
<td>Genotyping of WSSV prevalent in India</td>
<td>30</td>
</tr>
<tr>
<td>3.1.1</td>
<td>Collection of samples</td>
<td>30</td>
</tr>
<tr>
<td>3.1.2</td>
<td>DNA extraction</td>
<td>30</td>
</tr>
</tbody>
</table>
3.1.3 Diagnostic PCR for WSSV
3.1.4 Analysis of variable number tandem repeats (VNTRs)
3.1.5 Purification of PCR products
3.1.6 Cloning of purified PCR products
3.1.6.1 Ligation of PCR products into the cloning vector
3.1.6.2 Transformation
3.1.6.3 Screening of transformants
3.1.7 Plasmid DNA extraction
3.1.7.1 Plasmid extraction protocol
3.1.8 Sequence analysis
3.2 Epidemiological studies
3.2.1 Sampling
3.2.2 DNA Extraction and WSSV PCR
3.2.3 Genotyping PCR
3.3 Effect of environmental stress and hosts on WSSV minisatellite loci
3.3.1 Extraction of pure virus
3.3.2 WSSV passage through *Penaeus monodon*
3.3.3 Passaging of WSSV through different crustaceans
3.3.4 Infection of post larvae with WSSV
3.3.4.1 Effect of salinity stress on WSSV infected postlarvae of *P. monodon*
3.3.4.2 Effect of temperature stress on WSSV infected postlarvae of *P. monodon*
3.3.4.3 Effect of formalin stress on WSSV infected postlarvae of *P. monodon*
3.3.4.4 Effect of transportation stress on WSSV infected post larvae of *P. monodon*
3.3.4.5 Effect of EDTA stress on WSSV infected postlarvae of *P. monodon*
3.3.4.6 Effect of sodium hypochlorite stress on WSSV
infected post larvae of *P. monodon*

3.4 Comparison of virulence between different WSSV strains

3.4.1 Stains used for virulence study

3.4.2 Standardization of viral dose using real time PCR

3.4.3 WSSV virulence studies

3.4.4 Treatments in the study

3.4.5 Cumulative mortality and Real time PCR

3.4.6 Statistical analysis

3.5 To characterize the evolution of WSSV in India

3.5.1 Shrimp sampling and WSSV screening

3.5.2 Amplification of (ORF 23/24) and variable (ORF 14/15) regions

3.5.3 Amplification of transposase sequence

3.5.3.1 Dot blot hybridization

3.5.3.2 Labeling of PCR product

3.5.3.3 DNA spotting and hybridization

3.5.4 Cloning and sequence analysis

3.5.5 Bioinformatics studies and characterization of WSSV evolution

4. **EXPERIMENTAL RESULTS**

4.1 PCR for WSSV

4.2 Genotyping of WSSV prevalent in India

4.2.1 PCR for the 54 bp repeat unit (RU) in ORF 94

4.2.2 PCR for the 69 bp RU in ORF 125

4.2.3 PCR for the compound RU in ORF 75

4.3 Epidemiological studies

4.4 Effect of environmental conditions on WSSV minisatellite loci
4.4.1 WSSV passage through *Penaeus monodon* and other crustaceans 50

4.4.2 Effect of environmental stress on the WSSV infected PL 50

4.5 Comparison of virulence between three different WSSV strains 51

4.6 To characterize the evolution of WSSV in India 52

4.6.1 Analysis of ORF 23/24 region 52

4.6.2 Analysis of transposase region 53

4.6.3 Analysis of the ORF 14/15 regions 53

4.6.4 Bioinformatics studies and characterization of WSSV evolution 54

5. DISCUSSION 56

5.1 Genotyping of WSSV prevalent in India 57

5.2 Epidemiological studies 61

5.3 Effect of environmental conditions on WSSV minisatellite loci 62

5.4 Comparison of virulence between different WSSV strains 62

5.5 Characterizing the evolution of WSSV in India 64

6. SUMMARY 68

7. REFERENCES 70

8. APPENDIX (Publications and GenBank submissions) 83