Exploring the potential of *Sonneratia alba* leaf boiling extract as a natural preventive measure against causing white spot syndrome virus in tiger shrimp fingerlings

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**Abstract.** The possibility of mass mortality in farmed shrimp populations is a risk posed by WSSV. Vaccines and chemical drugs have been created to stop WSSV infection, but they can be harmful to the environment and human health. Therefore, natural prevention options are needed. 1% boiled *S. alba* extract was used in this study to test the technique of immersing tiger shrimp fry at different times before WSSV infection. The study was designed in RAL with eight treatments and three repetitions: These were A) 12-hour immersion; B) 24 hours; C) 36 hours; D) 48 hours; E) 60 hours; F) 72 hours; G) WSSV infection without soaking *S. alba* and H) without WSSV infection and *S. alba* extract. Treatment C had the best survival rate (85%) while the positive control had the lowest percentage (43.33%). Tiger prawns can increase resistance to WSSV infection by soaking them in 1% extract of boiled *S. alba* leaves. Water quality parameters were also measured, and the experimental results showed no significant changes. According to the results, immersion of tiger shrimp fry in 1% boiled *S. alba* extract for 36 hours was the most efficient way to increase fry survival from WSSV infection.

**1 Introduction**

*Penaeus monodon* is the primary species used for coastal aquaculture in Bangladesh and Southeast Asia [1,2]. Cultivation is experiencing problems due to the presence of a disease with typical clinical symptoms of white spots, White Spot Syndrome Virus (WSSV) is a common viral disease that is very deadly in Penaeid shrimp and many other crustaceans [3]. The WSSV virus, which is primarily responsible for outbreaks of White Spot Disease (WSD), has substantially contributed to the fall in shrimp output. [4,5,6]. When infecting shrimp post larvae and adults, WSSV can cause up to 80% mortality (attacks last several days to a week) [7]. Numerous hosts, including Penaeids, freshwater shrimp and crayfish, crabs, marine and brackish crustaceans, aquatic arthropods, and plankton, have been susceptible to this virus infection [8-11].

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Shrimp farming does not allow the use of antibiotics or pesticides because it causes environmental pollution, and the use of antibiotics has harmful effects and negative consequences, including the development of disease resistance at excessive doses [12-13]. In addition, excessive levels can increase pathogen resistance, among other undesirable effects [14]. Therefore, looking for alternatives to substitute pharmaceutical medications and antibiotics is very necessary.

A solution to the problem of disease attack, particularly WSSV in shrimp farming, is using natural materials that are quickly decomposed and environmentally acceptable. To be used as a substitute therapy for illnesses in aquaculture. The use of mangroves to treat diseases in aquaculture includes the treatment of WSSV, which first started in recent years [15-17].

Indonesia has abundant natural resources, including mangrove forests, so its utilization can be studied as an alternative treatment for shrimp ponds, including treating WSSV diseases. Previous research showed that several mangrove species growing in pond areas in South Sulawesi could be used to produce antibiotics, anti-vibriosis, and anti-WSSV [18-19]. *Exoecaria agallocha*, *Acanthus ilicifolius*, *Avicennia* sp., *Rhizophora mucronate*, *R. appiculata*, *Sonneratia* sp., and *Ceriops tagal* have all been mentioned as probable sources of protection against WSSV [20]. One of these mangrove species, *S. alba* has received widespread media attention for its alleged ability to create an anti-WSSV [21–22].

The use of *Sonneratia alba* for disease prevention in shrimp cultivation in ponds is designed by considering the environment and mangrove population. The approach used is that aquaculture activities can be guaranteed by maintaining the integrity of the mangrove ecosystem. Using *Sonneratia alba* for disease prevention measures is expected to reduce our dependence on dangerous chemicals or other practices that may harm the environment. This proactive strategy aims to ensure harmony between aquaculture and the mangrove ecosystem, thereby contributing to the health and sustainability of the mangrove ecosystem as a whole and encouraging sustainable and environmentally friendly aquaculture activities. This study aims to determine the potency of *S. alba* boiling extract with different soaking times for tiger prawns after WSS infection.

### 2 Research methods

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#### 2.1 Research procedure manufacture of WSSV suspension

This study procedure for making WSSV stock suspension white spots on shrimp carapace and WSSV positivity based on PCR detection are indicators of a WSSV outbreak in shrimp ponds in Takalar District, South Sulawesi. By altering the shrimp carapace and physiological solution, which were combined in a ratio of 1:3 and blended, shrimp samples were used for viral collection. The supernatant was centrifuged at 3000 x g for 20 minutes at 4°C. The supernatant was filtered using 0.4 L filter paper after being centrifuged at 8000 x g for 30 minutes at 4°C. For later usage, virus stocks were kept at -20°C [23].

#### 2.2 Research procedure

Glass jars with a 3L volume were employed as containers 40L of seawater with a salinity of 28 ppt was placed in 60L plastic containers with a 2L volume. When seawater is sterilized
with 150 ppm of chlorine and neutralized with 75 ppm of sodium thiosulfate, the salinity is 28 ppt-neutralized with 75 ppm of Sodium Thiosulphate.

The test animals used were PL35 tiger prawns at a density of 20 individuals per jar. S. alba boiling extract was used in the experiment at a concentration of 1% of rearing water. The immersion time variable was tested and evaluated using eight treatments. After immersion of 1% concentration of S. alba extract, tiger shrimp fingerlings were moved to an aquarium with saltwater with a salinity of 15 ppt for 15 minutes to perform the osmoregulation procedure. All treatments were infected with WSSV, except the negative control.

Rearing and observing tiger shrimp fingerlings were done for eight days, three times a day, with a dose of 20–40% of the daily biomass, as per the feeding schedule. Shrimp survival rate was monitored daily after WSSV infection, NH3-N, NO2-N, and Total Organic Matter, as well as other water quality indicators were measured at the start and end of the investigation.

2.3 Research design

This study design used a complete randomized design (RAL) consisting of eight treatments and three repeats. The treatments tested were as follows: A) Soaking for 12 hours; B) 24 hours; C) 36 hours; D) 48 hours; E) 60 hours; F) 72 hours; G) Positive control (infected with WSSV without soaking S. alba extract and; H) Negative control (without WSSV infection and S. alba extract)

2.4 Data analysis

An analysis of variance was performed on data about the survival rate of tiger shrimp fingerlings. This was followed by the Least Significant Difference test (Steel & Torrie, 1981). Furthermore, supplementary data analysis is descriptively examined and shown as tables and figures.

3 Results and discussion

3.1 Survival rate of tiger shrimp fingerling

Based on the investigation's findings into the application of S. alba boiling extract for WSSV disease prevention in tiger shrimp fingerlings, shown in Figure 1. The chart displays the impact of immersion in S. alba boiling extract at various intervals for each treatment on the survival rate of tiger shrimp larvae following WSSV infection from day one to day eight.
Fig. 1. The Survival Rate of tiger shrimp fingerlings after WSSV infection at various soaking durations in 1% *S. alba* extract. A) 12 hours of soaking; B) 24 hours; C) 36 hours; D) 48 hours; E) 60 hours; F) 72 hours; G) positive control (infected with WSSV without *S. alba* extract); and H) Negative control (without WSSV infection and *S. alba* extract)

Based on observations after WSSV infection by immersion, tiger shrimp fingerlings that had been soaked in 1% of the boiled extract of *S. alba* at different times, death occurred from the first day except in treatment D (48 hours of immersion) every day in all treatments until the fifth day with different percentage levels in each treatment with a range of 1.67-21.67%. On the sixth day of observation, the viability of the tiger shrimp fingerlings decreased. The death rate for treatment G (positive control of WSSV infection without soaking *S. alba* extract) was 31.67%.-At the same time, the treatment soaked with 1% boiled extract of *S. alba* had a lower mortality rate of 6.67-16.67%. After the trial ended on the eighth day, mortality continued to occur in each treatment with varying percentages. Tiger shrimp fingerlings immersed in *S. alba* extract survived between 70.0% and 85.00% after the WSSV challenge, whereas in the WSSV infection treatment without *S. alba* immersion, it was only 43.33%. The results of previous research on the use of *S. alba* extract via injection on adult tiger shrimp after being challenged with WSSV via injection showed that tiger shrimp death happened on the third day and had a 100% overall death rate. Statistical analysis revealed that it differed from other treatments (P< 0.05) [24.]

According to the findings of this investigation, soaking tiger shrimp fingerlings with *S. alba* extract can increase the survival of tiger prawns to fight WSSV infection attacks. This can be seen from the treatment with *S. alba* extract soaking, which had a lower mortality rate and better survival than the treatment without *S. alba* extract soaking. However, immersion's effectiveness also influences immersion's time and concentration.

PCR analysis of the test animals used in this study was negative for WSSV before the challenge test. At the end of the study (eighth day), live shrimp samples were analyzed to verify the mortality of shrimp larvae after testing for virus infection by immersion in the tiger shrimp fry-rearing containers. The results of PCR analysis formed a DNA band on agarose gel, which is a positive indicator of WSSV infection in the positive control treatment (G). In contrast, the other treatments were negative for WSSV.

The extract *S. alba* soaking treatment for 36 hours (Treatment C) showed the highest survival rate on the eighth day after WSSV infection. This research illustrates that *S. alba*
boiled extract has potential as an antiWSSV agent because it is effective in inhibiting WSSV infection, so it can help reduce the impact of this disease on tiger prawn cultivation [21-22].

### 3.2 Quality of water parameters

The outcomes of analyzing the parameters affecting the water quality of fingerlings rearing media, concentrations of ammonia, nitrite, and total organic matter at the study's beginning and ending points are shown in Table 1.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NH$_3$-N</th>
<th>NO$_2$-N</th>
<th>BOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>0,807</td>
<td>0,0022</td>
<td>24,650</td>
</tr>
<tr>
<td>A</td>
<td>9,676</td>
<td>0,0126</td>
<td>58,766</td>
</tr>
<tr>
<td>B</td>
<td>7,061</td>
<td>0,0065</td>
<td>54,450</td>
</tr>
<tr>
<td>C</td>
<td>7,7722</td>
<td>0,0057</td>
<td>60,520</td>
</tr>
<tr>
<td>D</td>
<td>6,1830</td>
<td>0,0060</td>
<td>54,636</td>
</tr>
<tr>
<td>E</td>
<td>7,0052</td>
<td>0,0061</td>
<td>56,535</td>
</tr>
<tr>
<td>F</td>
<td>6,9868</td>
<td>0,0059</td>
<td>57,230</td>
</tr>
<tr>
<td>G</td>
<td>6,7250</td>
<td>0,0060</td>
<td>56,134</td>
</tr>
<tr>
<td>H</td>
<td>6,9057</td>
<td>0,0060</td>
<td>56,633</td>
</tr>
</tbody>
</table>

Table 1 demonstrates that the concentrations of the water quality measure NH$_3$-N, NO$_2$-N, and BOT were initially low but increased in all treatments by the study's conclusion. The test animals did not consume the remaining meal during the trial, which could have harmed the test animals' health, which led to the high concentration of water quality in the tiger shrimp larvae rearing containers.

By preventing overfeeding and reducing the number of organic pollutants in culture water, proper water quality control in shrimp farming is essential to stop the spread of WSSV and maintain the health of shrimp fry. Unconsumed food and shrimp waste should be removed. In addition, sufficient aeration is also required to maintain dissolved oxygen levels in the water, which is necessary to keep the cultured organisms alive.

The study's results believed that the presence of feed residue from the rearing containers during the experiment played a role in poor water quality. These factors may have an impact on the health of the test animals. These compounds may have adverse effects on the health of the test animals.

### 4 Conclusion

One method to improve tiger shrimp fingerling's survival from WSSV infection is soaking in 1% boiled S. alba extract, with the most effective immersion period being 36 hours.

### Acknowledgments

The authors would like to express their gratitude to the research team, technicians, and contract workers of the fish and environmental health team of the Research Institute for Brackish Water Aquaculture and Fisheries Extension team at the Center for Brackish Water Aquaculture Research and Fisheries Extension who have the zeal to assist the author in conducting this research.
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