Survival and morphological performance of Black Tiger Shrimp larvae (*Penaeus monodon*) after immersion with extract of *Sargassum duplicatum*

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**Abstract.** This study aimed to evaluate the survival and morphological performance of Black Tiger Shrimp after immersion with *Sargassum duplicatum* extract as well after a *Vibrio harveyi* challenge. The experiment was performed in a completely randomized design with four treatments and 3 replicates each. Treatments consisted of immersions with *S. Duplicatum* extract (A) 600 ppm, (B) 700 ppm, (C) 800 ppm, and (D) control. Every unit contained 200 PL-4 shrimps. Shrimps were prior immersed into the extract for 15 minutes, then put into a plastic container for 7-day rearing. Survival and morphological performance (antennular, hepatopancreas, foregut, midgut, uropod, tail muscles, chromatophores, attachment, and stress) of larvae were observed at the end of the study. The challenge test was performed by an artificial infection with *Vibrio harveyi*. The results demonstrated no significant differences in immersion treatments on survival, yet it could indicate a non-toxic on 800 ppm of *S. duplicatum* for shrimp larvae. Hepatopancreas of shrimp *S. duplicatum* after the immersion was visibly better than not unless other observed organs that seemed no different. Further study is necessary for *S. duplicatum* extract in improving shrimp quality.

1. **Introduction**

Cultivation of Black Tiger Shrimp (*Penaeus monodon*) often suffers frustration in both hatchery and pond rearing as the ultimate effect of disease threats [1]. Vibriosis, a disease emerging from infection of *Vibrio harveyi* bacteria, is one of the common shrimp diseases. Vibriosis on hatcheries causes fluorescent tanks to glow at night. Vibriosis incident is possible in the phases of zoea, mysis, and early post larvae [2].

Efforts to control vibriosis are done using herbs, namely mangrove [3], seaweed [4], *Sargassum polycistum* [5]. *Sargassum duplicatum* has been utilized as an anticholesterol agent [6], biofuel [7], biofertilizer [8] antitumor [9]; anticancerantifouling [10,11], and antibacterial [12,13]. Therefore, this study intent to investigate the potential use of *S. duplicatum* to maintain the performance of Black Tiger Shrimp larvae.
2. Methods

2.1. PL-4 Shrimp immersion in the extract of Sargassum duplicatum
The experiment was conducted at the Black Tiger Shrimp Hatchery Facility in Barru regency owned by the Research Institute for Brackish Water Aquaculture and Fisheries Extension. The study was designed in a completely randomized design, 4 treatments 3 replications. Test animals used were PL4 stadia larvae.

The experiment was performed in a completely randomized design with four treatments and 3 replicates each. Treatments consisted of 15-minute immersions with extract of S. duplicatum (A) 600 ppm, (B) 700 ppm, (C) 800 ppm, and (D) control or no immersion. Every unit contained 200 PL-4 shrimps.

Shrimps were prior immersed into the extract for 15 minutes, then put into the plastic containers containing 30 liters sea water for 7-day. During the experiment, the media were kept owing water quality at the state condition of 30-31 ppt salinity, 29-30°C temperature, and 4 ppm dissolved oxygen (DO). At the end of the experiment, larval survival is calculated by the following formula:

\[
SR(\%) = \frac{n}{N} \times 100
\]

where \( SR \) = survival rate (%), \( n \) = number of shrimps survived at the end of the experiment, and \( N \) = stock number of shrimps.

2.2. Morphological performance
Observation of morphological performance was conducted at the end of the experiment. An amount of 6 shrimps were captured from every treatment. Several organs of the shrimps, namely antennular, hepatopancreas, foregut, midgut, uropod, tail muscles, chromatophores, attachment, and stress, were observed under a microscope with 4x magnification. Positive attribute (+) was awarded for normal organs unless abnormal ones got negative (-). The morphological score of shrimps was obtained by referring to the method of Harianti et al. (2005), with the following formula:

\[
NM = \frac{LN}{TI} \times PBM
\]

Where \( NM \) = morphological score, \( LN \) = number of normal shrimps observed, \( TI \) = total observed shrimps, \( PBM \) = percentage of morphological weights.

2.3. Challenge Test
The test was carried out in the Fish and Environmental Health Wet Lab Facility of Research Institute for Brackish Water Aquaculture and Fisheries Extension, Maros regency. The glass jars (prepared the same amount as the prior experimental units) were filled with 2 L chlorinated, aerated seawater at 28 ppt. An amount of 30 Shrimps, respectively, originated from the prior test were placed into the jars according to the prior unit. The test used Vibrio harveyi isolates obtained from the collection of the Research Institute for Brackish Water Aquaculture and Fisheries Extension in Maros. The Vibrio harveyi isolate, propagated in the Nutrient broth medium, then infusied into the jars with a final density of 3.05 x 106 cfu/mL. Shrimp survival was daily observed in 7 days.

Analysis of variance (Anova) was performed to the data of survival after the immersion and Vibrio challenge. The analysis was performed using Statistical Package for Social Science Version 16. While the morphological performance was analysed descriptively.
3. Results and discussion

3.1. Shrimp survival after immersion
Survival rates after the immersion were 95.33 ± 4.73 (B), 90 ± 7.94 (C), 87.5 ± 7 (A) respectively. While, and the control or no immersion was (D) 86.33 ± 18.04, exhibiting the lowest than others (Figure 1).

![Shrimp survival graph](image)

**Figure 1.** Shrimp survival (PL-4) after 7-day immersion in S. duplicatum EXTRACT. (A) 600 ppm, (B) 700 ppm, (C) 800 ppm, and (D) control or no immersion

The results demonstrated that no significant effect on survival observed. However, the values are still above 80%. This indicates a non-lethal concentration of overall treatments. After the treatment, shrimps could also survive until reaching 2-10 grams for approximately 1.5 months with the survival that still above 70%. The results also confirm a similar findings of Sudaryono [13], reporting the survival of White leg shrimp juvenile (83-93%) after being fed with hot water extracts of *Sargassum duplicatum*. While Yeh [12] revealed a very high survival (100%) after 3-h immersion with the similar extract.

3.2. Morphological performance
Observation on morphological performance resulted in the total scores were respectively 76.4 (A), 74.7 (B), 75.6 (C) and 74 (D) (table 1).

**Table 1.** Morphology score of Black Tiger Shrimp larvae after immersion with extract methanol/ aquades of *S. duplicatum*. (A) 600 ppm, (B) 700 ppm, (C) 800 ppm, and (D) control or no immersion

| Morphological attributes | Treatment | A  | B  | C  | D  |
|--------------------------|-----------|----|----|----|----|
| Antennular               | A         | 4.1| 5  | 5  | 5  |
| Hepatopancreas           | A         | 16.6| 16.6| 16.6| 13.3|
| Foregut                  | A         | 15 | 12.5| 12.5| 12.5|
| Midgut                   | A         | 6.6| 6.6| 6.6| 6.6|
| Uropod                   | A         | 10 | 8.3| 8.3| 10 |
| Tail Muscle              | A         | 4.1| 4.1| 5  | 5  |
| Chromatophore            | A         | 5  | 4.1| 4.1| 4.1|
| Attachment               | A         | 10 | 12.5| 12.5| 12.5|
| Stress                   | A         | 5  | 5  | 5  | 5  |
| Total                    | A         | 76.4| 74.7| 75.6| 74 |
The overall morphological attributes demonstrated that no differences were observed. This indicated that the immersion did not lead to any morphological abnormalities. Unless the only hepatopancreas showed any difference between treatments and control (Figure 2).

![Figure 2. Hepatopancreas of Black Tiger Shrimp larvae (a) without immersion of *S.duplicatum* EXTRACT, (b) with immersion](image)

Scores of hepatopancreas on treatment A, B and C are 16.6, while D (Control) is 13.3. This finding might be caused by the anti-microorganism active compound of *S.duplicatum* EXTRACT. The hepatopancreas of shrimp after immersion appeared to be relatively transparent, while the control has dark spots.

3.3. *Shrimp survival after Vibrio challenge*

Survival rates of Black Tiger Shrimp larvae after Vibrio challenge were 84.4±10.19% (C), 81.1±1.91% (B), 75.5±6.93% (D), and 72.2±1.91% (A) (Figure 3).

![Figure 3. The survival rate of Black Tiger Shrimp larvae after 7-day challenge with *V. harveyi*.](image)

A study of Rudi [4] tried to assay a feed containing active ingredients of *Gracilaria verrucose* to the performance of whiteleg shrimp after the 14-day challenge test, successfully resulted in the survival above 70%. Sudaryono [13] also conducted a study using hot water-extracting of *S. duplicatum*, obtaining 27–47% survival rate after the challenge with *V. parahaemolyticus*. The immersion of hot water-extracting of *S. duplicatum* to whiteleg shrimp juvenile could increase total hemocyte cells, phenoloxidase activity, respiratory burst, and clearance efficiency before and after being challenged with *V. alginolyticus* [12].
4. Conclusion
Based on the results and discussion, it can be concluded as follow:
1. Survival of Black Tiger Shrimp larvae after the immersion with S. duplicatum is more than 80%, which means the non-toxic for shrimp larvae.
2. Hepatopancreas of Black Tiger Shrimp larvae can improve better after the immersion with S. Duplicatum
3. Immersion with 800 ppm S. duplicatum EXTRACT promotes survival above 80% after the challenge with V. harveyi

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