Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(3): 2955 – 2969 (2025)
www.ejabf.journals.ekb.eg

The Effect of Different Media and Shelf Life on the Growth and Agar Content of Seaweed Seeds (*Gracilaria verrucosa*)

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ARTICLE INFO

Article History:
Received: Sept. 6, 2024
Accepted: May 19, 2025
Online: June 21, 2025

Keywords:
Agar content,
Media
Growth,
Sawdust,
Seaweed seeds,
Shelf life

ABSTRACT

Gracilaria is a type of seaweed that is widely cultivated around the world due to its antibacterial compounds and high economic value. This plant adapts quickly to ponds with fluctuating water quality conditions, making it easy to cultivate. However, there are several challenges related to seeds quality including media and shelf life which play a significant role. This research aimed to analyze effective media and optimal shelf life for *Gracillaria verrucosa* seeds.The method used was a laboratory experimental design with a randomized block design that includes two treatments and three replications of each. The difference in the first treatment was in media (straw, sawdust, and ice blokcs), while in the second treatment was shelf life (24, 48, and 72 hours). The parameters observed were the growth weight and agar content of *G. verrucosa*, as well as water quality (temperature, pH, dissolved oxygen, salinity, ammonia, nitrate, nitrite, and phosphate). The research results showed that the growing media and shelf life significantly affect the growth of *G. verrucosa* seaweed seedlings, as indicated by the difference in absolute growth rate and specific growth rate in each treatment. The use of sawdust and straw as media for seaweed seeds has a significantly higher effect on weight gain and growth rate of *G. verrucosa* compared to ice blocks. However, the best media was sawdust. Seaweed seeds stored for 72 hours exhibit higher weight gain and growth rates compared to those stored for 24 and 48 hours. Shelf life also affected the agar content of *G. verrucosa* seaweed. The longer the shelf life, the higher the agar content tended to be.

INTRODUCTION

Gracilaria is a type of seaweed that is widely cultivated in the world alongside Kappaphycus, Laminaria, Undaria, and Porphyra. Gracilaria is highly valued for its agar content, which is widely utilized as a feed and food additive **(Hadijah *et al.*, 2020)**, as well as a raw material in the pharmaceutical and cosmetic industries **(Peñalver *et al.*, 2020; Prasedya *et al.*, 2023)** as thickener, stabilizer, and emulsifying agent **(Liao *et al.*, 2021)**. Gracilaria also contains antibacterial compounds such as alkaloids, flavonoids, and steroids that have been proven to inhibit bacterial growth **(Zainuddin *et al.*, 2019)**. In addition to its economic value, Gracilaria plays an important ecological role by effectively binding nitrogen, phosphorus, and carbon, thereby contributing to the maintenance of aquatic ecosystem balance **(Arbit *et al.*, 2019; Gomez-Zavaglia *et al.*, 2019)**. Gracilaria readily adapts to ponds with fluctuating water quality and exhibits a high tolerance to various environmental factors, which makes it highly suitable for cultivation **(Purnomo *et al.*, 2021)**.

South Sulawesi ranks first for seaweed production in Indonesia, amounting to 1,623,302 million tons, with 19.11% cultivated from ponds and 80.89% from the sea **(BPS, 2021)**. One of the determining factors of cultivation success is the seeds, which can be obtained from nature, cultivation and tissue culture results. However, over time, the genetic quality is declining significantly since seaweed farmers typically plant seeds from previously cultivated seaweed (progeny seed). The decline in genetic quality is partly due to negative selection, making it harder to find high-quality seeds these days. This is due to the difficulty of bringing in tissue culture seedlings from outside the region because seaweed seedlings are prone to breaking and cannot survive for long at certain temperatures. Genetic quality can be improved through breeding and cultivation **(Hwang *et al*., 2019)**. Therefore, growing media are needed to maintain the freshness of seaweed seedlings for a certain period under various conditions, while also being easily obtainable and environmentally friendly.

MATERIALS AND METHODS

1. Study sites and data collection

This research was conducted in the Marana Experimental Pond Installation area of the Brackish Water Aquaculture Research and Fisheries Extension Center (BRPBAPPP), Marannu Village, Lau District, Maros Regency (Latitude 4°56'33.126"S, Longitude 119°34'55.1676"E) for 60 days. This was followed by the measurement of agar content at the Biotechnology Laboratory and the measurement of water quality parameters in the Water Quality Laboratory. The tools used in this research include: 47cm x 31cm x 26cm Styrofoam boxes, digital scales, adhesive tape, knives, scissors, secchi disk, water quality checker (WQC), thermometer, glass bottles, and labels. The materials used in this research were: seaweed (*G. verrucosa*), straw, sawdust, and ice blocks.

The method employed was a laboratory-based experiment using Randomized Block Design (RBD). The research activities were divided into three stages including:

a. Selection of *G. verrucosa* seedlings: This was conducted based on the Standard Operating Procedure (SOP) for providing quality seaweed seedlings, which states that the seedlings should be taken from healthy, fresh plants, free from spots, with a uniform color, and not easily broken.

b. Packaging of *G. verrucosa* seedlings: This was done with two treatments, each with three replications. Treatment (1): Growing media consisting of straw, sawdust, and ice blocks. Treatment (2): Shelf life of 24 hours, 48 hours, and 72 hours. In the first step, straw, sawdust, and ice were placed and arranged in each prepared styrofoam box until they reached a height of 4cm. Then, 100 grams of *G. verrucosa* seedlings were evenly distributed in the styrofoam boxes, and the growing media was added again in the same amount as before until the height reached 4cm. The styrofoam boxes were tightly closed and sealed with adhesive tape to prevent contamination from open air. For the second step, the firmly sealed styrofoam boxes were kept for 24 hours, 48 hours, and 72 hours. This treatment was replicated three times.
c. Planting of *G. verrucosa* seedlings: This was accomplished by directly sowing the seedlings into 1x1 m plots, with a total of 27 plots, each with 100 grams of seedlings. Each plot was surrounded by netting with a large mesh size to ensure smooth water circulation. Planting was done in the morning (Fig. 1).

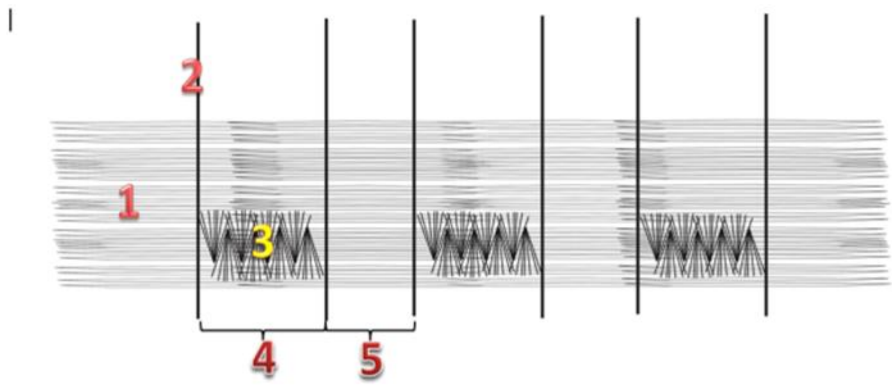

Fig. 1. Seaweed planting method in a pond plot. (1) Minimum water depth of 60cm, (2) Four 1m long bamboo poles are planted at the corners of the plot, inserted into the pond bottom about 10cm deep, (3) *G. verrucosa* seaweed, (4) 1x1m observation plot, (5) Each plot spaced 50cm apart

2. Observation parameters

2.1. Seaweed growth measurement

Seaweed growth was measured by weighing the seaweed. This was done by first taking seaweed samples from each plot, then weighing them before returning them to their original plots. The weight of the seaweed was measured every 7 days for a total of 7 times.

2.2. Water quality

The water quality parameters measured in the seaweed cultivation pond were pH, temperature, salinity, and dissolved oxygen. These measurements were taken before weighing the seaweed. Measurements of nitrite, nitrate, phosphate, and ammonia in the water were taken before and after the experiment. Water exchange in the pond was adjusted according to the tide and the condition of the water outside the pond when water was to be added.

2.3. Agar content

The *G. verrucosa* seaweed samples used were taken from the best growing media, which was sawdust, for all shelf life treatments. It is assumed that the shelf life affects the seaweed agar content. The seaweed samples were dried and weighed 10 grams, then soaked in a calcium hypochlorite solution (0.25%) for 3 days. Next, they were washed with running water and soaked again in freshwater for 3 hours until colorless. Then, they were soaked in a sulfuric acid solution (0.1%) for 15 minutes and rinsed with running water, then soaked in clean water for 15 minutes. The sample was supplemented with 500ml of distilled water in a hot plate at 150-200°C, 350 rpm until the volume reached 400ml. The extract was filtered, and the filtrate was oven-dried (at 60±5)°C for 3 days before being weighed **(Jayasinghe *et al.*, 2016)**.

3. Data analysis

The research data included growth rate of *G. verrucosa* calculated based on absolute growth rate (AGR) and specific growth rate (SGR). Supporting data on water quality parameters were described descriptively. ANOVA was used to analyze AGR and SGR, aiming to determine the effects of media and shelf life on the growth rate of *G. verrucosa*.

RESULTS

1. AGR (Absolute growth rate) of *Gracillaria verrucosa*

The results of the absolute growth rate (AGR) observation of *Gracilaria verrucosa* over a 49-day cultivation period with different growing media and shelf life showed an increase in wet weight growth in all treatments, except for the ice blocks media which experienced a weight decrease. The highest average absolute growth rate was obtained in the sawdust media with a shelf life of 72 hours (183.33±3.51g), followed by the straw media (163.00±4.00g). In contrast, the ice blocks media showed a decreasing trend in weight over time (51.33±3.51g) (Fig. 2).

The absolute growth rate of *G. verrucosa* can be influenced by the type of growing media and the length of seed shelf life. Sawdust and straw are two types of media that can be used for growing *G. verrucosa* seeds compared to ice blocks. However, sawdust is the most suitable media for growing seed. Sawdust acts as a medium that absorbs water, maintains humidity, and provides good aeration for a long period. This is in line with the findings of **Younis *et al.* (2022)** concerning sawdust having the ability to absorb water and maintain humidity, as well as providing sufficient aeration for seaweed.

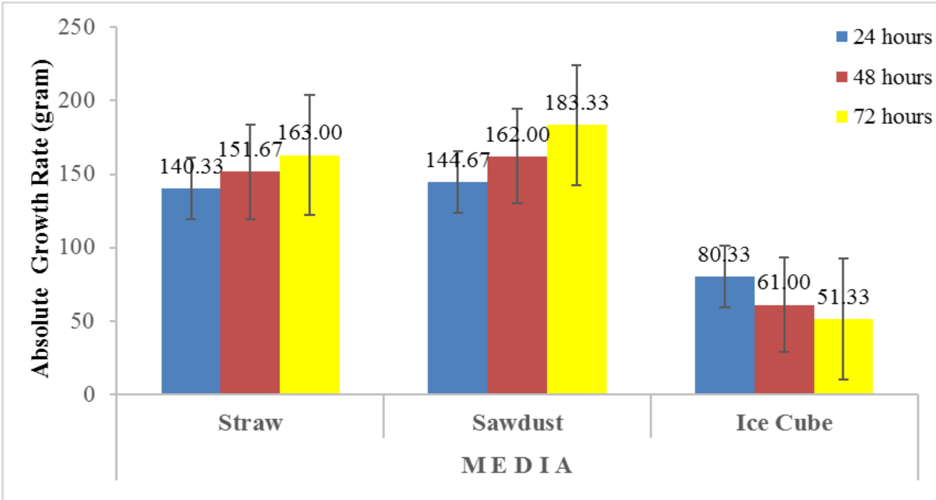

Fig. 2. Absolute growth rate of *G. verrucosa*

Although sawdust does not provide direct nutrients (nitrogen) like straw, it takes a longer time to decompose, making it suitable for growing media of seaweed seedlings **(Rominiyi *et al*., 2017)**. This is because sawdust is rich in cellulose and hemicellulose, which can strengthen cell walls and help maintain the shape and structure of seaweed **(Cosgrove, 2016)**. In addition, sawdust has high moisture retention and is available almost year-round, so if the wood species of the sawdust has high-quality, it can formulate a high-quality potting mix as well. Sawdust can hold more water, making it an excellent growing medium for seaweed seedlings. Sawdust is very effective for planting potted plants if composting of the sawdust residue is done before it is used in the potting mix **(Younis *et al*., 2022)**. In contrast, straw decomposes more quickly into organic matter **(Reichel *et al*., 2018)**.

The decrease in the absolute weight of seaweed in the ice block media is due to a combination of unfavorable physiological and environmental factors that do not support optimal growth. Low temperatures limit metabolic activity, causing physiological stress, reducing nutrient availability, and creating unsuitable environmental conditions. To achieve optimal growth, seaweed requires stable environmental conditions with appropriate temperature, nutrients, and light. The use of ice block media does not provide these conditions, resulting in a decrease in the absolute weight of seaweed over time **(Roleda & Hurd, 2019)**.

2. SGR (Specific growth rate) of *Gracillaria verrucosa*

Specific growth rate (SGR) was measured to evaluate the efficiency or relative growth rate compared to the initial biomass of the seaweed. The specific growth rate of

G. verrucosa over a 49-day cultivation period with different growing media and seed shelf life showed that the longer the shelf life, the higher the relative growth rate from the initial weight of the seaweed, except for the ice block media which showed a decrease. The specific growth rate with a seed shelf life of 24 hours in straw and sawdust media were 1.75 and 1.79% per day, respectively. The specific growth rate with a seed shelf life of 48 hours in straw media was 1.85% per day, compared to 1.91% per day in sawdust. The specific growth rate with a seed shelf life of 72 hours in straw was 1.93% per day, while in sawdust it was 2.08% per day. The specific growth rate of *G. verrucosa* in both straw and sawdust were considered normal. The results are consistent with the findings of **Susilowati *et al*. (2014)** who proved that the specific growth rate of *G. verrucosa* is in the range of 1.77 - 2.64% per day (Fig. 3).

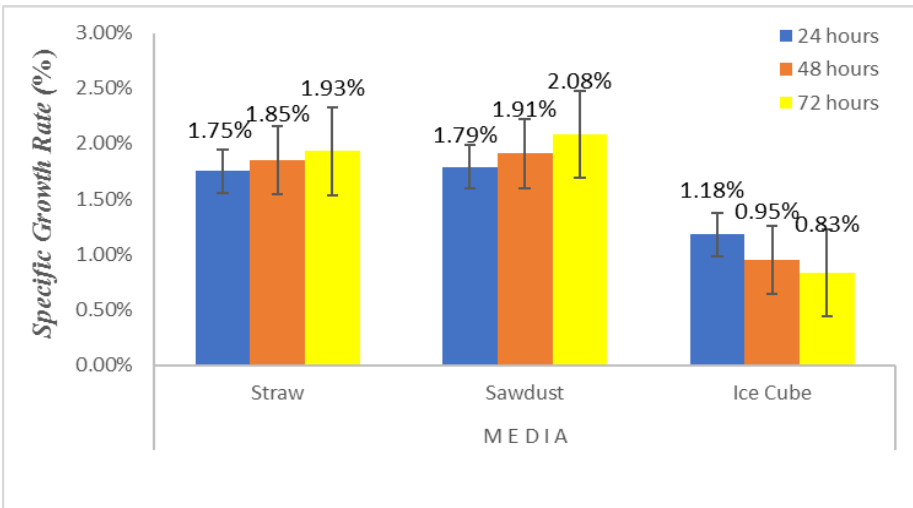

Fig. 3. Specific growth rate (SGR) of *G. verrucosa*

The specific growth rate (SGR) data of *G. verrucosa* under different growing media and shelf-life treatments showed a significant influence. While there was no significant difference between straw and sawdust media, the difference with ice blocks media was very significant. When using ice blocks, the longer shelf life, the lower the specific growth rate percentage of *G. verrucosa*. On the contrary, using straw and sawdust as growing media showed the longer the shelf life, the higher the specific growth rate percentage of *G. verrucosa*. Growing media is a key factor in providing a balance between water retention and good drainage for seaweed **(Bunt, 2012)**. The use of organic planting media such as straw and sawdust can enhance water usage efficiency and nutrient utilization efficiency, especially for farmers who are facing land shortages due to climate change **(Sahoo *et al*., 2023)**. A growing medium that is too dense or too loose can inhibit seaweed growth. Therefore, sawdust has a slightly higher specific growth rate percentage compared to straw, although the difference is not significant, because sawdust has better water retention and maintains aeration compared to straw **(Banitalebi *et al*., 2019)**.

The longer the shelf life of seaweed seeds, the more the straw and sawdust media undergo composting or biochar formation processes, resulting in organic substances and components similar to biostimulants, such as humic acid, which can enhance nutrient absorption efficiency, improve the quality of the growing media, and support the activity of microorganisms beneficial for the seaweed seedlings growth **(Gupta *et al*., 2021)**. Furthermore, the composting process produces beneficial bioactive compounds that enrich the nutrients content of the growing media and pave the way for more sustainable agriculture in the future **(Schmid, 2021)**. The use of straw and sawdust as planting media is an effective approach to utilize biomass waste that has the potential to be discarded. Both of these materials can support plant growth, such as seaweed, and can be processed into valuable products, such as compost, biochar, as well as effective biofertilizers and biopesticides. As a result, biological resources are becoming increasingly important in sustainable food production and environmental preservation. The utilization of straw and sawdust as biofertilizers is an example of the use of nutrient-rich agricultural solid waste, thus having the potential to create new beneficial products. This cutting-edge technology not only aids in the management of agricultural waste but also supports environmental preservation and strengthens the national economy **(Akanmu *et al.*, 2023; Rizwan *et al.*, 2023; Singh *et al.*, 2024)**.

On the other hand, the longer shelf life using ice blocks media, the lower the specific growth rate percentage. This is caused by the ice blocks media melting over time causing waterlogging, which risks root rot and inhibits specific growth in seaweed **(Buga & Petek, 2023)**. Another factor affecting the specific growth rate of seaweed is environmental temperature. Low temperatures produced by ice blocks affect the rate of enzymatic reactions in seaweed, causing metabolic disturbances and inhibiting seaweed growth **(Eggert, 2012)**.

3. Agar content of *G. verrucosa*

The agar content of *Gracilaria verrucosa* is closely related to the seaweed's growth rate. Optimal growth rates are often associated with higher agar content in terms of both quantity and quality, although many other factors also play a role in determining the final outcome. The results of the agar content analysis of *G. verrucosa* showed that the shelf life affected the agar content (Fig. 4). Descriptive analysis showed that the highest agar content was found at a shelf life of 72 hours, at 21.64%, followed by a shelf life of 48 hours at 18.23%, and the lowest at a shelf life of 24 hours at 15.21% with an initial agar content of 17.66%. There was no significant difference in the agar content of *G. verrucosa* using sawdust media with shelf life of 0, 24, 48, and 72 hours, where the agar content ranged from 15.21- 21.64%. This is in line with the findings of **Rahim *et al.***

(2016) elucidating that the agar content of *G. verrucosa* ranges from 9.98-26.52%. (Fig. 4).

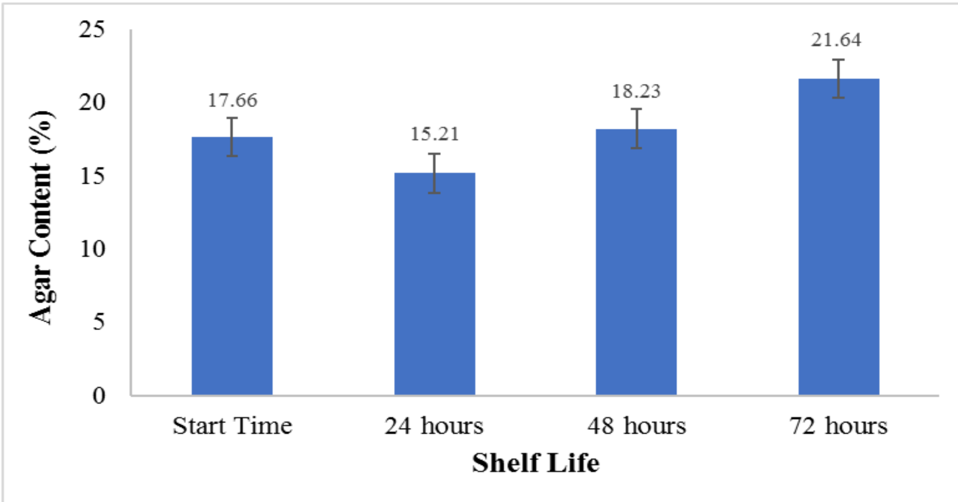

Fig. 4. The agar content of *Gracillaria verrucosa*

The growth and agar content of *G. verrucosa* are interconnected and influenced by environmental factors and physiological conditions. Generally, seaweed in active growing phase has a higher agar content compared to older seaweed. Optimal growth allows *G. verrucosa* to produce and store more agar. Longer shelf life also promotes agar production as a response to mild stress due to water deficiency, which strengthens cell walls and helps the plant survive **(Rejeki *et al.*, 2018; Gupta *et al.*, 2021)**.

Other factors influencing agar content include plant density and seaweed growth conditions in the pond. Seaweed initial weight does not directly affect agar content, but is more related to overall growth and productivity rather than specific agar content. High seaweed density when planting will create a competition for space, nutrients, and light, which can affect the quality and agar content **(Rahim *et al*., 2023)**.

The age of seaweed seedlings is also a factor affecting agar content. Older seaweed seedlings tend to have higher agar content compared to younger seaweed. This is due to the accumulation of polysaccharides (including agar) as the seaweed ages. However, too old an age can also cause a decrease in agar quality due to structural changes in the seaweed tissue that may affect gel strength or agar purity **(Baweja *et al*., 2016)**.

4. Water quality

Water quality is one of the factors that influence the growth and agar yield of seaweed. Water quality variables, especially temperature and light intensity, play an important role in agar formation. Agar content increases along with the rising of light intensity, which can affect photosynthesis in producing agar carbohydrates. In general, the water temperature in the pond during the study supported seaweed growth, ranging from 26.5- 31.6°C (Table 1). This is in line with that of **Lee *et al*. (2016)**, who noticed that the optimal temperature range of pond water for *Gracilaria* sp. cultivation is between 20 and 34°C. Further **Ya'la *et al.* (2024)** added that the optimal temperature for the growth of the seaweed *G. verrucosa* is between 25–33°C.

Dissolved oxygen (DO) plays an important role in seaweed growth and nutrient content. High DO levels are usually an indication of good water quality, helping to avoid conditions that can inhibit seaweed growth, such as the accumulation of toxic substances or nutrient deficiencies. DO levels during the study ranged from 2.83 – 4.8mg/ L (Table 1). The optimum DO range for seaweed growth in the sea is 4 – 8mg/ L **(Xin *et al*., 2019)**, however according to **Ya'la *et al.* (2024)**, it is between 4–14ppm. Meanwhile, the optimum DO range is 5– 7mg/ L for seaweed growth in the ponds **(Diana *et al.*, 2017)**. The minimum value in this study was below the optimal range, but still within the tolerance limit. This is likely due to the data collection being achieved when photosynthesis rate was declining, therefore the DO level was lower. However, the minimum level that can still be tolerated by most cultured organisms is usually around 3mg/ L.

Table 1. Water quality during the cultivation of *Gracillaria verrucosa* in the pond

Variable	Minimum	Maximum	Average	SNI Standard Value
Temperature (^{o}C)	26.5	31.6		25-30
Disolved Oxygen (mg/L)	2.83	4.8		Min. 3
Salinity (ppt)	24.81	29.65		28-34
pH	7.5	8.12		7-8.5
Ammonia (mg/L)			0.518	Max. 1
Phosphate (mg/L)			0.156	0.01-0.09
Nitrate (mg/L)			0.006	0.01-0.07
Nitrite (mg/L)			0.169	Max. 0.06

Source: Primary Data, 2024.

The salinity range of the pond water during the study was approximately 24.81–29.65ppt (Table 1). This range is below the SNI standard (7671.3:2010) which sets the optimal salinity at 28– 34ppt **(Hoq *et al.*, 2016)**. In fact, according to **Nguyen *et al.* (2016)** and **Ya'la *et al.* (2024)**, *G. verrucosa* can thrive well within a salinity range of 5–35ppt, with optimal range around between 15–25ppt. The pH value during the study was in the range of 7.5– 8.12 (Table 1). This range is in accordance with the SNI standard (7671.3:2010) of 7– 8.5 and according to **Simatupang *et al.* (2021)** and **Muchdar *et al.* (2024)**, the optimal pH value for seaweed growth is between 7.5- 8.5. The cultivated biota has adaptations to pH values. Therefore, the pH value for the *G. verrucosa* seaweed cultivation pond falls within the optimal range for seaweed growth and agar content.

Ammonia, nitrite, and nitrate are part of the nitrogen cycle, where nitrogen from organic matter is converted into various forms that can be used by aquatic organisms, with each stage affecting water composition and quality. The measured ammonia value in the research pond was 0.518mg/ L (Table 1). This value is still below the maximum threshold of the SNI standard (7671.3:2010) of 1mg/ L. The ammonia value in this study is still within a safe range, which is still below the threshold **(Zuldin & Shapawi, 2015)**. High ammonia concentration can be harmful to seaweed, causing tissue damage, inhibiting growth, or even death.

The nitrate content of the pond during the study was 0.006mg/ L (Table 1). This value is below the SNI standard range (7671.3:2010) of 0.01 – 0.07mg/ L, but still within the tolerance limit. Values below optimal range do not significantly affect agar content, but can reduce the growth rate of seaweed **(Jeliani *et al.*, 2018)**. Nitrate deficiency is indicated by the occurrence of bleaching on the red algae thallus. Nitrate is considered a limiting nutrient for algal growth if its content lower than phosphate in water bodies.

The nitrite content in the research pond was about 0.1687mg/ L (Table 1). This value exceeds the maximum limit of the SNI standard (7671.3:2010) of 0.06mg/ L, but is still within the tolerance limit and does not have a significant negative effect on seaweed growth or physiology because it is still less than 1mg/ L. At this level, nitrite can still be assimilated or converted into other forms of nitrogen, without causing damage to the seaweed (**Ciji & Akhtar, 2020)**.

Similar to nitrite which exceeded the SNI standard range (0.01-0.09mg/ L), the phosphate content of the research pond was 0.1561mg/ L. However, the phosphate range is still tolerable for the growth of *G. verrucosa*. Therefore, although it exceeds the SNI standard, the phosphate value in this study is still within the optimal range for seaweed growth. Phosphate is a major nutrient factor for algae and plays a role in protein formation and photosynthesis **(Sulistiawati *et al.*, 2020)**.

CONCLUSION

Growing media and shelf life proved to have a significant impact on the growth of *Gracilaria verrucosa* seedlings, as reflected by differences in absolute growth rate and specific growth rate in each treatments. The use of sawdust and straw as growing media for seaweed seedlings had a significantly greater effect on weight gain and specific growth rate of *G. verrucosa* compared to the use ice blocks. However, the best medium was sawdust. Seaweed seedlings shelf life of 72 hours had higher weight gain and specific growth rate compared to 24 and 48 hours. Shelf life also affected the agar content of *G. verrucosa*, with longer shelf durations, generally associated with increased agar content.

Acknowledgements

We would like to thank the Ministry of Education, Culture, Research, and Technology for providing financial support through Master's Thesis Research scheme. We also thank the Brackish Water Aquaculture Research and Fisheries Extension Center (BRPBAP3) Maros for their data support and facilities, enabling the successful completion of this research. Special thanks to all staff and employees of the Marana Experimental Pond Facility (ITP) for their assistance throughout this research.

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