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# GROWTH RESPONSE *OF Caulerpa racemosa* ON DIFFERENT CULTURE MEDIA (CONTAINERS VS. TARPAULINS) UNDER CONTROLLED CONDITIONS

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## ABSTRACT

*Caulerpa racemosa* is a commercially valuable macroalga, and cultivation conditions can influence its growth performance. Differences in environmental stability across cultivation media can lead to variations in stolon growth, ramification, and biomass accumulation, necessitating an evaluation of the optimal cultivation system under controlled conditions. The objective of this study was to compare the growth of primary stolons, ramules, and fresh biomass of *C. racemosa* cultivated in containers and tarpaulin tanks, and to determine the most effective medium for supporting vegetative growth in a controlled environment. This study employed a Completely Randomized Design (CRD) with two cultivation medium treatments (recycled water containers and tarpaulin tanks) over 30 days, with the containers maintained under similar environmental conditions, including controlled light intensity, salinity, and water circulation. Growth parameters (stolon length, ramification, and fresh biomass) were measured and compared across treatments. Stolon growth in the container medium reached an average length of 21.7–24.0 cm, whereas in the tarpaulin trough it ranged from 11.0 to 20.6 cm. Growth in the container medium was optimal due to more stable environmental conditions (smaller fluctuations in temperature and pH) and was statistically significantly higher than other treatments ($p < 0.05$). Containers can be recommended as an efficient cultivation medium for *C. racemosa* at the laboratory and pre-production scales, owing to their greater environmental stability and improved growth performance. Further research is recommended to evaluate nutrient optimization and scalability for coastal aquaculture applications.

**Keywords:** Caulerpa; Growth; Media; Biomass

## INTRODUCTION

*Caulerpa racemosa* is an economically important green seaweed widely used as a functional food, as a natural feed in aquaculture, and as a source of bioactive compounds with strong antioxidant and antibacterial activities (Yap *et al*., 2019). This seaweed also plays a crucial ecological role in coastal ecosystems, including nutrient uptake, oxygen provision, and substrate stabilization (Dermawan *et al*., 2024; Yilmaz & Aksu, 2023). However, *C. racemosa* is vulnerable to environmental changes, such as rising temperatures and pollution, making controlled cultivation systems essential (Landi *et al.,* 2022). With increasing commercial and biotechnological demand, the development of efficient and sustainable cultivation systems has become critically important. However, the growth of *C. racemosa* is highly influenced by environmental stability , particularly light intensity, temperature, salinity, and water circulation. Cultivation systems that fail to maintain stable conditions often result in reduced vegetative regeneration and lower biomass productivity.

Previous studies have shown that variations in light intensity can significantly affect the growth rate and biomass *accumulation in C. racemosa* (Wulandari *et al*., 2020). Stable environmental conditions, including temperature, salinity, and nutrient availability, are also known to enhance biomass productivity (Sari *et al*., 2023). The development of stolons and ramules, the primary vegetative structures responsible for nutrient uptake and regeneration, is highly dependent on the culture medium's ability to maintain these parameters (Valentine *et al*., 2021). Various cultivation systems have been explored, ranging from open systems such as tarpaulin ponds and tidal ponds to closed systems such as small containers or photobioreactors (Chen *et al*., 2019; Kim *et al*., 2017). Closed systems offer better environmental control and reduce the risk of contamination, but are limited by higher costs and smaller cultivation volumes (Schmitz *et al*., 2022). Conversely, open systems allow for large-scale biomass production but are more susceptible to environmental fluctuations and contamination (Zhang *et al*., 2022).

Although the influence of environmental conditions on macroalgal growth has been extensively studied, direct comparative research on *C. racemosa* cultivated in closed-container systems versus open-tarpaulin systems remains limited, particularly regarding stolon elongation and ramification. This knowledge gap presents a challenge in selecting the optimal cultivation system for early seedling development and biomass enhancement. The novelty of this study lies in the experimental comparison of two cultivation media under controlled conditions, enabling a clear assessment of which system better supports vegetative growth. These findings are significant for improving propagation efficiency, increasing biomass yields, and supporting sustainable and scalable seaweed cultivation strategies in tropical coastal regions.

This study aims to analyze differences in primary stolon growth, ramification, and fresh biomass of *Caulerpa racemosa* cultivated in containers and tarpaulin tanks under controlled environmental conditions. The expected outcome is to identify the most effective cultivation medium for supporting optimal vegetative growth. The results of this study are expected



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to contribute to the development of efficient, environmentally friendly, and large-scale cultivation techniques for *C. racemosa*, particularly for seedling production and sustainable aquaculture applications in tropical coastal ecosystems.

## RESEARCH METHODS

### Time and Location of the Study

This study was conducted over 30 days, from October 1 to 30, 2025, at Un Taar Bay, South Dullah Island, Tual City, Maluku.

### Equipment and Materials

The equipment used in this study included an aerator pump (Resun LP-40, Resun, China), a salinity refractometer (ATC Handheld Salinity Refractometer, RHS-10ATC, China), a pH meter (Hanna Instruments HI98107, USA), a digital thermometer (Xiaomi Mi Digital Thermometer, China), an analytical balance (Ohaus Pioneer PA214, USA), a tarpaulin tub (80 L, local supplier, Indonesia), and a container culture vessel (5 L, local supplier, Indonesia).

The materials used in this study were healthy *Caulerpa racemosa* seedling cultures (local coastal stock, Tual, Indonesia), natural seawater (salinity 30–32 ppt), young coconut water (local supplier, Indonesia), Moringa oleifera leaf extract (local supplier, Indonesia), and rice washing water (household source, Indonesia).

Coconut water and plant extracts are used as organic materials and natural nutrient sources in algal cultivation because they contain macro- and micronutrients that support growth (Hernandez-Herrera *et al.,* 2023).

### Research Design

This study employed a Completely Randomized Design (CRD) with two cultivation treatments: P1: Container culture medium (5 L volume) and P2: Tarpaulin tank culture medium (80 L volume). Each treatment was conducted in triplicate, resulting in a total of 6 experimental units consistently coded as: P1A, P1B, P1C (for containers) and P2A, P2B, P2C (for tarpaulins). The layout of each experimental unit was determined entirely at random through a randomization method to comply with RAL principles (Table 1). This was done to ensure that each experimental unit received equal environmental exposure and to minimize bias from external factors, such as differences in light intensity or air circulation at the research site. Seawater with a salinity of 30–32 ppt was used in each container, accompanied by continuous moderate aeration. Seawater with a salinity of 30–32 ppt is the optimal condition for the growth of *Caulerpa racemosa* (Kumar *et al.,* 2023).

**Table 1.** Layout of Experimental Units at the Randomized Location (RAL)

| | Column 1 | Column 2 | Column 3 |
|---|---|---|---|
| Row 1 | P2 -B | P1 -A | P2 -A |
| Row 2 | P1 -C | P2 -C | Pi -B |

Notes: P1 = Container; P2 = Tarpaulin Tub; A, B, C = Replicates.

### Preparation and Maintenance of Cultures

*Caulerpa racemosa* seedlings (±150 g per container) were cleaned and placed into their respective culture media. An organic fertilizer consisting of young coconut water, Moringa oleifera leaf extract, and rice washing water was applied. The method used was a combination of seedling soaking (*pretreatment*) and media fertilization. In the container treatment, 2 liters of fertilizer solution were soaked for 30 minutes, followed by an additional 2 liters. Water quality stability was maintained by replacing 30% of the culture water every three days. Weekly measurements were taken for primary stolon length (cm), number of ramules, and fresh biomass (g), along with pH, salinity, and temperature.

### Statistical Analysis

Data were analyzed using one-way ANOVA to determine the effect of treatments, followed by post-hoc tests (t-test or Duncan's test) at a 95% confidence level. Statistical analysis was performed using SPSS version 26.0. Growth trends were visualized using graphs generated from the weekly measurement data.

## RESULTS AND DISCUSSION

### Growth Performance of *Caulerpa racemosa* in Different Cultivation Containers

Observations of the growth of *Caulerpa racemosa* cultivated in two types of containers (containers and tarps) showed differences in the mean values of stolon length (SP), number of ramules (Ram), and fresh weight (W) over the four-week observation period. The mean values and standard deviations are presented in Table 2.

**Table 2.** Average Stolon Length (SP) and Number of Ramules (Ram) of *Caulerpa racemosa* Cultivated in Different Containers.

| Location | Week | SP_mean (cm) | SP_sd | Ram_ mean | Ram_ sd |
|---|---|---|---|---|---|
| Container | I | 21.93 | 9.15 | 13.75 | 6.99 |
| Container | II | 22.18 | 9.57 | 16.25 | 9.74 |
| Container | III | 24.00 | 8.22 | 11.25 | 7.93 |
| Container | IV | 21.73 | 11.66 | 22.67 | 8.08 |
| Tarpaulin | I | 20.63 | 6.05 | 14.00 | 7.26 |
| Tarpaulin | II | 15.75 | 4.84 | 16.00 | 3.16 |
| Tarpaulin | III | 18.25 | 4.92 | 20.25 | 5.12 |
| Tarpaulin | IV | 11.00 | 6.06 | 14.25 | 7.68 |

The average stolon length in containers ranged from 21.73 to 24.00 cm (Figure 1), while in tarpaulin trays it ranged from 11.00 to 20.63 cm. The highest stolon growth in the container occurred in Week III (24.00 cm), while the tarpaulin trough showed its maximum stolon length in Week I (20.63 cm), followed by a decline through Week IV. Higher standard deviation values in the container indicate greater variation in growth among replicates. This pattern indicates that container conditions influence stolon elongation, with more stable volume and controlled depth supporting more consistent elongation (Rahmawanti, 2021).

The slowdown in stolon growth in week IV in the tarpaulin basin is suspected to be related to a decrease in salinity to 20 ppt. *Caulerpa racemosa* is known to be sensitive to sudden changes in salinity, which can disrupt osmotic balance and reduce metabolic activity. Consequently, more energy is expended on adaptation rather than growth. Additionally, tarpaulin tanks are more susceptible to environmental fluctuations, leading to greater salinity changes and slowing stolon growth (Guo *et al*., 2022; Rani *et al*., 2022).

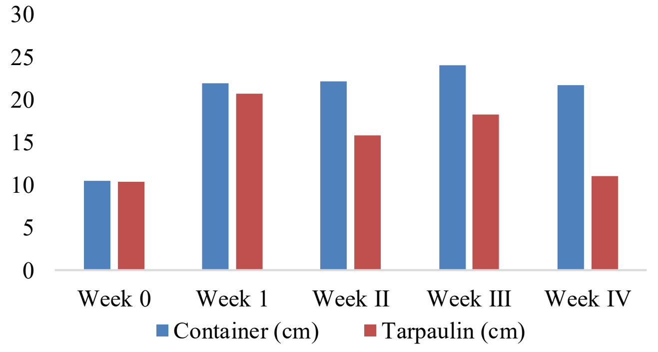


**Figure 1.** Average Stolon Length of *C. racemosa*

The average number of ramules ranged from 11.25 to 22.67 units in the container and from 14.00 to 20.25 units in the tarpaulin tank. Containers showed peak ramule development in Week IV (22.67 units), while tarpaulin tanks reached their peak in Week III (20.25 units) and then declined in Week IV (Table 3). The increase in ramule count reflects active vegetative branching, which is influenced by light intensity and nutrient availability. A similar trend has been reported in *Caulerpa* lentillifera, where branching density increases under higher light exposure to prevent self-shading (Apriliyanti *et al*., 2024). The decrease in the number of ramules in Week IV in the tarpaulin tank may indicate nutrient limitations or increased intra-thallus competition, consistent with previous findings on the growth *physiology of Caulerpa* (Robles & Tahiluddin, 2022).

**Fresh Weight (Biomass)**

The average fresh weight (W) of *Caulerpa racemosa* cultivated in different container media is presented in Table 3, showing that fresh weight increased from 172 g (Week II) to 241 g (Week IV). In the tarpaulin tank, fresh weight reached 215.50 g by Week IV. This increase indicates effective accumulation of vegetative biomass in both culture systems, although the patterns of early stolon and ramification development differed among treatments.

Wet weight values marked with a dash (-) indicate that data is unavailable or cannot be calculated. This may be due to the absence of measurements, an insufficient sample size, or the condition of the *Caulerpa racemosa* thallus being damaged or degraded by environmental stress. This is caused by fluctuations in salinity resulting from continuous rainfall over several days during cultivation, leading to a sudden drop in salinity. *C. racemosa* is an alga sensitive to changes in osmotic pressure; excessive freshwater entering the algal cells causes the cells to swell and eventually burst (lysis), which visually appears as a thallus that turns white, becomes soft, and is damaged (Prasetyo and Prayitno, 2014). Additionally, a decrease in light intensity—due to overcast conditions and rain reduces the sunlight penetration required for photosynthesis. This hinders energy production in the algae, thereby reducing the thallus tissue's resilience. Another factor is changes in pH and water quality, as rainwater tends to be acidic and can carry sediment particles from land (if cultivation is conducted in coastal areas), which can adhere to the thallus and disrupt algal respiration. Additionally, the absence of replicates indicated by the standard deviation bars means that data variability cannot be calculated.

**Table 3.** Average Fresh Weight (W) of *Caulerpa racemosa* Cultivated in Different Container Media

| Location | Week | W_mean (g) | W_sd |
|---|---|---|---|
| Container | I | - | - |
| Container | II | 172 | - |
| Container | III | - | - |
| Container | IV | 241 | 47.06 |
| Tarpaulin | I | - | - |
| Tarpaulin | II | - | - |
| Tarpaulin | III | - | - |
| Tarpaulin | IV | 215.5 | 7.59 |

Previous research by Nguyen *et al.* (2023) showed that *Caulerpa* species can produce substantial biomass under favorable cultivation conditions (nutrient availability, light intensity, salinity, and temperature) and are even used as biofilters in aquaculture systems due to their high nutrient-absorption efficiency. Biomass production in *C. racemosa* is strongly influenced by morphological traits (e.g., stolon elongation and branching) and environmental conditions. Based on this study's results, although the tarpaulin system initially showed slower stolon elongation, it ultimately produced biomass comparable to that of the container system. These results may be related to higher plant density and a larger cultivation area, which potentially promote increased ramification and accumulation of vegetative mass under favorable conditions. Comparison graphs of stolon length, number of ramules, and fresh weight for *Caulerpa racemosa* cultivated in container and tarpaulin systems are presented in Figures 2, 3, and 4, respectively. The comparison of *Caulerpa racemosa* stolon length showed a significant difference ($p < 0.05$). In contrast, the comparison of *Caulerpa racemosa* ramule number showed no significant difference (ns), and the comparison of the fresh weight of *Caulerpa racemosa* showed no significant difference.

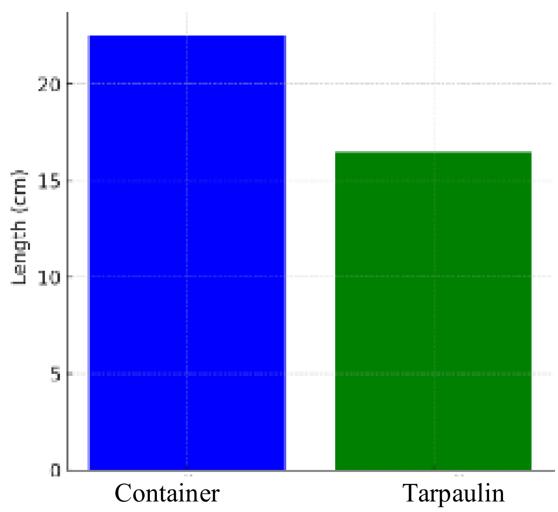


**Figure 2.** Comparison of *Caulerpa racemosa* Stolon Length Cultivated in Container and Tarpaulin Systems.

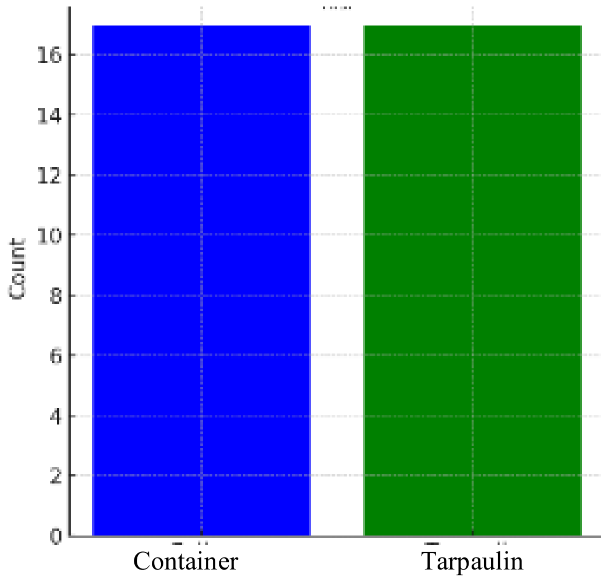


**Figure 3.** Comparison of the Number of *Caulerpa racemosa* Shoots Cultivated in Container and Tarpaulin Systems

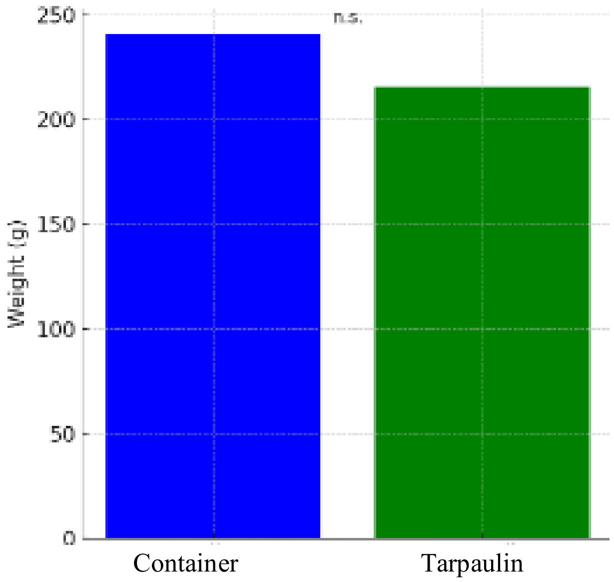


**Figure 4.** Comparison of the Fresh Weight of *Caulerpa racemosa* Cultivated in Container and Tarpaulin Systems.

**Analysis of Variance (ANOVA) for Stolon Length**

A two-way ANOVA for stolon length (SP) is presented in Table 4. It shows that the type of cultivation container significantly affects stolon elongation (F = 4.497; p = 0.044 < 0.05). These results indicate that differences in culture media (containers vs. tarps) significantly affect stolon growth. Conversely, the observation period (weeks) did not significantly affect stolon length (p > 0.05), and no significant interaction between location and observation time was observed (p > 0.05).

Descriptively, the longest stolon was recorded in Container III (24.00 ± 8.22 cm), followed by Container II (22.18 ± 9.57 cm) and Container I (21.93 ± 9.15 cm). The shortest stolon was recorded in Tarpaulin IV (11.00 ± 6.06 cm).

The highest average number of ramules (Ram_mean) was recorded in Tarpaulin III (20.25 ± 5.12 units) and Container IV (22.67 ± 8.08 units). The highest fresh weight (W_mean) was observed in Container IV (241.00 ± 47.06 g), followed by Tarpaulin IV (215.50 ± 7.59 g) and Container II (172.00 g).

**Table 4.** Results of Two-Way ANOVA for Stolon Length (SP) of *Caulerpa racemosa*.

| Factor | Sum of Squares | df | F-value | p-value |
|---|---|---|---|---|
| Location | 266.5992 | 1 | 4.4979 | 0.0449 |
| Week | 132.9692 | 3 | 0.7478 | 0.5347 |
| Location × Week | 82.9604 | 3 | 0.4666 | 0.7084 |
| Residual | 1,363.249 | 23 | — | — |

These findings indicate that although the observation period had no significant effect, container type influenced the morphometric growth patterns *of C. racemosa*, with container cultivation systems generally supporting the development of larger stolons.

**Analysis of Variance (ANOVA) for Number of Ramules**

The ANOVA results for the number of ramules (Ram) are presented in Table 5. They show no significant effect of container type (p = 0.8599 > 0.05) or observation period (p = 0.769 > 0.05). The interaction between these two factors was also not significant (p = 0.173 > 0.05). These results indicate that ramuli development was relatively stable across all treatments and weeks, suggesting that environmental conditions in both cultivation systems remained within the appropriate tolerance range for *C. racemosa* growth.

The independent t-test presented in Table 6 shows no significant difference in fresh weight between the container and tarpaulin cultivation systems (p = 0.326 > 0.05). The assumptions of normality and homogeneity were met (Shapiro–Wilk and Levene tests, p > 0.05). Therefore, the test results are valid, and the fresh biomass produced in both cultivation systems is statistically similar.

**Table 5.** Results of Two-Way ANOVA for *Caulerpa racemosa* Shoot Length (SL).

| Factor | Sum of Squares | df | F-value | p-value |
|---|---|---|---|---|
| Location | 1.6534 | 1 | 0.0318 | 0.8599 |
| Sunday | 59.0296 | 3 | 0.3789 | 0.7691 |
| Location × Week | 282.0370 | 3 | 1.8103 | 0.1734 |
| Residual | 1,194.417 | 23 | — | — |

**Table 6.** Independent t-Test for Wet Weight

| Test | t-value | p-value | Shapiro-Container | Shapiro-Wrap | Levene |
|---|---|---|---|---|---|
| Independent t-test (equal variances assumed) | 1.0699 | 0.3258 | 0.0728 | 0.4582 | 0.2553 |

Statistically, this indicates that the type of cultivation container has a specific effect on primary stolon growth but does not significantly influence ramule length or fresh weight. Thus, differences in growing space have a greater impact on horizontal growth (stolon elongation) than on vertical growth or biomass accumulation. The longer primary stolon growth on the tarpaulin medium indicates that a wider growing space and more uniform light exposure provide *C. racemosa* with the opportunity to crawl and expand its photosynthetic surface. In container media, limitations in volume and horizontal space caused stolons to overlap and grow more vertically. A similar phenomenon was reported by Maharani *et al*. (2022), who stated that the growth of *C. racemosa* stolons is strongly influenced by spatial configuration and lateral light distribution in cultivation media.

Unlike stolons, ramule length showed relatively constant growth across containers. This is thought to be because ramule formation is more influenced by internal plant factors, such as meristem activity and photosynthetic efficiency, than by external factors such as container type. As long as nutrients and light are available in sufficient quantities, ramule growth will proceed steadily (Peterson *et al*., 2018; Handayani *et al*., 2021). Furthermore, the absence of significant differences in fresh weight indicates that biomass accumulation proceeds at a relatively similar rate in both systems. Well-controlled dissolved nutrient and environmental conditions allow *C. racemosa* to utilize resources efficiently, so that total fresh weight does not differ significantly, even though stolon growth patterns differ. These findings support the findings of studies by Sulistiani *et al*. (2020) and Wirasatriya & Subekti (2021), which reported that *C. racemosa* can adapt to variations in container shape as long as the primary environmental factors (nutrients, temperature, and light) remain within optimal ranges. The differing effects on morphological growth (stolon and ramule length) and biomass growth (fresh weight) indicate that *C. racemosa*'s physiological response is not always linear. According to Nursid *et al*. (2022), an increase in biomass can occur through thickening of the stolons or stolon branches without being accompanied by an extension of the morphological structures. This explains why, in container systems, fresh weight can increase even when stolon length is shorter than in tarp systems.

**Water Quality**

The results of water quality research in the container and tarpaulin tanks are presented in Table 7, which shows that water quality in both tanks remained relatively stable during the four-week monitoring period. Temperature and pH showed only minor fluctuations, while salinity gradually decreased in both systems. Nutrient levels, including phosphate, nitrate, nitrite, and ammonia, remained low or below the detection limit throughout the entire observation period. Overall, the measured parameters indicate that both cultivation environments maintained conditions suitable for aquatic organisms.

Based on Table 7, the decrease in salinity in both containers (from 27 to 23 ppt in the container and from 27 to 20 ppt in the tarpaulin) indicates the presence of significant confounding variables. This decrease is suspected to have occurred due to the entry of freshwater, such as rainwater, the addition of water without salinity control, or leakage. The more open tarpaulin container tends to be more susceptible to these changes. These salinity changes may affect the growth of *Caulerpa racemosa* stolons, as they are sensitive to salinity fluctuations. A decrease in salinity can disrupt osmotic balance and reduce metabolic activity, thereby slowing growth (Guo *et al*., 2022; Sudirman *et al*., 2023).

The results indicate that the effect of the cultivation container on stolon growth does not change over time. In other words, the container's physical characteristics are not strong enough to significantly alter growth rate over the relatively short observation period. The study by Nurhayati *et al*. (2020) also supports these findings, showing that the growth of *C. racemosa* in various containers (buckets, fiberglass tanks, and glass aquariums) did not differ significantly when environmental conditions and nutrient levels were uniformly controlled. This reinforces the hypothesis that water quality stability is more critical than the container's shape or material. Overall, these results indicate that *Caulerpa racemosa* exhibits broad tolerance to variations in the physical conditions of cultivation containers. The relatively uniform growth across different locations signifies the species' potential for flexible cultivation at various scales and systems—whether in simple containers such as used containers or in large containers like tarps or fiberglass tanks—provided that key environmental factors remain within optimal ranges.

**Table 7.** Water Quality Parameters in Container Tanks and Tarpaulin Tanks

| Parameters | Week 1 | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|
| **(Tank Container)** | | | | |
| Temperature (°C) | 30.2 | 30.1 | 30.3 | 30.2 |
| pH | 7.90 | 7.85 | 7.88 | 7.82 |
| Salinity (ppt) | 27 | 26 | 25 | 23 |
| Phosphate (mg/L) | <0.25 | <0.25 | <0.25 | <0.25 |
| Nitrate (mg/L) | <2 | <2 | <2 | <2 |
| Ammonia (mg/L) | <5 | <5 | <2 | <1 |
| Nitrite (mg/L) | <0.1 | <0.1 | <0.1 | <0.1 |
| **(Tarpaulin Tank)** | | | | |
| Temperature (°C) | 30.2 | 30.1 | 30.2 | 30.2 |
| pH | 7.81 | 7.79 | 7.83 | 7.80 |
| Salinity (ppt) | 27 | 25 | 22 | 20 |
| Phosphate (mg/L) | <0.1 | <0.1 | <0.1 | <0.1 |
| Nitrate (mg/L) | <2 | <2 | <2 | <2 |
| Ammonia (mg/L) | <5 | <5 | <5 | <5 |
| Nitrite (mg/L) | <0.1 | <0.1 | <0.1 | <0.1 |

Beyond biological aspects, differences in cost and ease of management between container and tarpaulin systems are also important considerations. Containers are generally cheaper, easier to obtain, and allow better control of water quality due to their small volume, making them more efficient for experimental or beginner-scale operations. Conversely, tarpaulin tanks require higher initial costs and more complex management, particularly in maintaining the stability of parameters such as salinity and water quality.

From a management perspective, tarpaulin tanks are more susceptible to environmental fluctuations (e.g., rain and evaporation), thus requiring more intensive monitoring. This can impact the growth *of Caulerpa racemosa* if not managed properly. The choice of container should be tailored to cultivation objectives, production scale, and management capabilities to ensure optimal, efficient results. Thus, it can be concluded that *Caulerpa racemosa* can grow well in various simple containers, such as used containers or large-scale tarpaulin-based systems, provided that environmental factors and nutrients are maintained within optimal ranges. This opens opportunities for the development of low-cost seaweed cultivation using local materials, particularly for coastal SMEs that require simple yet efficient cultivation systems.

## CONCLUSION

The research results indicate that container type influences *Caulerpa racemosa* growth. In initial observations, the container produced longer stolons than the tarpaulin. However, this difference was no longer significant in subsequent periods, suggesting that environmental conditions in both containers remained relatively similar and conducive to growth. A t-test for fresh weight also showed no significant difference between the two containers. This indicates that *C. racemosa* has good adaptability to simple cultivation media. Both types of containers can be used effectively for small-scale cultivation if water quality, light intensity, and nutrient availability are maintained.

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