

Variation of N and P Fertilizer Combination Ratios on the Growth and Morphology of *Caulerpa racemosa* in a Closed System Cultivation

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Abstract

Caulerpa racemosa lives in waters with sand, coral and mud substrates, and is widely used by coastal communities as food. The purpose of this study was to evaluate the effect of nitrogen (N) and phosphorus (P) fertilizers using Urea (NH_2CONH_2) and SP-36 (P_2O_5) with different concentration variations on the growth and morphology of *C. racemosa*. This research was conducted at the Brackish Water Aquaculture Fisheries Center (BPBAP) Takalar. *C. racemosa* was maintained in basin containers equipped with a water circulation system in each container. This research design uses a complete randomized design (CRD) with 5 treatments and 3 replicates. The treatment used were the following ratios of Urea and SP-36 fertilizer: A (100 : 0%), B (75 : 25%), C (50 : 50%), D (25 : 75%), and E (0 : 100%). The results indicate that the addition of nutrients to the closed cultivation medium using a combination of N and P fertilizers resulted in the highest growth rate of 2.2% per day in treatment C and the lowest growth rate of 1.64% per day in treatment E. While the stolon diameter and rachis length showed no significant differences, the distance between rachises in treatment B was significantly different ($P < 0.05$) compared to other treatments. These data are useful for considering the optimal combination of urea and SP-36 fertilizers for the cultivation of *C. racemosa*.

Introduction

Seaweed is a type of algae and lives in sea waters with various species that provide many economic benefits for people living in coastal areas. In addition, seaweed can support aquatic productivity and the global seaweed industry, whose value can reach billions of dollars (Monagail et al., 2017). One type of algae has important economic potential is *C. racemosa*, which belongs to green algae. *C. racemosa* can be found throughout the year because it can grow naturally and adapts easily to waters (Tapotubun et al., 2020 ; Zaw et al., 2020) and generally lives in waters with sand, coral, and mud substrates (Sunaryo et al., 2015).

C. racemosa has quite good potential to be developed because it has economic value as a food ingredient and medicine (Yudasmara, 2015). Coastal communities use *Caulerpa* by eating it raw as a vegetable or fresh vegetable (Novianti et al., 2015; Rahayu et al., 2019). Due to its high nutritional content, secondary metabolites, crude fiber, protein, fat, and carbohydrates, this algae is used as a functional food ingredient (Sinurat & Fadrijah, 2019; Tapotubun et al., 2020). This algae also contains fatty acids and omega-3 (Syakilla et al., 2022). Apart from being used as a food ingredient, *C. racemosa* can also be used as a biofilter in wastewater treatment in the cultivation of aquatic organisms Chen et al. (2019) and Liu et al., (2016).

Caulerpa cultivation provides benefits to cultivation, especially in waste processing. *Caulerpa*, is used as a food ingredient that is bought and sold in the market today, mostly comes from nature because its availability from cultivation is still very limited. Excessive exploitation of nature can cause damage to *C. racemosa* populations in nature therefore, cultivation is necessary to meet market needs and preserve this algae in waters.

Caulerpa cultivation can guarantee the availability of products that are good in quality and quantity and reduce threats to nature. Cultivation can be carried out in open or closed waters while still paying attention to factors that influence their lives. Environmental conditions, such as the availability of nutrients, are factors that influence the success of cultivation. Nitrogen (N) and phosphorus (P) are essential macronutrients that play a vital role in the growth and development of algae. Nitrogen has an important role in the synthesis of proteins, nucleic acids, and chlorophyll, which are very necessary for the photosynthesis and growth of *Caulerpa*. Meanwhile, phosphorus is very important to maintain metabolic activity and increase *Caulerpa* growth efficiency. According to Xiao et al. (2017) and Ismail et al. (2024), the availability of nitrogen and phosphorus in an optimal cultivation environment has a very significant impact on the physiological performance and growth of *Caulerpa* so that it is healthier and more productive. Research (Budiyan et al., 2012) resulted in the specific growth rate of *C. racemosa* var *uvifera*. The highest was achieved at a nitrogen concentration of 100 µM. The addition of 8.26 ppm NPK and 16.26 ppm urea resulted in a growth rate of *C. racemosa* var. *uvifera* good (Setiaji et al., 2012). liquid fertilizer concentration of 0.34 mL/L of water gets a growth rate of 9.2 cm in length (Pramita et al., 2022), increasing nitrogen in the media can increase the chlorophyll content of *C. racemosa* (Zainuddin et al., 2024). Nutrients N and P have a real influence on the growth and morphology of *Caulerpa*. Nitrogen can increase stolon formation so that biomass also increases (Stuthmann et al., 2023), while phosphorus can accelerate growth and strengthen root structure (Windarto et al., 2024).

The balance between N and P in the media is very important because the interaction between the two will affect nutrient absorption (Windarto et al., 2024). Excess or deficiency of nitrogen is not balanced by the availability of phosphorus will inhibit growth and conditions will not be ideal for *Caulerpa* (Stuthmann et al., 2023; Windarto et al., 2024). Although research on the effect of N and P on the growth and morphology of seaweed has been carried out, further research is needed to better understand the effect of adding N and P fertilizers with varying concentrations on growth and morphology of *C. racemosa*. The aim of this research was to evaluate the effect of applying N and P fertilizer using urea fertilizer (NH₂CONH₂) and SP-36 (P₂O₅) with different concentration variations on growth and morphology of *C. racemosa*

Materials and Methods

This study was conducted from May to September 2022. Maintenance of *C. racemosa* was carried out at the Brackish Water Aquaculture Fisheries Center (BPBAP) Takalar. This research uses basins as research containers and is equipped with a water circulation device in each container. Observations of the growth and morphology of *C. racemosa* were carried out directly at the location during the study.

Research Design

This study used a completely randomized design (CRD) of 5 treatments and 3 replications. The treatment used is the ratio of urea and SP36 fertilizer as follows: A (100% : 0%), B (75% : 25%), C (50% : 50%), D (25% : 75%), and E (0% : 100%). Urea and SP36 fertilizers were used as sources of N and P added to the media. Next, *C. racemosa* seedlings are placed into the maintenance medium, with 50 grams in each container. Maintenance of *C. racemosa* was carried out for 45 days, and water changes were carried out every 3 days for as much as 40–60% of the water volume. The water change is carried out using a hose (siphon system), starting by turning off the aeration. The process is performed in the morning by lowering the water level in all containers to the predetermined height. Once the water reduction is complete, the next step is to refill the containers with seawater from the prepared water reservoir.

Caulerpa Racemosa Seaweed

This study uses *C. racemosa* obtained from *Caulerpa* cultivator pond farmers in Puntondo Hamlet, Manggara Bombang District, Takalar Regency. *C. racemosa* was brought by car to the research site, then placed in the tub that had been prepared for acclimatization, cleaning of dirt that sticks, as well as the selection of seaweed needed. The selection is based on cleanliness, color brightness, integrity, wounds, and hardness of *C. racemosa*.

Growth and Morphometrics of *Caulerpa racemosa*

The daily growth rate of *C. racemosa* was determined by measuring the weight of seaweed every 15 days using a digital scale. Morphometric observations of *Caulerpa* were carried out by measuring the distance between the rachis, rachis length and stolon diameter using a digital caliper. The daily growth rate is calculated by the equation:

$$DGR = \frac{\ln Wt - \ln W0}{t} \times 100\%$$

Where :

DGR= Daily Growth Rate (%)

Wt = Initial weight (g)

Wo = Final weight (g)

t= Maintenance period (days)

Statistical Analysis

The data obtained from all treatments were statistically analyzed using one-way analysis of variance (ANOVA) to determine the effect of the treatments on the growth and morphology of *C. racemosa*. When significant differences were found, Tukey's test was performed to assess the significance of these differences at a 0.05 significance level. All results were analyzed using SmartstatXL V.3.6.5.3.

Result

Daily Growth Rate

The research results showed that the highest daily growth rate of *C. racemosa* was observed in treatment C at 2.121%, while the lowest was in treatment E at 1.642%. The variance analysis of *C. racemosa* indicated differences in daily growth rates across the treatments applied (Figure 1). The daily growth rates in treatments B, C, and D were significantly different from all other

treatments, whereas treatments A and E showed no significant differences between them, with lower daily growth rates compared to the other treatments.

Weight Gain

Growth in *C. racemosa* based on weight gain showed an increase from the beginning to the end of the study. At the beginning of the maintenance, the weight increased rapidly and decreased as the research time increased, especially at the end of the study. The highest weight in *C. racemosa* was obtained in treatment C, which was 129.83 grams, and the lowest in treatment E, which was 104.73 grams (Figure 2).

Morphology

Observations of *C. racemosa* morphology in this study were devoted to the diameter of the talus, the distance between the rachis, and the assimulator. Analysis of variance showed that the talus size and rachis length of *C. racemosa* were not significantly

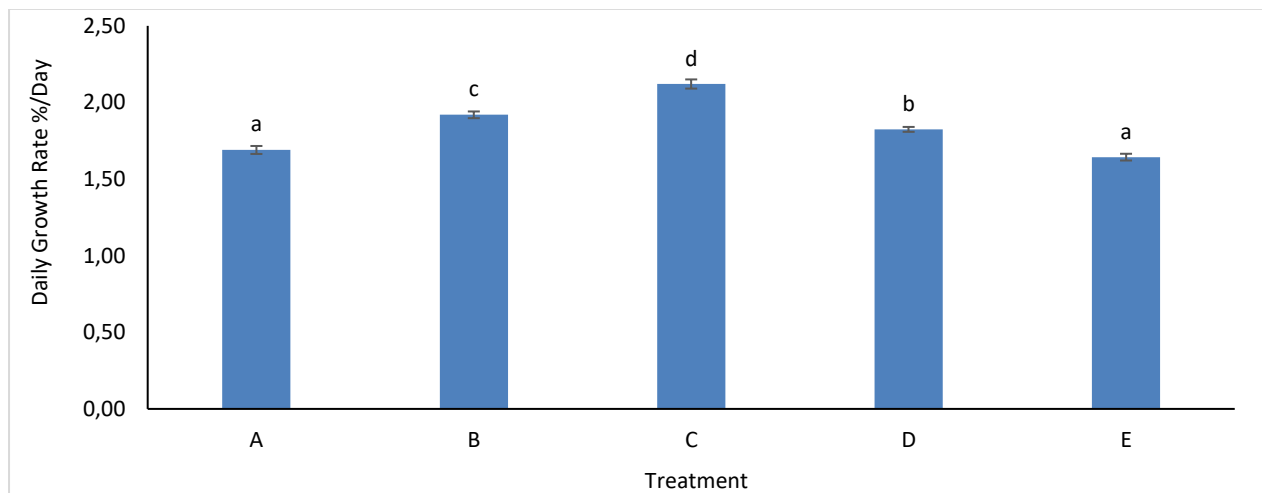


Figure 1. Daily Growth Rate of *C. racemosa* Given N and P Fertilizers with Different Ratio Combinations.

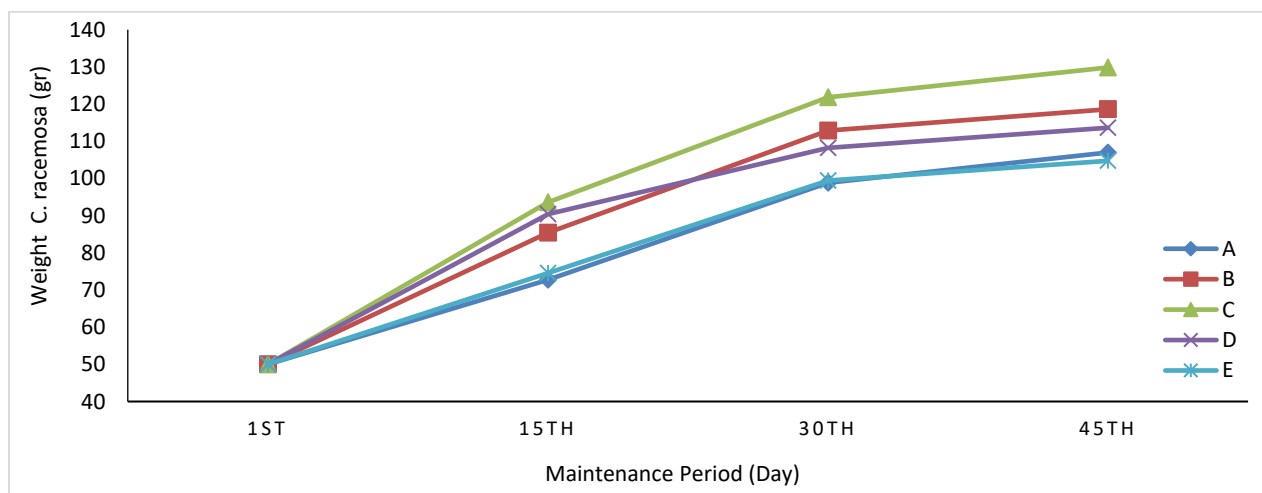


Figure 2. Weight Increase of *C. racemosa* Given N and P Fertilizers With Different Ratio Combinations.

different, while the distance between rachis was significantly different at the 0.05 level. (Table 1). Treatment B was significantly different from the other treatments. Treatments C and D also had a high distance between rachis, but the value was still lower than treatment B. The low distance between rachis was obtained in treatments A and E with values of 12.11 mm and 12.22 mm, respectively. Rachis or branches are where the ramuli are arranged. The lowest length of *Caulerpa* rachis was obtained in treatment E with a value of 57.80 mm and the highest in treatment B with a value of 66.40 mm.

Discussion

The application of N and P fertilizers in the cultivation of *C. racemosa* under a closed system resulted in a continuous increase in the growth rate until the end of the cultivation period. The significant differences in growth observed in the treatments indicate that N and P play a crucial role in stimulating cell division, leading to growth; however, they must be adjusted to the required levels so that the N and P nutrients can be optimally absorbed. Treatment C, with the addition of 50% Urea and 50% SP-36 fertilizers, showed the highest daily growth rate. Thus, the concentration of N and P in the treatment medium supports good growth in *C. racemosa*. Rendiansyah et al. (2024) stated that proper fertilization can increase the growth rate, and different amounts of fertilizer can affect the growth of *C. lentillifera*. The study by Budiyan et al. (2012) showed that varying concentrations of Urea nitrogen resulted in significantly different growth, with the addition of 100 μ M nitrogen producing the highest specific growth rate of 1.36% per day.

The growth obtained in treatments B and D is high but lower compared with treatment C. Nitrogen concentrations that are too high and phosphorus concentrations that are too low or vice versa can cause the growth rate of *C. racemosa* to be inhibited. High concentrations of $\text{NO}_3\text{-N}$ can inhibit growth rates (Guo et al., 2015), and excessive accumulation of P hurts *Caulerpa* growth (Reef et al., 2012). High growth rates are an indication that the ratio of the combination of N and P fertilizers used is optimal enough to support the growth of *C. racemosa*. Alketife et al. (2017) stated that the addition of N and P often produces good nutrient absorption and optimal growth.

The lowest growth of *C. racemosa* was obtained in treatments A and D, proving that to get good growth, N and P fertilizers need to be added together into the media. The presence of N and P greatly affects growth because each has a very important role. According to Alexandre & Santos (2020), N and P are needed during growth; if one of the elements is not available, it can disrupt other essential functions. When N is present in the water but not accompanied by the availability of P, the absorption of nitrogen still increases and growth still occurs at the beginning of growth, but growth does not occur optimally because ATP synthesis is disrupted (Pedersen et al., 2019) (Alexandre & Santos, 2020); conversely, the availability of phosphorus without nitrogen will reduce the ability of algae to synthesize protein (Pedersen et al., 2019), resulting in decreased growth.

Macroalgal growth is influenced by environmental factors such as temperature and salinity (Ciasico, 2023). In addition, the availability of N and P nutrients significantly the growth of *C. racemosa*. The use of both organic and non-organic fertilizers to enrich the cultivation media has been commonly done and proven to provide good results on seaweed growth. Lideman et al. (2024) suggested that the provision of organic and inorganic fertilizers in the cultivation of *C. racemosa* has a positive effect on growth and chlorophyll content. Increasing the concentration of NPK fertilizer (nitrogen, phosphorus, and potassium) can increase the growth rate and biomass of *C. racemosa* (Subur et al., 2021).

The growth pattern of *C. racemosa* maintained with the addition of nutrients N and P in different dosage combinations results in rapid growth initially, followed by a slowdown towards the end of maintenance. A decrease in cell growth capacity due to the saturation of cell division leads to a reduction in growth, which is initially rapid during the early growth phase (Nadlir et al., 2019). Pakidi & Suwoyo (2017) added that growth will not increase further when cell enlargement reaches its limit under optimal conditions. Supriyantini et al. (2018) stated that the decline in growth rate is caused by the lower increase in thallus weight as maintenance duration increases, leading to competition for nutrients and sunlight absorption for photosynthesis.

Environmental conditions where macroalgae live influence their morphology due to the adaptation process, allowing them to adjust to their surroundings.

Table 1. Results of Morphological Measurement of *C. racemosa* Given N and P Fertilizers with Different Ratio Combinations

Treatment	Stolon Diameter (mm)	Distance Between Rachis (mm)	Rachis Length (mm)
A	2,39 \pm 0,38	12,11 ^a \pm 1,63	61,13 \pm 4,21
B	2,38 \pm 0,40	16,94 ^c \pm 0,37	66,40 \pm 6,37
C	2,25 \pm 0,26	15,67 ^{bc} \pm 0,90	63,86 \pm 4,77
D	2,20 \pm 0,15	13,14 ^{ab} \pm 1,41	64,28 \pm 2,18
E	2,23 \pm 0,08	12,22 ^a \pm 0,83	57,77 \pm 1,70

Environmental conditions such as the availability of N and P are the ones that affect the stolons and rachis of the *C. racemosa*. The distance between the rachis was found to be the largest in treatment B. The addition of N fertilizer in combination with a larger amount of P fertilizer results in a higher concentration of N than P, which affects the distance between *C. racemosa* rachis. Mo et al. (2020) suggest that the interaction of N and P affects plant morphology, where high concentrations of N without sufficient P produce unbalanced growth to increase the distance between rachis.

The availability of nutrients, particularly N and P in the medium, can influence the development of stolons and rachis in the *C. racemosa*. According to Malta et al. (2005), high growth rates are supported by high nutrient availability; however, the allocation for biomass in stolons and rachis will vary depending on the conditions of nutrients N and P. The diameter of the stolons and the length of the rachis in *C. racemosa* showed no significant differences across all treatments used. Research by Antara et al. (2022) demonstrated that growth rates increased with higher concentrations of fertilizers applied, but there were no significant differences in thallus regeneration rates. The longest rachis of *C. racemosa* was found in treatment B, measuring 66.40 mm with a stolon diameter of 2.40 mm. The addition of N fertilizer in much higher than P increased the high N levels in the medium, impacting the growth of stolons and rachis. Fernández et al. (2020) stated that increased N promotes faster vertical growth. However, while it can enhance growth, an excess of N without a balance of other nutrients can lead to damage in stolons (Kartika et al., 2023). Variations in nutrient concentrations need to be carefully considered to have a positive effect on the stolons and rachis of *Caulerpa*. Research by Arimoto et al. (2019) showed that the morphological development of fronds and stolons in *C. lentillifera* is influenced by variations in nutrient availability.

Conclusion

The results showed that the addition of nutrients in closed cultivation media using a combination of N and P fertilizers with different ratios had a significant effect on the growth of *C. racemosa*. However, the treatment used to assess the effect on the morphology of *C. racemosa* did not result in significant different in stolon diameter and rachis length, except for the distance between rachis. The research results obtained can provide technical information about the addition of N and P nutrients to the media using a combination of urea and SP-36 in the cultivation of *C. racemosa*.

Ethical Statement

This study was conducted ethically, professionally, and responsibly. No ethics committee approval or special permission was required.

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No funding was received for conducting this study.

Author Contribution

The first author is responsible for conducting the research, analyzing the data, and writing the article. The second author provides input on interpreting the results and preparing the manuscript. The third and fourth authors provide input on data analysis and proofread the manuscript before submission.

Conflict of Interest

The authors declare that there are no potential commercial or financial conflicts of interest related to the research reported in this article.

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