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Chlorophyll of *Caulerpa racemosa* in Indoor Cultivation with Varying Concentrations of Nitrogen and Phosphorus Fertilizers

Fadli Zainuddin[1, 2*], Sri Andayani[3], Mohammad Mahmudi[4], Muhamad Firdaus[4]

[1]Doctoral Program in Fisheries Science and Marine, Faculty of Fisheries and Marine Sciences, Brawijaya University, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Papua University, Indonesia
[3]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Brawijaya University, Indonesia
[4]Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Brawijaya University, Indonesia

***Corresponding Author : temmicki@yahoo.com**

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ABSTRACT

Caulerpa racemosa belongs to a group of green algae commonly called sea grapes. The green color as a characteristic of *C. racemosa* is closely related to the chlorophyll pigment it contains. The concentration of chlorophyll in algae is influenced by the addition of nutrients to the rearing medium, such as nitrate (N) and phosphate (P) nutrients. To figure out the effect of adding N and P using urea and SP36 fertilizers with different concentrations on the chlorophyll content of *C.racemosa* was the main target of the current study. The research was conducted indoors, using a completely randomized design (CRD) consisting of 5 treatments and 3 replications. The treatment was a comparison of urea and SP36 as follows: A (100 : 0 %), B (75 25 %), C (50 : 50 %), D (25 : 75 %), and E (0 : 100 %). The addition of N and P into the media using urea and SP 36 fertilizers in a ratio (50: 50%) resulted in the highest concentrations of chlorophyll a, chlorophyll b, and total chlorophyll. The lowest chlorophyll was found in treatment E, namely the addition of P without N (Urea 0 : SP 36 100 %).

INTRODUCTION

Caulerpa racemosa belongs to a group of green algae commonly called sea grapes, which is one of the macroalgae currently being developed. This seaweed is known to the public by the names *lawi-lawi* or *latoh*, and can be cultivated. Seaweed cultivation is generally carried out in open water, so it is not necessary to provide nutrients, but when cultivation is carried out in closed ponds or containers, the addition of nutrients is absolutely necessary. Increasing seaweed production requires intervention and adaptation in aquaculture practices such as nutrient enrichment **(Luhan *et al*., 2015)**.

Enriched rearing media exhibit high growth rates and photosynthetic activity, which is due to the high content of nitrogen and chlorophyll in *Saccharina japonica* **(Guo *et al*., 2015)**. The supply of nutrients to the media affects the concentration of photosynthetic pigments greatly **(Figueroa *et al.*, 2009)**. The green color, a characteristic of *C. racemosa*, is closely related to the chlorophyll pigment contained therein, which plays an important role in photosynthesis. *C. racemosa* has abundant pigments, especially chlorophyll a and chlorophyll b **(Balasubramaniam *et al*., 2020)**. Environmental conditions, especially the availability of nutrients N and P in waters that are not in accordance with the needs of algae, can affect chlorophyll concentrations. **Ismail and Osman (2016)** stated that the total nitrogen and total phosphorus contained in the cultivation media had a direct impact on the content of photosynthetic pigments.

Nitrogen and phosphorus are considered limiting nutrients because they are needed by algae in the ecosystem **(Martins *et al*., 2011; Shakouri & Balouch, 2020)**; therefore, they must be added to water conditions that are less fertile or nutrient-poor. The application of phosphate and nitrogen in water is necessary because they have an important role **(Harrison & Hurd, 2001; Pickering *et al*., 2007)**. The more nutrients that can be absorbed and utilized by seaweed, the better it is to support the biological activity of pigments in tissues. Talus *Caulerpa* absorbs N and P from the media, and if absorption can occur optimally, chlorophyll formation and tissue development will occur **(Gao *et al*., 2017)**. A deficiency in the nutrients N and P in the media has an unfavorable impact on seaweed, because of their very important role in the survival process. Nitrogen is needed to help metabolic processes, while phosphorus is required for photosynthesis **(Pantjara & Sahib, 2008; Setiaji *et al*., 2012; Noorjahan *et al*., 2019)**. The optimal absorption of nitrate and phosphate is consistent with the formation of chlorophyll. Chlorophyll has a very important function in seaweed, especially in the process of photosynthesis. According to **Lee *et al*. (2014)** the process of photosynthesis takes place optimally when the chlorophyll-a and chlorophyll-b content of sea grapes is high.

The amount of N and P available must be balanced and sufficient, according to the needs of *C. racemosa*, so that it can stimulate the formation of chlorophyll, which is necessary for photosynthesis. The concentration and balance of the application of these two nutrients must be considered in order to obtain the appropriate results **(Roleda & Hurd, 2019)**. Usually, plants need a greater amount of nitrate than phosphate, so that the absorbed nutrients can be utilized properly. In general, the ratio of N and P as a requirement is 10:1 **(Suthar *et al*., 2019)**.

The nutritional needs of N and P *C. racemosa* in relation to the appropriate balance need to be considered in cultivation; therefore, this study aimed to determine the effect of applying N and P using urea (NH_2CONH_2) and SP-36 (P_2O_5) fertilizers in different combinations on the chlorophyll content of *C. racemosa.*

MATERIALS AND METHODS

This research was conducted in May – September 2022. Seaweed maintenance was carried out at the Brackish Water Aquaculture Fisheries Center in Takalar, while chlorophyll analysis was carried out in the Productivity and Water Quality Laboratory, Faculty of Fisheries and Marine Sciences, Hasanuddin University Makassar.

Research design

The study used a completely randomized design (CRD) with five treatments and three replications. The treatment was a comparison of urea and SP36 as follows: A (100 : 0 %), B (75 : 25 %), C (50 : 50 %), D (25 : 75 %), and E (0 : 100 %). Urea and SP36 were used as sources of N and P. *C. racemosa* was reared for 45 days. During maintenance, water changes was performed every 3 days for as much as 40 – 60 % of the water volume.

C. racemosa seaweed

The *C. racemosa* used in this study was obtained from *Caulerpa* cultivator pond farmers in Puntondo Hamlet, Manggara Bombang District, Takalar Regency. Seaweed was transported by car to the research site and then accommodated in a tub that has been prepared to be cleaned of all adhering dirt while the required seaweed was selected.

Chlorophyll analysis

Chlorophyll content was analyzed using a combination of the methods of **Arnon (1949)** and **Song *et al*. (2021)** as follows: 2g of sample was crushed or pulled and then added to 8ml of acetone, extracted using a shaker for ± 1h in a room with limited light conditions, and then centrifuged at 1000 rpm for 30min. The results were then measured using a spectrophotometer to determine the absorbance value at a wavelength of 663 and 645nm. Chlorophyll content was calculated using the following equation:

$$Ca = 12.7 \times A663 - 2.69 \times A645$$

$$Cb = 25.8 \times A645 - 4.68 \times A663$$

$$Ct = Ca + Cb$$

Where,

Ca = Chlorophyll a

Cb = Chlorophyll b

Ct = Total Chlorophyll

RESULTS

Caulerpa Chlorophyll per rearing period

Seaweed chlorophyll measured in rearing with different N and P fertilizer concentrations showed an increase in all treatments at the beginning of rearing, but after 15 days of rearing, there was a decrease in chlorophyll content in treatments D and E. While at the end of rearing, a decrease was detected in chlorophyll in all treatments (Fig. 1). The increase in chlorophyll in the initial period of rearing was an indication that the N and P nutrients added to the media could be utilized by *C. racemosa*. **Ji *et al*. (2019)** stated that macroalgae during the photosynthesis process absorb N and P from the water. Nutrients that are balanced and available in sufficient quantities help the process of needing chlorophyll for photosynthesis during the growth period of seaweed. According to **Astuti *et al.* (2021)**, the formation of new tissue and talus in seaweed requires nutrients in sufficient quantities and balance with their needs. In the final period of *C. racemosa* maintenance, all treatments showed decreased chlorophyll concentration. Old seaweed uses nutrients no longer for growth but to maintain its life. According to **Lamohamad *et al*. (2021)**, the decrease in chlorophyll content is caused by microalgae retaining chlorophyll to sustain their lives.

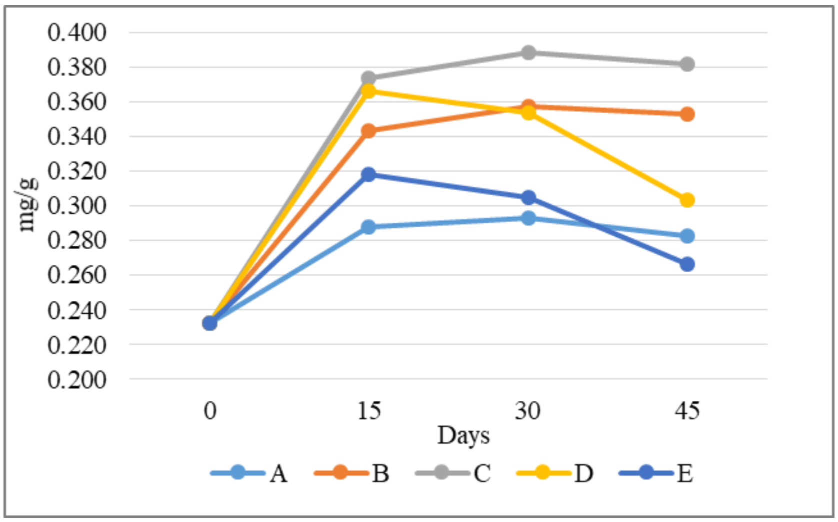

Fig. 1. *C. racemosa* chlorophyll is given every 15 days with N and P fertilizers in different combinations

Total chlorophyll of *C. racemosa*

The chlorophyll a and b concentrations found in algae show photosynthetic activity. The highest average of chlorophyll a, chlorophyll b, and total chlorophyll in this study were obtained in treatment C (Fig. 2). The chlorophyll b content obtained from *C. racemosa* was higher in treatments B, C, and D than chlorophyll a; this indicates the synthesis of chlorophyll b from chlorophyll a during the development of seaweed in that treatment. **Setiari and Nurchayati (2009)** suggested that increased chlorophyll b content in plants could increase the functional efficiency of the photosynthetic antenna in Light Harvesting Complex II (LHC II). The highest concentration of total chlorophyll was obtained in treatment C (Figure 2), namely by adding N and P using 50% urea and 50% SP36. This shows that the N and P added to the media in treatment C had a balance of concentrations that suited the needs. Nitrogen (N) and phosphorus (P) present in the media can be utilized by seaweed when their concentrations are in accordance with their requirements, and generally, plants utilize nitrogen in larger quantities than phosphorus **(Suthar *et al*., 2019)**.

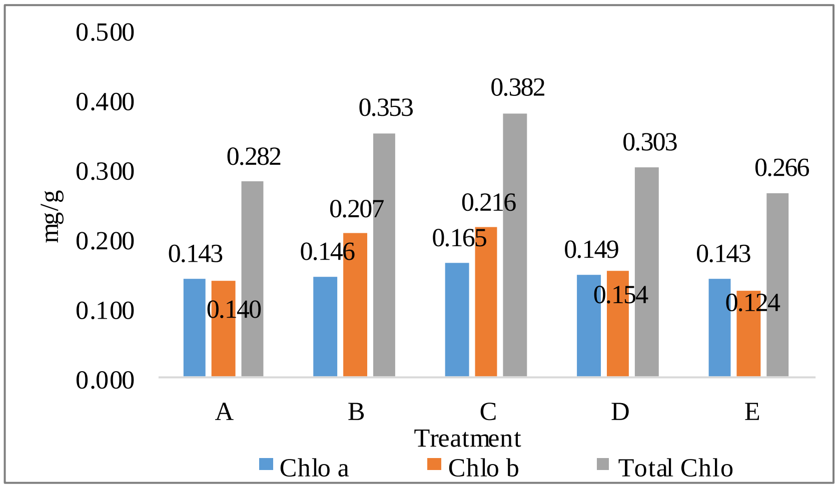

Fig. 2. Concentration of chlorophyll a, chlorophyll b, and total chlorophyll of *C.racemosa*

DISCUSSION

Seaweed is a photosynthetic organism with a high pigment content. Different pigment combinations and different environmental conditions cause color variations between species. Chlorophyll is the most abundant pigment found in algae. Chlorophyll a, chlorophyll b, antheraxanthin, lutein, neoxanthin, violaxanthin, zeaxanthin, and β-carotene are found in green seaweed, and the green color of seagrass is caused by the presence of chlorophyll a **(Dumay & Morançais, 2016)**. Chlorophyll is influenced by the presence of N nutrients, which can act as both inhibitors and enhancers of chlorophyll concentration **(Buapet *et al*., 2008)**; nitrate is a form of nitrogen which has an important role in the formation of chlorophyll **(Setyanti *et al*., 2013; Ismail & Osman, 2016)**, and increasing N in the media can increase chlorophyll **(Setyanti *et al.*, 2013)**. While, **Martins *et a*l. (2011)** suggested that the photosynthetic pigment content of *Hypnea musciformis* is positively correlated with the phosphate concentration in the rearing medium.

Chlorophyll requires balanced N and P elements so that it can be formed optimally. If there are elements that are too high or low in these nutrients, there will be disturbances in the development of seaweed. In the second period of maintenance, it was seen that treatments D and E experienced a decrease in chlorophyll content while the other treatments still increased. Adding phosphate elements to the medium is necessary to increase growth, but when the amount exceeds the requirement, it has a negative impact. Research by **Bergstrom *et al*. (2003)** showed that seeds of *Fucus vesiculosus* L enriched with phosphate at high concentrations caused 50% mortality. The existence of high nutrients that exceed the needs that occur in the waters can be accepted by seaweed for a limited time. Several types of macroalgae can store nutrients when there is a jump in nutrients and use them temporarily **(Buapet *et al*., 2008)**. The nutrients stored by seaweed will be utilized when the nutrients are reduced under favorable conditions.

The need for nitrogen and phosphorus elements in algae is crucial for chlorophyll synthesis. The deficiency of either nitrogen or phosphorus in macroalgae can lead to a reduction in chlorophyll production due to the vital role of both elements. Nitrogen is one of the macro elements that plays a role in forming chlorophyll molecules **(Roleda & Hurd, 2019)**, and phosphorus is involved in shaping the molecular structure of chlorophyll and also functions as a part of adenosine triphosphate (ATP) molecules, which serve as the primary energy source in living cells **(Hurd *et al*., 2014)**. The lack of necessary nutrients is clearly evident in treatments A and E, where only one type of fertilizer was added, resulting in the lowest chlorophyll content. The results of **Chrismadha *et al.* (2006)** clearly demonstrate a significant impact of low nitrogen and phosphorus on the reduction of chlorophyll content in *S. fusiformis.*

Chlorophyll content is significantly influenced by the N and P conditions in the medium. The presence of high concentrations of N and P can increase chlorophyll content but can also have negative effects depending on the ratio of N and P nutrients. The N/P nutrient ratio to chlorophyll has a positive correlation **(Maslukah *et al*., 2017)**. Nitrate and phosphate levels show a positive linear relationship with chlorophyll-a content **(Ningrum *et al*., 2022)**. The chlorophyll concentration obtained in this study with high N fertilizer and low P fertilizer, and vice versa, shows a less favorable concentration. This may be because the N and P ratio provided is not suitable for the needs of the cultivated *C. racemosa.*

CONCLUSION

In conclusion, the addition of N and P nutrients to the rearing medium using urea and SP36 fertilizers showed the highest chlorophyll content in the application of fertilizers with a ratio of 50 % urea and 50 % SP 36. Chlorophyll b was higher than chlorophyll an in the treatment with N and P nutrients added, and vice versa in the treatment with only N or P nutrients added to the rearing medium.

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