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Original Article

Effect of coconut (*Cocos nucifera*) water, and aqueous extract of Mung bean (*Vigna radiata*) sprouts and Moringa (*Moringa oleifera*) leaf on the growth and nutrition of *Caulerpa racemosa*

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Abstract: *Caulerpa racemosa,* or sea grape, has a high nutritional content and offers many health benefits to humans. The problem faced in cultivating sea grapes is growth, which affects production. This study aimed to determine the effect of different times of soaking *C. racemosa* in (*Cocos nucifera*) water, and aqueous extract of Mung bean (*Vigna radiata*) sprouts and Moringa (*Moringa oleifera*) leaf on its morphology and growth performance. Fifty grams of *C. racemosa* was soaked in coconut water and an aqueous extract of moringa leaves and mung bean sprouts at different times and was cultivated for 30 days. This study used a complete randomized design (CRD) with four treatments and three replicates: A (without soaking), B (extracts of Mung bean sprouts and Moringa leaves soaked for 2 hours), C (crude extracts of Mung bean sprouts and Moringa leaves soaked for 4 hours), D (coconut water soaked for 40 minutes), and E (coconut water soaked for 50 minutes). The results showed that the soaking process of *C. racemosa* in coconut water and aqueous extract of moringa and mung bean sprouts significantly affected the growth and nutrition of *C. racemosa*. *Caulerpa racemosa* soaked in coconut water for 40 minutes showed the highest absolute growth (206.66±7.96 g), specific growth (5.45±0.10 %/day), protein (9.11±0.29%), and fiber (1.02±0.20). The morphology of *C. racemosa* showed that the ramulli, rhizoid, fronds, and stolon were fresh, healthy, and clean. Soaking sea grape seeds in natural PGRs can increase the growth, production, and nutritional content. Further research can be conducted, such as identifying the bioactive substance and its amount in coconut water.

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Introduction

Caulerpa, *Caulerpa racemose*, is a seaweed that can be consumed and is rich in protein, minerals, fiber, and vitamins (Ma'aruf et al., 2013; Chen et al., 2019). Due to its high demand in world markets, it has high economic value and it is an export commodity in the Indonesian aquaculture sector (Simanjuntak et al., 2017). *Caulerpa racemosa* is a green seaweed with antibacterial activity (Kandhasamy and Arunachalam, 2008). It contains nutrients such as nitrogen, magnesium, and iron (Gabrielsen, 1996). In addition, *C. racemosa* has high nutritional value, including polyunsaturated fatty acids (PUFA), essential amino acids, minerals, dietary fiber, vitamins, and naturally bioactive compounds (Kumar et al., 2011; Nagappan and Vairappan, 2014). However, its production capability must continue to be increased to meet market demands (Hermawan, 2015), and it currently needs a way to increase its growth.

One way to increase its growth is by adding plant growth regulators (PGRs), hormones, or fertilizer. Artificial hormones can have negative effects on plant growth. Plant growth regulators (PGRs) are synthetic compounds that mimic naturally occurring plant hormones and are widely used in agroforestry. The residues of PGRs in agricultural products have been found to have hepatotoxicity, nephrotoxicity, genotoxicity, neurotoxicity, carcinogenicity, and teratogenicity, which are seriously detrimental to human health. Furthermore, PGRs are suspected to

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disrupt the function of human and animal reproductive systems (Xu et al., 2018). Experiments on adding natural growth regulators to plants have been carried out and are known to increase growth. Using natural materials is an environmentally friendly step that saves production costs. The growth regulators that will be applied to increase growth are auxins and cytokinins. The hormone cytokinin stimulates branch formation, while shoot growth is stimulated by the hormone auxin (Brault and Maldiney, 1999; Moubayidin et al., 2009).

Plant hormones can be used to increase crop yields, which affect every phase of the growth and development of a plant. Zeatin is one of the most common forms of cytokinin hormones in plants. Young Moringa (*Moringa oleifera*) leaves are known to have a high zeatin content. The zeatin content found in young Moringa leaves is reported to be more than 5 mcg/g (Mona, 2013). Mung bean (*Vigna radiata*) sprouts contain phytohormones such as auxin, gibberellins, and cytokinins (Marliah et al., 2010). Fatimah (2008) explained that cytokinins, auxins, and gibberellins accelerate the process of cell division and development, and stimulate the growth of shoots and roots. In addition, natural growth hormones that can be used are cytokinins and auxins contained in coconut (*Cocos nucifera*) water (Aisa et al., 2020). Coconut water is a natural growth stimulant in propagation by cuttings with an auxin content of up to 60% and cytokinins of up to 20% (Ayyubi et al., 2019). Coconut water contains the cytokinin hormones 0.0017% and 0.0039% auxin, which play a role in optimizing metabolism and growth (Rosniawaty et al., 2018, Rosniawaty et al., 2020). Besides being easy to obtain, this plant growth regulator (PGR) is natural. However, research on soaking *C. racemosa* in coconut water, crude extracts of Moringa leaves, and Mung bean sprouts as natural PGRs have rarely been done. Hence, this study aims to determine the effect of different times soaking of *C. racemosa* in coconut water, moringa leaves, and Mung bean sprouts on its morphology, growth performance, and nutrient content.

Materials and Methods

This research was conducted at the Center for Brackish Water Aquaculture Fisheries (BBPBAP) in Jepara, Central Java, Indonesia. The *C. racemosa* seeds used as research material came from Jepara, Indonesia. The weight of the seeds used in each treatment was 50 g. The container used for this study was a concrete pond with a sandy mud substrate (Windarto et al., 2021), a seawater level of 80 cm, and an off-bottom planting method with a maintenance period of 30 days.

This study used five treatments each with three replications. The treatment was natural PGR of Mung bean sprouts, Moringa leaf extract, and coconut water with different soaking times, including treatment A (without soaking), treatment B (aqueous extracts of mung bean sprouts and Moringa leaves soaked for 2 hours), treatment C (aqueous extracts of mung bean sprouts and Moringa leaves soaked for 4 hours), treatment D (coconut water soaked for 40 minutes), and treatment E (coconut water soaked for 50 minutes).

Preparation of the natural PGRs: The treatment used in the study refers to the research of Warohmah and Karyanto (2018) that the solution was made with green bean seeds, which germinated for two days. The Mung beansprouts were weighed as much as 250 g to be blended by adding 500 ml of distilled water and filtered using filter paper. The Moringa leaf solution was prepared by blending 100 g of Moringa leaves in 500 ml of distilled water and filtering using filter paper. Meanwhile, green coconut water was used for treatments D and E.

Soaking process: According to the treatments, seaweed weighing 50 g/treatment was soaked in a mixture of sprout solution (5 ml) and Moringa leaves (5 ml) in 1 L seawater. At the same time, for the coconut water treatment, the amount of coconut water used was 250 ml of coconut water and 750 ml of seawater.

Morphology of *C. racemosa*: Morphological observations of *C. racemosa* were carried out before and after rearing for 30 days, including observing stolons, ramuli, fronds, and color.

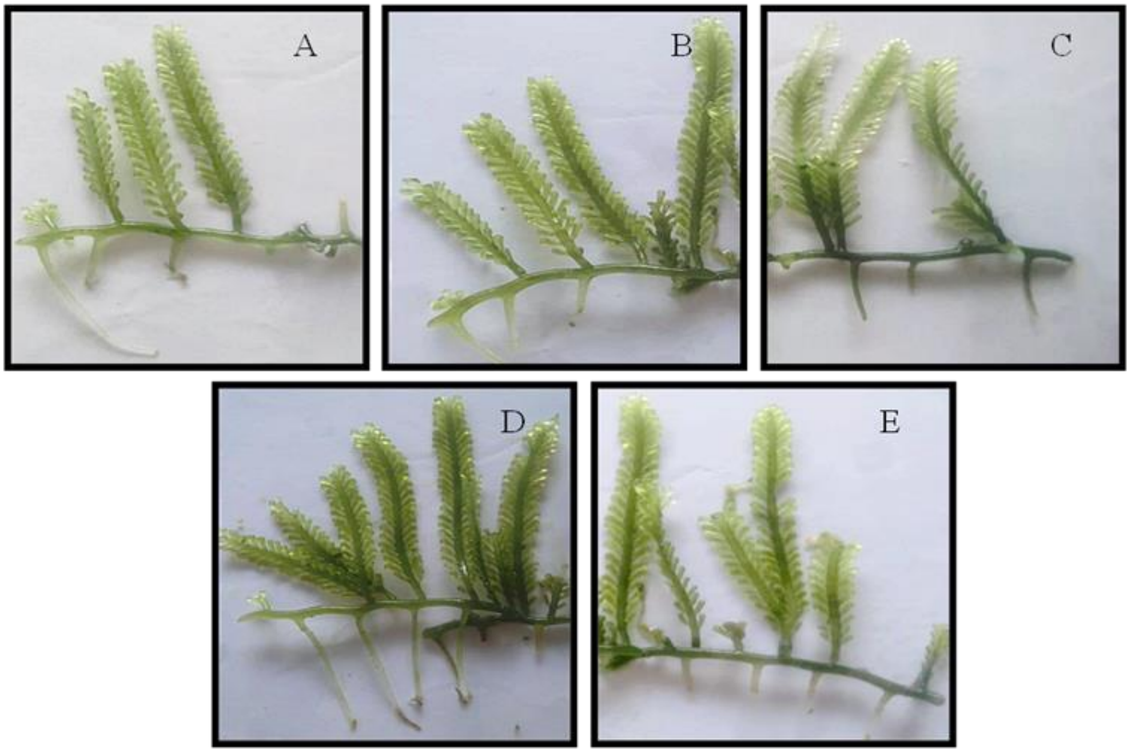

Figure 1. Morphology of *Caulerpa racemosa* during treatment. (A) without soaking; treatment B (aqueous extracts of mung bean sprouts and Moringa leaves soaked for 2 hours); treatment C (aqueous extracts of mung bean sprouts and Moringa leaves soaked for 4 hours); treatment D (coconut water soaked for 40 minutes) and treatment E (coconut water soaked for 50 minutes).

Absolute weight growth: According to Effendi (1997), the calculation of absolute growth was calculated using the formula of $W = W_t – W_0$, where W = absolute growth test(g), W_0 = Test weight at the start of maintenance (g), and W_t = Test weight at the end of maintenance (g).

Specific growth rate: The Specific Growth Rate (SGR) was calculated based on the formula according to Effendi (1997): SGR = (Ln W_t-Ln W_0)/t x 100%, where SGR = Specific growth rate (%/day), W_t = Final research weight (g), W_0 =Initial research weight (g), and t = length of time of research (days).

Proximate analysis: The sample's protein, fat, ash, fiber, and water content were determined using a proximate analysis (AOAC, 2005). The Kjeldahl technique was used to analyze the protein content. The Soxhlet method was used to analyze the fat content. The water and ash content were assessed using gravimetric principles. The carbohydrate content was manually determined based on the findings of the approximate analysis.

Water quality: Water quality in each variable was measured using different tools. Dissolved oxygen (DO) and temperature were measured using a Water Quality Checker, salinity using a refractometer, pH using a pH meter, and temperature using a thermometer. Water quality measurements were carried out two times per day.

Data analysis: The data is analyzed using analysis of variance (ANOVA), after a normality test, a uniformity test, and an additivity test are performed to ensure that the data is normal, homogeneous, and additive. Assuming a known significance (P<0.05), proceed to Duncan's multi-region test to determine the mean difference between treatments. Morphological and water quality data were analyzed descriptively.

Results

was determined by comparing the growth increment readings on scales by two readers. It was observed that the number of annuli

Morphology: Figure 1 shows *C. racemosa* morphology. Ramulli, fronds, stolon, rhizoid, color, fouling, and disease were observed. The used *C. racemosa* were healthy and clean.

Growth performances: After 30 days, *C. racemosa*

Table 1. Nutrition of *Caulerpa racemosa* (wet weight).

Parameter (%)	Treatment				
	A	B	C	D	E
Moist	87.45±0.16	86.54±0.31	87.09±0.66	84.34±0.41	86.79±0.09
Ash	5.90±0.25	4.46±0.46	4.78±0.08	5.14±0.81	3.66±0.21
Fat	0.38±0.16	0.37±0.03	0.29±0.02	0.39±0.18	0.34±0.07
Fiber	1.01±0.13	0.82±0.16	0.72±0.22	1.02±0.20	0.98±0.03
Protein	4.89±0.24	7.81±0.11	7.12±0.17	9.11±0.29	8.23±0.05

Description: A (without soaking), B (aqueous extracts of mung bean sprouts and Moringa leaves soaked for 2 hours), C (aqueous extracts of mung bean sprouts and Moringa leaves soaked for 4 hours), D (coconut water soaked for 40 minutes), and E (coconut water soaked for 50 minutes).

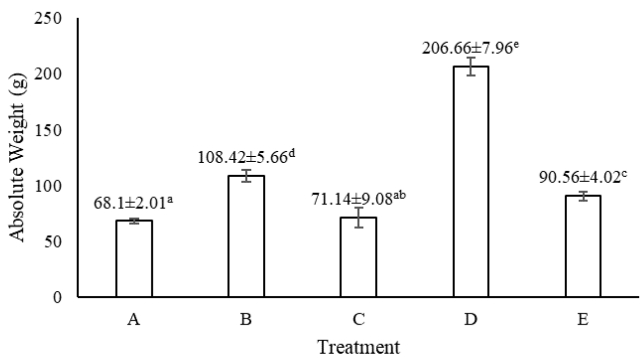

Figure 2. Absolute growth of *Caulerpa racemosa*.

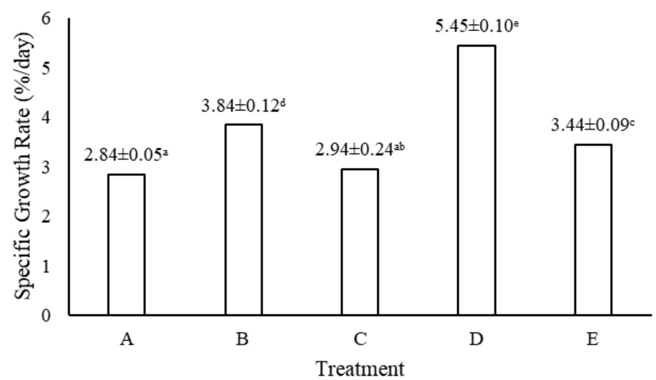

Figure 3. The specific growth rate of *Caulerpa racemosa*.

weight increased from 50g. Figure 2 shows the absolute growth of *C. racemosa*. Based on the result, treatment D showed the highest absolute weight (206.66±7.96 g); meanwhile, treatment E showed the lowest absolute weight (90.56±4.02 g).

Figure 3 shows the specific growth rate of *C. racemosa* under different treatments. Based on the result, the highest specific growth rate of *C. racemosa* was treatment D (5.45±0.10 %/day). The lowest specific growth rate was recorded in treatment A (2.84±0.05 %/day). Based on the result, soaking *C. racemosa* in the natural PGRs had a significant effect on the absolute growth and specific growth rate. *Caulerpa racemosa* soaked in coconut water for 40 minutes (D) showed the best result (W: 206.66±7.96 g, SGR: 5.45±0.10%/day), followed by treatments B, E, C, and A. It showed that natural PGRs influence the growth performance of Caulerpa. *Caulerpa racemosa* soaked in coconut water (D) grew better than soaking with moringa leaf extract and mung bean sprouts.

Nutrition of *C. racemosa*: Table 1 shows the nutrition composition of *C. racemosa* 30-day treatment (wet weight). The nutrient analysis showed that the soaking of *C. racemosa* on natural PGRs significantly affected their nutrients ($P<0.05$). The highest protein and fiber were found in treatment D at 9.11±0.29 and 1.02±0.20%, respectively, and the lowest nutrients were found in treatment A.

Water Quality: Water quality is one factor that supports Caulerpa's growth. The results of water quality are presented in Table 2.

Discussions

Based on the results, *C. racemosa* exhibited a favorable state of health and cleanliness, devoid of any indications of pathological conditions. The ramulli of each treatment had favorable characteristics, displaying abundant growth on the branches and overall good health. The morphological condition demonstrated that plant growth regulators (PGRs) impacted various plant structures, including the ramulli, fronds, stolon, rhizoid, and coloration. Treatment A had some rotten parts. When exposed to natural PGRs, *C. racemosa* had a greater number of ramulli than treatment A. Both the fronds and the stolon were normal. However, the stolon of treatment D was the longest and thickest, while the stolon of

Table 2. Water quality data during maintenance.

Treatment	Variable			
	Temperature (°C)	DO (mg/l)	pH	Salinity
A	26.0-28.4	4.23-5.64	7.71-8.13	29-31
B	26.3-28.6	4.27-5.67	7.70-8.15	29-31
C	26.5-28.9	4.22-5.66	7.78-8.16	29-31
D	26.2-28.4	4.26-5.68	7.77-8.14	29-31
E	26.0-28.5	4.25-5.66	7.76-8.11	29-31
References	25-30[a]	3-8[b]	7.70-8.30[c]	28-33[d]

Note: [a]Nana *et al*. (2012); [b]Wantasen and Tamrin (2012); [c]Ilustrisimoo *et al*. (2013); [d]Anggadiredja *et al*. (2008)

treatment A was the smallest and thinnest. The morphological differences between *C. racemosa* that had not been immersed in PGRs (A) and *C. racemosa* that had been immersed in PGRs were reported to be due to the hormone content in natural ZPT such as auxin, gibberellins, cytokinins, and their derivatives. These hormones are effective in stimulating growth, strengthening stems, and regulating cell development (Miransari and Smith 2014; Heyl et al., 2012; Mamaril, 1988; Mintah et al., 2018; Aisa et al., 2020; Estevez, 2021). Treatment A, which did not incorporate the soaking process, yielded more stems with a lack of frond growth.

Additionally, the fronds in this treatment did not possess ramulli at their distal ends. In contrast, other treatments exhibited a tallus characterized by a profusion of ramulli towards the distal portions of the fronds. Wahyono et al. (2021) stated that Auxin, an agent that prevents leaf rot, is found in coconut water. In plants, auxins can undergo a reaction that results in the production of inhibitors. These inhibitors can then work to prevent the creation of ethylene, which leads to plant organ rotting.

Since the auxin hormone affects the size and length of plant cells, it will significantly impact seaweed growth. Treatment A resulted in more stems that do not produce fronds and few ramulli. A tallus is present in other treatments, but the fronds do not have any ramuli at their tips. According to Ariyanti et al. (2020), Auxin regulates cell enlargement and elongation and increases plant growth. Additionally, Auxin promotes plant development.

The rhizoid was normal, and the color of each treatment was green. *Caulerpa racemosa* soaked in natural PGRs showed a greener color than the control group. It is presumably due to the interaction of growth regulators auxin and cytokinins with *C. racemosa*, which can increase the amount of chlorophyll and slow down chlorophyll degradation. Using growth regulators auxins and cytokinins can increase the amount of chlorophyll in aging plant tissues, thereby slowing down the degradation of chlorophyll and the aging process of plants (Fu et al., 2000; Costa et al., 2005).

It was hypothesized that the disparity in results was attributable to the soaking time being either less or longer duration. An excessive amount of soaking will produce excessive growth additives, which will not be suitable for growth requirements. It has been demonstrated by Septiyaningrum et al. (2020) that the ideal pH range for sea grape growth is between 7.3 and 8.2. Suppose the soaking process is longer than the maximum recommended time. In that case, the thallus will be damaged (it will bleach), and the color will change. This condition arises due to an excessively high level of PGRs in the thallus of the seaweed. The addition of natural plant growth regulators has a substantial effect on the growth of seaweed. This is believed because coconut water, moringa leaves, and Mung bean sprouts contain growth hormones such as auxin, gibberellins, cytokinins, and their derivatives. These hormones help stimulate growth, cell division, elongation, and cell development. It is suspected that soaking for too long will reduce growth performance. This is thought to be because the phytohormones contained in growth regulators are easily damaged non-enzymatically due to the influence of light intensity and high temperatures (Dascaliuc, 2002;

Sukmadi, 2013).

According to the findings of the current work, sea grapes that did not receive any treatment or were not given any additional hormones developed more slowly than those that did. According to Aisa et al, (2020), soaking seaweed seeds in a solution containing growth hormones is one method that can be used to increase the quality of seaweed seeds. Coconut water includes numerous growth hormones, including cytokinin and auxin. The view of Owusu (2020), asserts that the influence of the auxin hormone in the water of the coconut can be traced to the increased plant growth that occurs as a result of the application of coconut water. The auxin hormone in coconut water speeds up cell division and plant development in length. Wahyono et al. (2021) pointed out that coconut water contains growth regulators such as the hormone auxin; the hormone auxin can help trigger the growth of blood vessel tissue and cell division so that it can support growth in stem diameter. Coconut water contains the hormone auxin, which inhibits leaf rot. Auxin can react in plants to produce inhibitors, which function as barriers to the formation of ethylene, which causes rot in plant organs. Ayyubi et al. (2019) stated that coconut water is also known as a natural growth stimulant in propagation by cuttings.

Aqueous extract of moringa leaf and mung bean sprouts containing exogenous growth hormones applied to *C. racemosa* by soaking affect the growth of *C. racemosa*. Natural growth regulators play an important role in controlling biological processes in plant tissues (Gaba, 2005; Davies, 2010). The role of growth regulators includes regulating the growth speed of each tissue. The activity of growth regulators depends on the type, chemical structure, concentration, plant genotype, and plant physiological phase (George, 1993; Dodds and Roberts, 1982; Satyavathi et al., 2004).

The effect of soaking natural growth regulators in the form of moringa leaves and mung bean sprouts on the growth of *C. racemosa* can occur because they each contain the growth hormones auxin and cytokinin. In forming organs such as shoots or roots, there is an interaction between exogenous growth regulators added to the media and endogenous growth regulators (Winata, 1987). Adding auxin or cytokinin to the culture medium can increase the concentration of endogenous growth regulators in cells, thereby becoming a "trigger factor" in tissue growth and development (Poonsapaya et al., 1989). Cytokinins are not able to carry out their functions well. Cytokinins can play an active role in shoot formation when working with auxin. The formation of shoots and differentiation occurs if there is an interaction between auxin and cytokinin (Widyastuti and Tjokrokusumo, 2007). Mung bean sprouts have several organic compounds, one of which can be used as a growth regulator, namely, auxin. Auxin content in sprouts is 3.24 ppm (Sukmadi, 2013). Moringa leaves have a high cytokinin content; cytokinin content in moringa leaves ranges from 5-200 ppm (Culver et al., 2012). PGRs increase the activity of the enzyme nitrate reductase, which is necessary for converting nitrate to nitrite, which is a critical step in creating protein. PGRs could also be involved in enhancing the activity of the -aminolevulinic acid dehydratase (ALAD) enzyme, lowering the concentration of chlorophyll-degrading enzymes, and improving nutritional content (Shah et al., 2021; Shah et al., 2022; Shah et al., 2023). According to Khan et al. (2020), chlorophyll, protein, and sugar levels in plants treated with PGRs were dramatically increased. According to Patil et al. (2022), the application of PGRs affects the metabolism or synthesis of carbohydrates.

Caulerpa has a high nutritional value and is beneficial for humans. The results showed that *C. racemosa* in this study had high moisture (84.34-87.45%, wet weight), ash (3.66-5.90%), fat (0.29-0.39%), fiber (0.72-1.02), and protein (4.89-9.11%). Fresh seaweed commonly has a high moisture content. *Caulerpa racemosa* is a potential dietary option that offers numerous advantages for human health. These species have earned the name "Green Caviar" due to the high regard in which their consumers hold both their flavour and texture. Caulerpa exhibits numerous applications as a potential food source for humans and animals, owing to its composition of essential macronutrients, including carbohydrates, proteins, dietary fiber, and fats. It also contains vital minerals such as calcium, potassium, magnesium, salt, copper, iron, and zinc. The culinary preparation methods exhibit variations based on the species and geographical origin. Seaweeds are versatile and can be ingested in various forms, including dried, fresh, boiled, in salads, utilized as sauces, or subjected to cooking methods.

Fithriani (2015) stated that the Indonesian *C. racemosa* species is characterized by its significant content of insoluble dietary fiber. Insoluble dietary fiber comprises cellulose and hemicellulose, which are crucial in preventing constipation, colitis, and hemorrhoids. The functional capabilities of seaweed proteins have been demonstrated to include antihypertensive, antidiabetic, antioxidant, anti-inflammatory, antitumoral, antiviral, antibacterial, and various other therapeutic effects. Hence, it may be posited that proteins derived from seaweed possess the potential to serve as a viable and natural substitute to develop functional food products (Thiviya et al., 2022).

The growth of *C. racemosa* is influenced by water quality. Water quality measurements are based on temperature, DO, pH, and salinity. The temperature range obtained during the research was 26.0-28.9℃. According to Nana et al. (2012), seaweed can live at 25-30℃. According to Hui et al. (2014), *C. racemosa* grows well at a temperature of 27.5°C.

Dissolved oxygen is a limiting factor for all living organisms. According to Susilowati et al. (2012), dissolved oxygen is generally found in the surface layer because oxygen gas comes from the air. Phytoplankton also helps increase dissolved oxygen levels in the layers during the day. This addition is caused by the release of oxygen gas due to photosynthesis. Oxygen solubility is significant in influencing the chemical balance of water and the life of organisms. During the *C. racemosa* seaweed cultivation period, dissolved oxygen in the maintenance container was found to be 4.22-5.68 mg/L. According to Wantasen and Tamrin (2012), the dissolved oxygen content to support seaweed cultivation is 3-8 mg/l.

Measurements of the pH in the current study ranged from 7.70-8.16. This range qualifies for the cultivation of *C. racemosa* seaweed. According to Ilustrisimo et al. (2013), the suitable pH range for seaweed cultivation is between 7.7 and 8.3. Water conditions that are very acidic or very alkaline will endanger the survival of organisms because they will cause metabolic and respiration disorders. According to Pescod (1973), pH values are influenced by several factors, including biological activities such as photosynthesis and respiration of organisms, temperature, and the presence of ions in water.

The salinity measured during the present work ranged from 29-31 ppt. This range follows research by Anggadiredja et al. (2008) that sea grapes can grow optimally at salinity between 28-33 ppt. Salinity is one of the water quality parameters that play an essential role in stimulating the growth rate of the biota being maintained (Sulistijo, 2002).

Conclusion

Using plant growth regulators (PGR) such as coconut water and aqueous extract of moringa leaves and mung bean sprouts significantly affected the growth and nutrition of *C. racemosa*. *Caulerpa racemosa* soaked in coconut water for 40 minutes showed the highest absolute growth (206.66±7.96 g), specific growth (5.45±0.10 %/day), protein (9.11±0.29%), and fiber (1.02±0.20). The morphology of *C. racemosa* showed that the ramulli, rhizoid, fronds, and stolon were fresh, healthy, and clean. It suggested that the Caulerpa farmer soak the *C. racemosa* in coconut water for 40 minutes to improve their production. Further research can be conducted, such as identifying the bioactive substance and its amount in the natural PGRs.

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