

Effect of Exogenous Natural Plant Growth Regulators (Pgrs) on the Morphology, Growth, and Nutrient of Sea Grapes (*Caulerpa Racemosa*)

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Abstract

This study aimed to determine the effectiveness of aqueous extract from various plants as an exogenous plant growth regulator (PGRs) in the morphology, growth, and nutrition of *C. racemosa*. The natural PGRs used were coconut water (CW), shallots and coconut water (AESCW), maize (AEM), moringa leaves and mung beansprout (AEMBS), and *E. cottonii* (AEEC). A 50g of *C. racemosa* was soaked on the natural PGRs and was cultivated for 30 days with a sandy mud substrate. The results showed that the soaking of *C. racemosa* on natural PGRs had a significant effect ($P < 0.05$) on absolute growth, specific growth rate, and nutrient. *C. racemosa* soaked in aqueous shallot and coconut water showed the best absolute growth (214.32 ± 7.68 g) and SGR ($5.55 \pm 0.08\%$ /day), meanwhile *C. racemosa* soaked in coconut water had the best nutrition (protein: $9.54 \pm 0.16\%$; fibre: $1.37 \pm 0.21\%$; and fat $0.42 \pm 0.04\%$). The morphological analysis showed that soaking *C. racemosa* on natural PGRs resulted in long fronds, bigger stolons, a greener and fresh colour, and more ramulli.

Introduction

Caulerpa racemosa is a green seaweed or well-known sea grape that is an economically important macroalgae species of the genus *Caulerpa* (Caulerpaceae, Chlorophyta) utilized for local consumption and international trade in Southeast Asian countries (Kandhasamy and Arunachalam 2008; Pereira 2016; Dumilag 2019). Sea grapes contain nutrients such as nitrogen, magnesium, and iron. Seagrasses contain high mineral content, nitrogen, magnesium, iron, protein, polyunsaturated fatty acids (PUFAs), and vitamins (Gabrielsen 1996; Saito et al. 2010; Peña-Rodriguez et al. 2011; Nagappan and Vairappan 2014). Based on nutrient analyses, *C. racemosa* thalli contain 3.60% total nitrogen, 0.938% potassium, 0.614% phosphorous, 16.79% calcium, 0.75% magnesium, 0.018% sulphur, 1.05 ppm iron, 14.95 ppm copper, and 36.13 ppm zinc (Chapman and Chapman 1980).

In Indonesia, locally known as “Lato”, it is used as “pecel” or salads. *C. racemosa* cultivation has been carried out in Indonesia, including in Sulawesi, Nusa Tenggara and Jepara, Central Java. According to DJPB data (2017), the demand for *Caulerpa* increases yearly, both nationally and internationally. National seaweed data for five years (2011–2015) shows a positive upward trend, with an average increase of 22.25%. Increased demand must be accompanied by increased production as well. The problem that arises is that *C. racemosa* has not been maximized. Improving the quality of seeds to boost growth is one strategy to solve this challenge. Seaweed grows due to the processes of respiration and photosynthesis that it performs.

Synthetic plant growth regulators alter the plant's quality and potentially harm human consumption. Plant growth regulators (PGRs) can improve physiological efficiency, including photosynthetic ability, thereby helping in effective flower formation, seed, and fruit development and ultimately enhancing the productivity of the crops (Solaimalai et al. 2001). However, the excessive use of PGRs and their degradation products have side effects on human health, animals, and plants. The residues of PGRs in agricultural products are seriously detrimental to human health because they have been found with

hepatotoxicity, neurotoxicity, carcinogenicity, and other side effects. Furthermore, PGRs are suspected of disrupting human and animal reproductive systems (Xu 2018; Chen et al. 2022).

Currently, efforts are being made to boost growth by making plant growth regulators from natural materials. The role of growth regulators has been documented in hundreds of herbaceous crops (Kavina et al. 2011). Some marine natural materials have been used as PGRs, such as *Ulva*, *Padina*, and *Sargassum* (Garcia et al. 2020). In addition to using marine natural materials, natural PGRs from land plants are widely used. Coconut water has auxins, gibberellins, and cytokinins (Mamaril 1988; Mintah et al. 2018; Aisa et al. 2020; Estevez 2021); Moringa leaves containing zeatin (Culver et al. 2012; Maishanu et al. 2017); mung bean sprouts containing abscisic acid (ABA), gibberellin (GA), jasmonic acid (JA), and indole-3-acetic acid (IAA) (Holdsworth et al. 2008; Linkies and Leubner 2012; Miransari and Smith 2014); maize containing cytokinin (Schmülling et al. 2003; Heyl et al. 2012; Murai et al. 2014); shallots containing auxin (Yunindanova 2018), and *Eucheuma cottonii* containing auxin, gibberellin, and cytokinin (Sedayu et al. 2013;). These natural materials, such as PGRs, are mainly used on higher plants, but it has rarely been investigated on low-level plants such as seaweed. Hence, this study aimed to determine the effectiveness of aqueous extract from various plants as exogenous plant growth regulators (PGRs) in the morphology, growth, and nutrition of *C. racemosa*.

Materials And Methods

Experimental setup and natural plant growth regulators preparation

C. racemosa used was from Jepara, Central Java, weighing 50g/treatment. *C. racemosa* seeds are selected, which are still fresh, healthy, and clean of dirt. The PGRs used were coconut water, shallots, maize, moringa leaves, mung bean sprouts, and *Eucheuma cottonii*. Coconut water (CW) added as much as 25% and 75% seawater. An aqueous extract of shallots and coconut water (AESCW) was made by blending shallots with distilled water (150 g/L). The solution is filtered and taken as 10% plus 25% coconut water and 65% seawater. Seagrasses were soaked in each natural PGRs with the following treatment: aqueous extract of maize (AEM) was made by mixing 50g of young maize kernels, 50g of sugar, and distilled water and fermented for 24 hours, and the crude extract was taken and then mixed with seawater. Aqueous extract of moringa leaves and mung bean sprouts (AEMBS) was made by blending 250g of sprouts and adding 500ml of distilled water and filtering; 100 g of Moringa leaves were blended added 500 ml of distilled water and filtered. The two solutions were mixed, and 5mL was taken to be mixed into 1L of seawater. Aqueous *E. cottonii* (AEEC) extract was made by blending *E. cottonii* with distilled water (1:1), filtering, and extracting. The aqueous extract was taken 5% and mixed into 1L of seawater.

TC: without any PGRs (treatment control)

CW: *C. racemosa* was soaked in coconut water for 40 minutes

AESCW: *C. racemosa* was soaked in an aqueous extract of shallots + coconut water for 40 minutes

AEM: *C. racemosa* was soaked in an aqueous extract of maize for 40 minutes

AEMBS: *C. racemosa* was soaked in an aqueous extract of moringa leaves + mung beansprout for 120 minutes

AEEC: *C. racemosa* was soaked in an aqueous extract of *Eucheuma cottonii* for 90 minutes

Previously, a preliminary test was carried out for each treatment to determine the concentration and duration of immersion that did not make *C. racemosa* wither or even die. After soaking, *C. racemosa* was cultured in cement ponds with sandy mud substrate (Windarto et al. 2021) using the off-bottom method with a 6:1 concentration of nitrate and phosphorus (Harwanto et al. 2020). The cultivation process was carried out for 30 days.

Morphological analysis

The samples were taken out of the pond one at a time, cleaned, and preserved in wet seawater until weighed. After the study, morphology and appearance were noted, particularly ramulli, fronds, stolon, rhizoid, colour, fouling, and disease signs.

Measurement of growth performances

Absolute growth of *C. racemosa*

Absolute growth of *C. racemosa* has used the formula of Togatorop et al. (2017):

$$G = W_t - W_0$$

Where:

G: absolute growth (g)

W_0 : weight at the start of culture (g)

W_t : weight at the end of culture (g)

Specific Growth Rate

The following formula (Syahlun et al. 2013) was used to calculate specific growth rate (SGR) values:

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100\%$$

Which:

SGR: specific growth rate (% day⁻¹);

W_t : weight of *C. racemosa* at the end of culture (g);

W_0 : weight *C. racemosa* at the start of culture (g);

t: Culture duration (days).

Analysis of *C. racemosa* nutrient

A standard technique was used to determine the samples' proximate chemical composition (AOAC 2005). The parameters for proximate analysis were protein, fat, ash, crude fibre, and moisture. Protein analysis was performed using the Kjeldahl method. Lipid samples were analyzed using the gravimetric method using the weight of the sample to find the lipid levels.

Water Quality

Different devices were used to determine the water quality for each characteristic. A water quality checker (YSI environmental 550A) was used to assess DO, temperature, and pH; a refractometer (Atago S/Mill-E) was used to detect salinity.

Data Analysis

The data collected throughout the trial was evaluated using Analysis of Variance (ANOVA) and the Duncan test to see significant differences between treatments. Water quality was analyzed descriptively.

Results

Morphology of *C. racemosa*

The result of the morphology of *C. racemosa* under treatment can be seen in Fig. 1. Parameters observed were ramulli, fronds, stolon, rhizoid, colour, fouling, and disease signs. Overall, *C. racemosa* was in good condition and clean, with no signs of disease.

The fronds and stolons of each *C. racemosa* have different sizes. Based on observations made after 30 days of the study, data on the longest fronds and the largest stolon size were obtained, as seen in Table 1.

Table 1
The longest fronds and the largest stolon size of *C. racemosa*

Treatment	Fronds length (mm)	Stolons diameter (mm)
TC	10	68.5
CW	39	535.2
AESCW	17	70.9
AEM	19	135.6
AEMBS	13	92.3
AEEC	11	101.2

The result showed that *C. racemosa* soaked in coconut water (CW) had the longest fronds and the most oversized diameter of stolons; meanwhile, the *C. racemosa* without any treatments showed the shortest fronds and stolons.

Absolute Growth of *C. racemosa*

The initial weight of *C. racemosa* planting was 50g; during 30 days of maintenance, the weight increased. The initial weight and final weight data can be seen in Table 2.

Table 2
The initial weight and final weight of *C. racemosa*

Replication	Initial weight (g)	Final weight (g)					
		TC	CW	AESCW	AEM	AEMBS	AEEC
1	50	118.30	247.80	259.10	186.65	159.40	134.66
2	50	116.10	263.20	275.20	168.44	163.54	140.77
3	50	120.18	259.00	258.70	165.80	152.34	129.23
4	50	111.34	255.30	264.30	170.50	160.61	125.34
Average		116.48 ± 3.81	256.33 ± 6.54	264.33 ± 7.69	172.85 ± 9.40	158.97 ± 4.75	132.50 ± 6.71

The result showed an increase of *C. racemosa* during the culture (30 days); treatment AESCW showed the best result; on the other hand, the TC showed the slightest result.

The absolute growth of *C. racemosa* under different treatments can be seen in Fig. 2.

Specific Growth Rate (SGR) of *C. racemosa*

The specific growth rate (SGR) of *C. racemosa* under different treatments can be seen in Fig. 3.

C. racemosa Nutrient

The nutrient composition of *C. racemosa* under treatment after 30 days is presented in Table 3.

Table 3
Nutrient of *C. racemosa*

Parameter (%)	Treatment					
	TC	CW	AESCW	AEM	AEMBS	AEEC
Moist	87.23 ± 0.45	80.96 ± 0.11	87.51 ± 0.36	87.20 ± 0.43	88.07 ± 0.25	87.00 ± 0.42
Ash	5.83 ± 0.08	7.72 ± 0.29	2.61 ± 0.04	3.10 ± 0.03	3.09 ± 0.04	4.19 ± 0.05
Fat	0.42 ± 0.05	0.42 ± 0.04	0.72 ± 0.13	0.54 ± 0.05	0.41 ± 0.02	0.60 ± 0.10
Fiber	1.13 ± 0.07	1.37 ± 0.21	1.06 ± 0.07	0.94 ± 0.16	0.87 ± 0.16	1.58 ± 0.06
Protein	5.40 ± 0.41	9.54 ± 0.16	8.12 ± 0.12	8.23 ± 0.20	7.57 ± 0.04	6.64 ± 0.22

The nutrient analysis showed that the soaking of *C. racemosa* on natural PGRs had a significant effect ($P < 0.05$) on the nutrient. The highest protein was found in the treatment CW ($9.54 \pm 0.16\%$), and the highest fibre was found in the treatment AEEC ($1.58 \pm 0.06\%$); meanwhile, the lowest nutrient was found in the treatment TC.

Water quality

Results of water quality measurement for *C. racemosa* culture during the research were presented in Table 4.

Table 4
Water quality of *C. racemosa* culture media

Parameter	Value	Optimum value	References
DO (mg/L)	5,3–5,383	> 3,5	Sunaryo et al. (2015)
Salinity (ppt)	30–32	25–33	Hui et al. (2015)
Temperature (°C)	26–28	26–30	Gao et al. (2018)
pH	7,5–8	6,5–9	Sunaryo et al. (2015)

The data showed that the water quality during research was suitable for culturing the *C. racemosa*.

Discussion

Morphology

Based on the morphology analysis, *C. racemosa* was in good condition and clean, with no signs of disease. Each treatment's ramulli was good, with lots of ramulli on the branches and healthy. However, TC treatment found some rotten parts. *C. racemosa* soaked in natural PGRs showed more ramulli than the TC. Fronds and stolon were normal; treatment CW showed the longest and thickest stolon, while TC showed the shortest and thinnest stolon. The morphological differences between *C. racemosa* that were not immersed in PGRs (TC) and *C. racemosa* that were immersed in PGRs were due to coconut water, shallots, maize, moringa leaves, mung beansprouts, and *E. cottonii* possessing growth hormones such as auxin, gibberellin, cytokinins and its derivatives which are effective for stimulating growth, strengthening stems, and regulating cell development. Coconut water's use focuses on the content of the auxin hormone in coconut water. The hormone auxin affects the size and length of cells in plants, so it will significantly affect seaweed growth. The TC treatment, which did not involve soaking, resulted in more stems that did not grow fronds, while the existing fronds did not have ramulli at the ends of the fronds. In other treatments, there is a tallus with lots of ramulli at the ends of the fronds. Wahyono et al. (2021) stated that coconut water contains auxin, a leaf rot inhibitor. Auxins can react in plants to produce inhibitors that function as inhibitors of the formation of ethylene, which causes the rotting of plant organs. Ariyanti et al. (2020) and Indriyanti et al. (2020) stated that Auxin regulates cell enlargement and elongation and stimulates plant growth. The rhizoid was normal, and the colour of each treatment was green. *C. racemosa* soaked in natural PGRs showed greener colour than TC (without soaking). 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 in *C. racemosa*. 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). The morphology condition showed that the natural PGRs affect the ramulli, fronds, stolon, rhizoid, and colour.

In addition to the role of hormones, the environment is an external factor that causes seaweed to have a good morphology. A suitable environment will make seaweed absorb the nutrients in the media, either in the form of fertilizers or hormones derived from natural ingredients. Brightness is an important factor related to sunlight's availability that affects photosynthesis to support its growth so that rhizoids, assimilators (fronds), and seaweed ramulli can grow well (Yuniarsih et al. 2014). Sandy mud substrate is a suitable medium because *C. racemosa* can stick and propagate well so that the morphology of the *C. racemosa* is not damaged (Windarto et al. 2021).

Growth Performances

Adding hormones through soaking in natural PGRs produces significant effects on seaweed growth. This is thought to be because coconut water, shallots, maize, moringa leaves, mung beansprouts, and *E. cottonii* possess growth hormones such as auxin, gibberellin, cytokinins, and its derivatives which are effective for stimulating growth, cell division, elongation, and cell development.

Based on the result of the study, soaking *C. racemosa* in the natural PGRs had a significant effect ($P < 0.05$) on the absolute growth and specific growth rate. *C. racemosa* soaked in aqueous shallots and

coconut water (AESCW) showed the best result (G: 214.32 ± 7.68 ; SGR: $5.55 \pm 0.08\%/day$), followed by treatment CW, AEM, AEMBS, AEEC, and TC. AESCW showed the best result because shallot and coconut water contain auxin, gibberellin, and cytokinin. Auxin in coconut water is absorbed by seaweed. Auxin stimulates cells to divide rapidly and develop into shoots and stems (Ariyanti et al. 2018; Aisa et al. 2020). The hormone auxin itself influences the formation of fronds and cell division in seaweed. Auxin hormone helps accelerate the occurrence of cell division in the growth process. The auxin concentration of 0.0039% in young coconut water causes differentiation and shoot formation because coconut water has better auxin and cytokinin reserves. The content of auxin and cytokinin in coconut water has an essential role in cell division, thus helping the formation of shoots and stem elongation. Coconut water contains 60% auxin and 20% cytokinin. Indriyanti et al. (2020) stated that auxin regulates physiological processes and stimulates plants to grow at low levels. This is confirmed by Ayyubi et al. (2019) that coconut water is also known as a natural growth stimulant in cuttings propagation. Shallot was known to contain growth hormones such as auxin and gibberellin, which could enhance the growth of seeds. Plant growth and development were stimulated by the environment or hormones (Yunindanova et al. 2018). The combination (AESCW) will work synergistically compared to treatments that use only one ingredient (CW).

C. racemosa soaked in an aqueous extract of maize (AEM) showed better results compared to the control because the crude maize extract contains the hormone gibberellins, which causes thickening of the thallus, the cytokinin group that functions in cell division, inhibits chlorophyll degradation, and aging increasing stem height (Damiska et al. 2015; Pandeey et al. 2017; Sundari et al. 2016). *C. racemosa* soaked in an aqueous extract of moringa leaves and mung beansprout (AEMBS) and soaked in an aqueous extract of *E. cottonii* had a better result than the control moringa leaves, mung beansprout, and *E. cottonii* containing exogenous growth hormones, such as auxin, cytokinin and gibberellin. The auxin content in mung beansprouts is 3.24 ppm (Sukmadi 2013). Moringa leaves have a high cytokinin content; the cytokinin content in Moringa leaves ranges from 5-200 ppm (Culver et al. 2012). Adding auxin or cytokinin to the culture media can increase the concentration of endogenous growth regulators in cells to become a "trigger factor" in growth and tissue development (Poonsapaya et al. 1989). Cytokinins are not able to carry out their functions properly alone. Cytokinins can play an active role in shoot formation when they work together with auxin. Shoot formation and differentiation occur if there is an interaction between auxin and cytokinin (Widyastuti and Tjokrokusumo 2007). Natural growth regulators play an important role in controlling biological processes in plant tissues (Gaba 2005; Davies 2010). The role of growth regulators, among others, regulates the growth rate of each tissue. The activity of growth regulators in growth depends on the type, chemical structure, concentration, plant genotype and plant physiological phase (Dodds and Roberts 1982; George 1993; Satyavathi et al. 2004).

Water Quality

Caulerpa spp is native to warm tropical waters and is relatively easy to propagate. Several factors influence *Caulerpa* spp growth, including age, phenotype, genotype, salinity, temperature, nutrient availability, and light (Mosquera and Salamanca 2016; Kasim et al. 2017; Aris et al. 2021). The results of

temperature measurements obtained a value of 26-28°C, which is considered optimal to support the growth of *C. racemosa*; according to Gao et al. (2018), the optimal temperature for the growth of *C. racemosa* is 26°C-30°C this is because at that temperature *C. racemosa* will form new stolon growth. The salinity value obtained during the maintenance period was 30–32 ppt. The salinity range suitable for the growth of *Caulerpa* sp. is 25–30 ppt (Bambaranda et al. 2019; Hui et al. 2015), a relatively high growth rate is in the salinity range of 29–37 ppt (Rabia 2016) and will not experience growth if the salinity is below 25 ppt (Mosquera et al. 2016; Rabia 2016). The measurements in the rearing pond showed that the average dissolved oxygen value obtained was 5.3–5.83 mg/L. This value follows the opinion of Sunaryo et al. (2015) that the value of dissolved oxygen in the cultivation of *Caulerpa* sp. is preferably more than 3.5 mg/L. The pH measured during the study was 7–8, where the range was still suitable for the growth of *C. racemosa*. Illustrisimo et al. (2013) stated that the pH 7-8.3 growth of *Caulerpa* sp. has increased, Susilowati et al. (2012) said that alkaline waters (7–9) are productive waters and play a role in encouraging the process of changing organic matter in water into minerals that can be assimilated by phytoplankton.

Conclusion

The results showed that the soaking of *C. racemosa* on natural PGRs had a significant effect ($P < 0.05$) on absolute growth, specific growth rate, and nutrient. *C. racemosa* soaked in an aqueous of shallot and coconut water at 40 minutes showed the best absolute growth (214.32 ± 7.68 g) and SGR ($5.55 \pm 0.08\%$ /day), but *C. racemosa* soaked in coconut water had the best nutrition (protein: $9.54 \pm 0.16\%$; fibre: $1.37 \pm 0.21\%$; and fat $0.42 \pm 0.04\%$). The morphological analysis showed that soaking *C. racemosa* on natural PGRs resulted in long fronds, bigger stolons, a greener and fresh colour, and more ramulli.

Statements and Declarations

Data availability

All data generated or analyzed during this study are included in this published article.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Conceptualization: Seto Windarto, Titik Susilowati; Methodology: Seto Windarto, Titik Susilowati; Formal analysis and investigation: Seto Windarto, Dicky Harwanto, Alfabetian Harjuno Condro Haditomo; Writing - original draft preparation: Seto Windarto; Writing - review and editing: Seto Windarto; Funding acquisition: Seto Windarto, Alfabetian Harjuno Condro Haditomo, ; Resources: Seto Windarto, Dicky Harwanto; Supervision: Seto Windarto, Titik Susilowati

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Figures

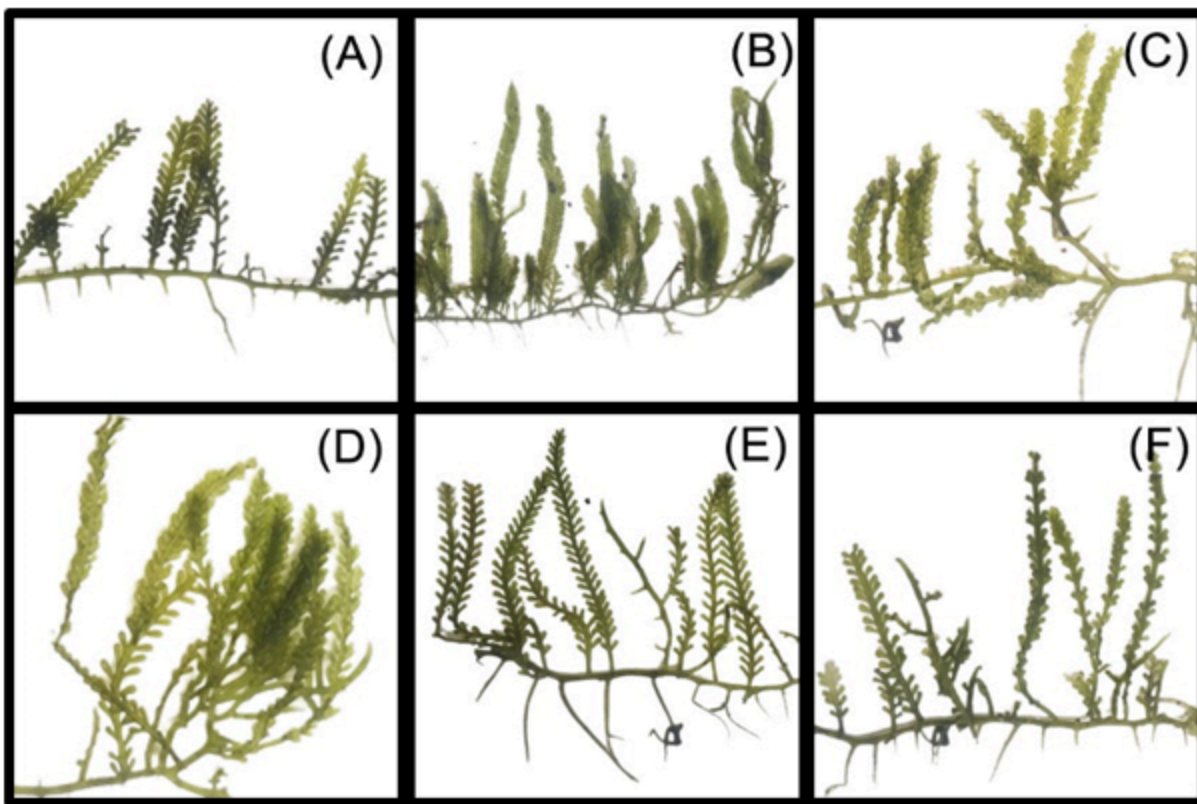


Figure 1

Morphology of *C. racemosa* under treatment for 30 days (A) Control; (B) Soaked in coconut water (CW); (C) Soaked in an aqueous extract of shallots and coconut water (AESCW); (D) Soaked in an aqueous extract of maize (AEM); (E) Soaked in an aqueous extract of moringa leaves and mung beansprout (AEMBS); and (F) Soaked in an aqueous extract of *E. cottonii* (AEEC).

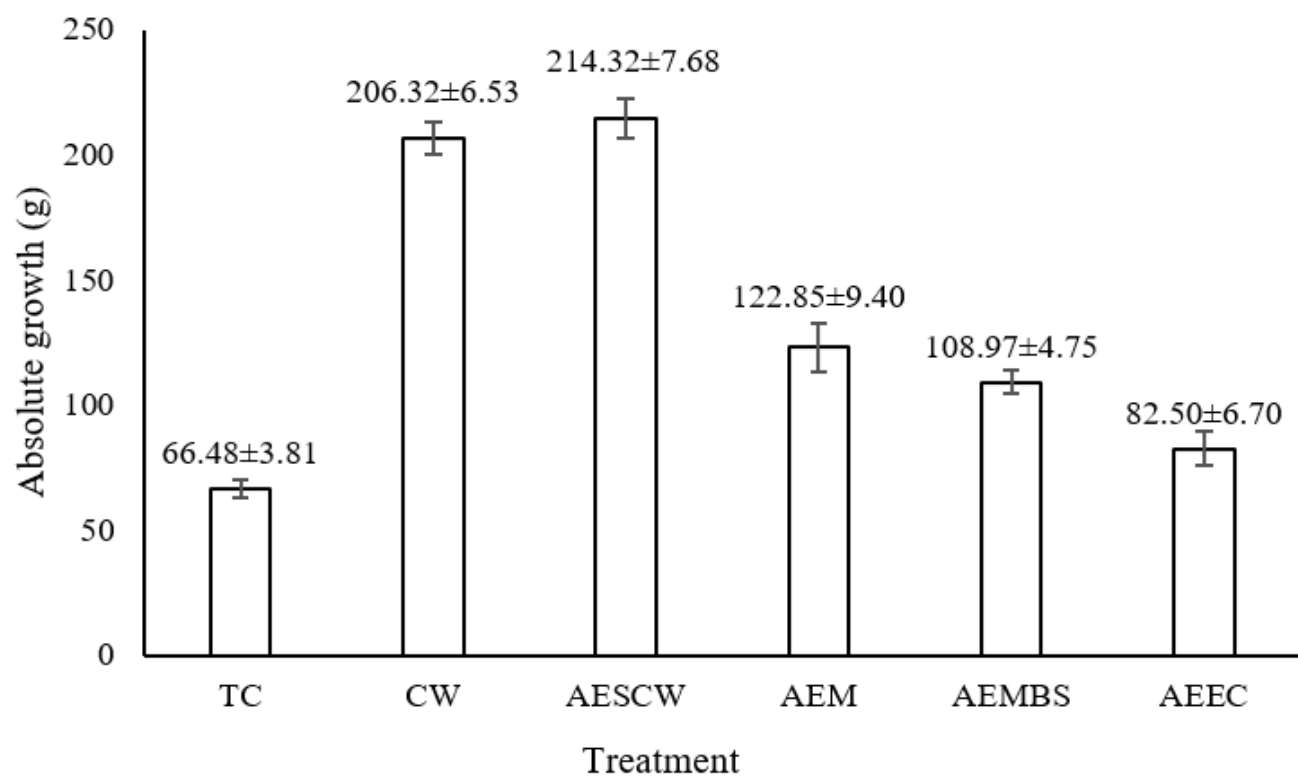


Figure 2

Absolute growth of *C. racemosa*

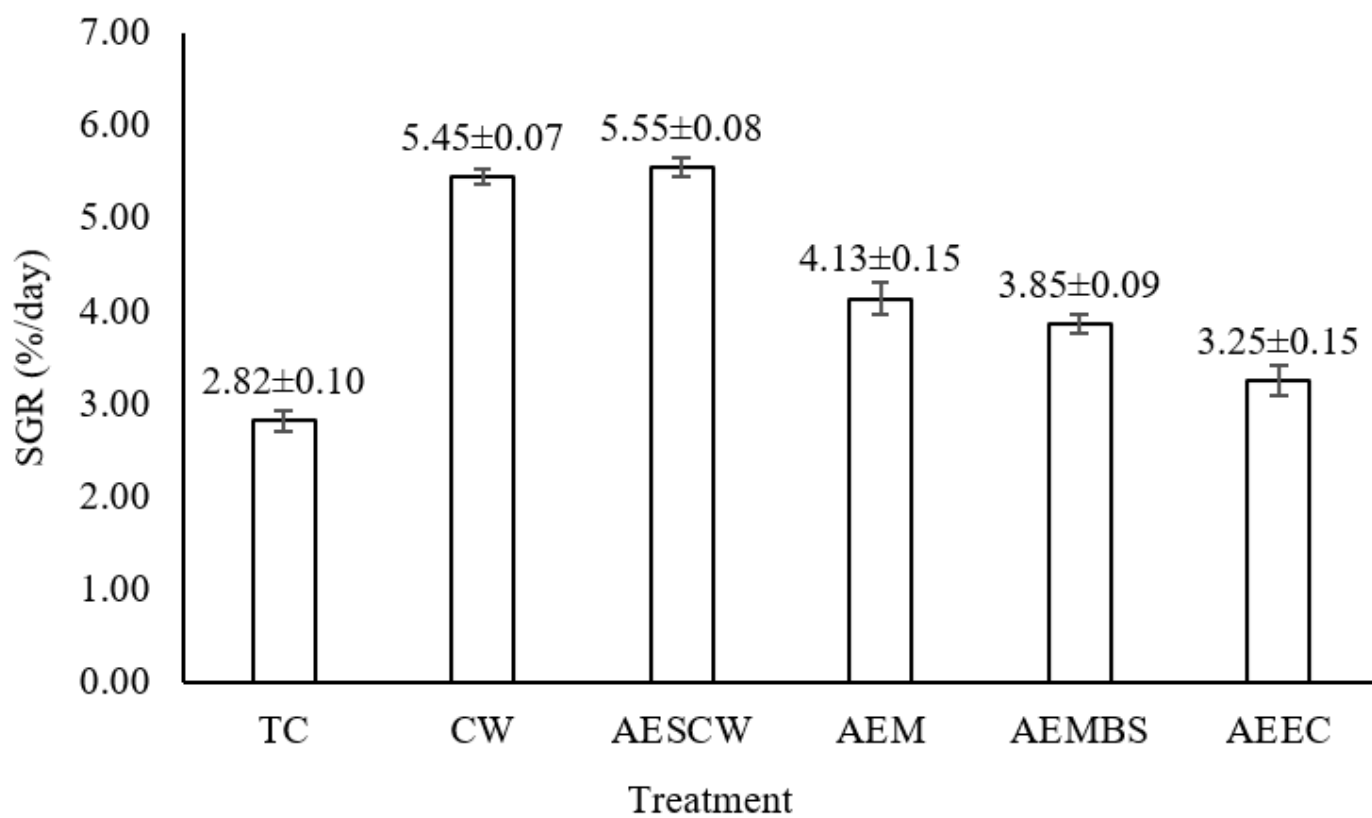


Figure 3

The specific growth rate of *C. racemosa* (%/day) during research

During the research, the specific growth rate (SGR) of *C. racemosa* resulted in *C. racemosa*, which soaked in an aqueous extract of shallots and coconut water had the best result ($5.55 \pm 0.08\%$ /day), followed by CW, AEM AEMBS, AEEC, and TC. The statistical test showed that the soaking of *C. racemosa* on natural PGRs had a significant effect ($P < 0.05$) on its specific growth rate.