Effect of liquid organic fertilizer on growth and carrageenan of *Eucheuma denticulatum* (Solieriaceae: Rhodophyta)

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Abstract. The present research was conducted to determine the effect of liquid organic fertilizer on the growth and carrageenan of seaweed. *Eucheuma denticulatum* seedlings were selected and soaked in a liquid organic fertilizer solution at different doses and soaking times. The study was conducted in 2 cultivation cycles for 35 days, from September to November 2021, using the off-base method in the waters of Bolok Village. The daily growth rate (DGR), absolute growth rate (AGR), relative growth (RG), and carrageenan yield of *E. denticulatum* ranged from 2.40±0.04 to 3.21±0.13% day^{-1}, 32.90±0.45 to 51.26±1.05 g day^{-1} and 65.79±0.89 to 102.53±0.13%, 0.40±0.01 to 1.11±0.02%, respectively. The results of *E. denticulatum* carrageenan extraction showed the presence of a significant difference between treatments ($p<0.05$). These values are influenced by changes in the water quality variables during the cultivation period. In conclusion, the application of liquid organic fertilizer can increase the volume and quality of the carrageenan content of *E. denticulatum* cultivated by the off-base method.
Key Words: fertilizer immersion, growth rates, micronutrients, seaweed.

Introduction. Kupang Regency, East Nusa Tenggara Province has the potential for seaweed cultivation area of 9,015 ha with a total production reaching 305,333 tons year^{-1} of the genus *Eucheuma* sp. and *Kappaphycus* sp. (Kusuma et al 2021). The community in Bolok Village, Kupang, Indonesia mostly cultivated *Eucheuma denticulatum* seaweed traditionally, where growth is left to natural conditions without any treatment, so that the results cannot be maximized. Apart from the influence of environmental conditions, the quality of the seaweed seeds used is also a determining factor for seaweed productivity at the cultivator level. The limited quality of seeds is one of the factors that often cause a decrease in production (Serihollo et al 2021).

Increased production can be achieved through the use of the latest technology that is easy and adaptive, by adding nutrients to seaweed to increase growth and regeneration of seaweed cells. Liquid organic fertilizer (LOF) is enriched with macro and micro nutrients, amino acids, and growth hormones that are formulated in a balanced manner and are very beneficial in every phase of plant growth (Daud et al 2014). The addition of LOF at a dose of 0.5 mg L^{-1} was able to increase the growth of *E. denticulatum* up to 2.78% day^{-1} (Sarira et al 2018) and increased the carrageenan content of *Eucheuma cottonii* by 66.52%, with a treatment time of 2 hours and a fertilization concentration level of 2 g (Rijoly et al 2020).

The study investigates the growth and carrageenan production of *E. denticulatum* to provide critical baseline information for the future aquaculture of this species. This study aimed to determine the growth and carrageenan yield based on the treatment of *E. denticulatum* (cultivated with the off-base system) with LOF. The results of the study are expected to be used as information and comparison for further research that wants to explore more deeply the positive effect of LOF on seaweed in the waters of Kupang Regency, Indonesia.

Material and Method

Description of the study sites. The current study was conducted at Bolok Village, Kupang Regency, Indonesia, about 2 nautical miles from the mainland. The location of the waters is easily accessible and has coral and sandy bottom conditions. The research activity was carried out close to the location of the Polytechnic of Marine and Fisheries Kupang (Figure 1).

Figure 1. Map of study location, Kupang, Indonesia.

Donor reef and nursery setup. *E. denticulatum* used for this study came from Bolok Village waters, which were purchased from fishers. *E. denticulatum* used for research was first adapted in the teaching factory of Marine and Fishery Polytechnic Kupang. In total, 1,200 g of *E. denticulatum* and 150 mL of LOF were used: the LOF was soaked in 25 jars with 50 g of *E. denticulatum*, each. The method used in this research is an experimental method with a factorial, completely randomized design. There are two main factors, namely the LOF dose with four levels and the LOF soaking time for seaweed seeds with two levels. Each treatment was repeated three times. The study also used control (A0B0) as a comparison. The type of treatment, LOF dose, and soaking time can be seen in Table 1.

Table 1

Kind of treatments, doses, and application of liquid organic fertilizer

Treatment	*Doses*	*Time*
No LOF (A0B0)	-	-
A1B1	3 mL L^{-1} of seawater	15 minutes
A1B2	3 mL L^{-1} of seawater	30 minutes
A2B1	5 mL L^{-1} of seawater	15 minutes
A2B2	5 mL L^{-1} of seawater	30 minutes
A3B1	7 mL L^{-1} of seawater	15 minutes
A3B2	7 mL L^{-1} of seawater	30 minutes
A4B1	10 mL L^{-1} of seawater	15 minutes
A4B2	10 mL L^{-1} of seawater	30 minutes

The research began with the preparation of a container in the form of a jar (volume 2 L), totaling 25 units. The container is filled with a volume of 1.5 L seawater with a salinity of 34 ppt, sourced from the waters of Bolok Village. The next step was to weigh the LOF according to the dose of each treatment. Each container was equipped with a hose and an aeration stone, which served as a mixer so that the LOF in the jar was homogeneous. The steps taken after weighing the LOF were: determining the initial weight of *E.*

denticulatum which to be used in this study, using a digital scale. 50 g of *E. denticulatum* were used in each treatment. Cultivation of *E. denticulatum* was carried out for 35 days from September to November 2021. Daily growth rate (DGR) and absolute growth rate (AGR) were calculated using the formula of Dawes et al (1994). Relative growth (RG) was calculated using the formula of Yong et al (2013). The harvested *E. denticulatum* were then sun-dried, and the carrageenan yield was calculated using the formula of Rijoly et al 2020. To determine the carrageenan content of *E. denticulatum*, tests were carried out at the analytical laboratory, Artha Wacana Christian University Kupang.

The daily growth rate was calculated using the following formula (Dawes et al 1994):

$$DGR\ (\%\ day^{-1}) = \mathit{ln}[(W_f/W_o)/t] \times 100$$

Where:
ln - the natural logarithm;
W_f - the final fresh weight (g) at *t* day;
W_o - the initial fresh weight (g);
t - the number of culture days.

The absolute growth rate of *E. denticulatum* was calculated using the formula of Dawes et al (1994):

$$AGR\ (g) = W_t - W_o$$

Where:
G - the absolute growth of seaweed (g);
W_t - the weight of seaweed seedlings at the end of the study (g);
W_o - the initial weight of seaweed seedlings at the beginning of the study (g).

The relative growth was calculated using the following formula (Yong et al 2013):

$$RG\ (\%) = [(W_t/W_o)^{1/t} - 1] \times 100$$

Where:
W_t - the average seaweed wet W_t at time *t*;
W_o - average wet weight of seaweed at day 0;
t - the time intervals (days).

The harvested *E. denticulatum* were then sun-dried, and the carrageenan yield was calculated using the following formula (Rijoly et al 2020):

$$\text{Carrageenan yield } (\%) = (\text{isolated carrageenan weight}/\text{dried sample weight}) \times 100$$

***Environmental parameters**.* Observations of water quality parameters were carried out once every seven days. Water samples were collected at the beginning and at the end of the study for carrageenan content, nitrate concentration, and turbidity measurements. Water quality in each variable was measured using different instruments. Dissolved oxygen, temperature, and pH were measured using an oxygen meter (Lutron DO-5510), and salinity using a refractometer (Atago S/Mill-E). Everything is measured on the spot. Seawater nitrate concentration was measured using a spectrophotometer (Optima sp-3000), which was carried out at the Technical Implementation Unit of the Environmental Laboratory, Department of the Environment and Forestry of East Nusa Tenggara Province.

***Statistical analysis**.* All data were tested for assumptions of normality and homogeneity. The quantitative data obtained were processed and analyzed as descriptive statistics using the Statistical Package for the Social Sciences (SPSS) version 18 (International Business Machines Corporation). A significant difference was considered at the level of $p<0.05$. Secondary data (water quality data) were analyzed as descriptive statistics using Microsoft Excel.

Results. The daily growth rate of *E. denticulatum* ranged from 2.40±0.04 to 3.21±0.13% day^{-1}. *E. denticulatum* seed soaking treatment at a dose of 7 mL L^{-1} of seawater and a soaking time of 30 minutes resulted in the best daily growth rate (Figure 2). Duncan's test results showed that several treatments were significantly different from each other (Table 2).

Table 2

The daily growth rate of *Eucheuma denticulatum* in all treatments (mean±SD)

Treatment	*Average daily growth rate (% day^{-1}) ± SD*
No LOF (A0B0)	2.40^{a}±0.04
A1B1	2.50^{ab}±0.07
A1B2	2.49^{ab}±0.22
A2B1	2.52^{ab}±0.17
A2B2	2.62^{bc}±0.22
A3B1	2.98^{d}±0.13
A3B2	3.21^{e}±0.13
A4B1	2.55^{ab}±0.14
A4B2	2.66^{bc}±0.16

Values (mean of duplicates ±SD) in same row with different letters are significantly different (P<0.05).

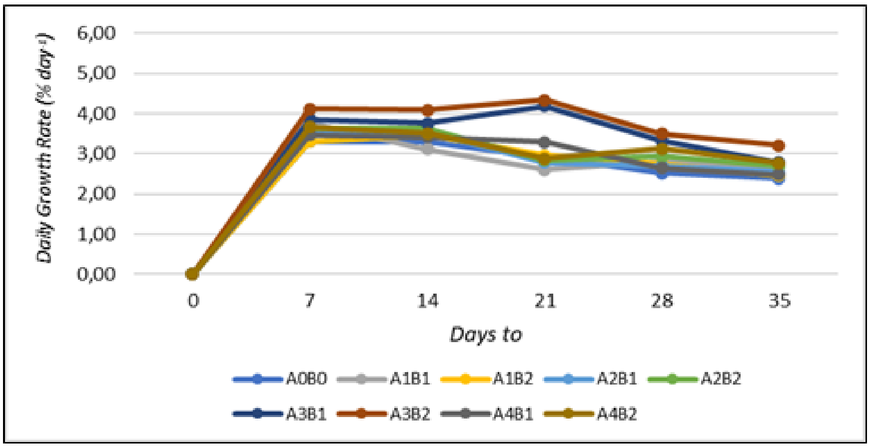

Figure 2. The daily growth rate of *Eucheuma denticulatum* in each treatment.

Absolute growth. The absolute growth rate of *E. denticulatum* ranged from 32.90±0.45 to 51.26±1.05 g. The highest absolute growth was obtained in the A3B2 treatment and the lowest was obtained in the control. The absolute growth of *E. denticulatum* over 35 days is presented in Figure 3. Duncan's further test results showed that several treatments were significantly different from each other (Table 3).

Table 3

Absolute growth of *Eucheuma denticulatum* in all treatments

Treatment	*Average absolute growth rate (g) ± SD*
No LOF (A0B0)	32.90^{a}±0.45
A1B1	35.33^{bc}±1.18
A1B2	34.94^{b}±1.29
A2B1	35.01^{bc}±1.52
A2B2	37.59^{d}±1.95
A3B1	45.43^{f}±1.09
A3B2	51.26^{g}±1.05
A4B1	35.68^{c}±1.27
A4B2	39.11^{e}±1.03

Values (mean of duplicates ±SD) in same row with different letters are significantly different (P<0.05).

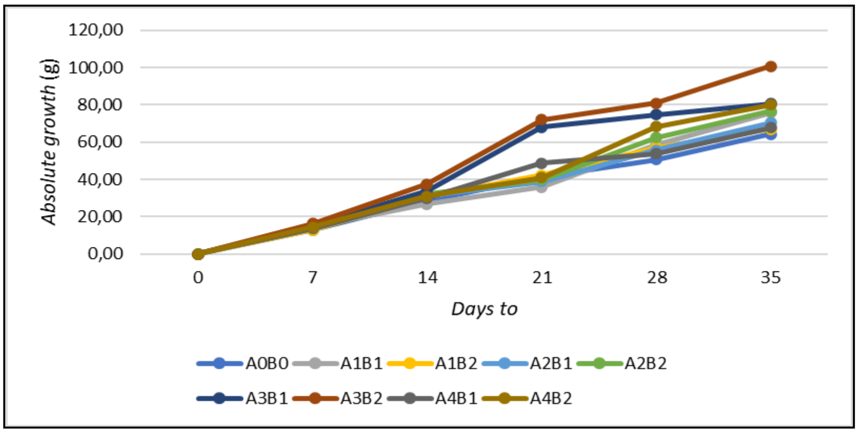

Figure 3. Absolute growth of *Eucheuma denticulatum* in each treatment.

Relative growth. The relative growth of *E. denticulatum* ranged from 65.79±0.89 to 102.53±0.13%. Duncan's further test results showed that several treatments were significantly different from each other (Table 4). The relative growth of *E. denticulatum* over 35 days is presented in Figure 4.

Table 4

Relative growth of *Eucheuma denticulatum* in all treatments

Treatment	*Average relative growth (%) ± SD*
No LOF (A0B0)	65.79^{a}±0.89
A1B1	70.67^{cd}±2.35
A1B2	69.88^{b}±2.57
A2B1	70.03^{bc}±3.04
A2B2	75.17^{e}±3.90
A3B1	90.86^{g}±2.18
A3B2	102.53^{h}±0.13
A4B1	71.36^{d}±2.54
A4B2	78.21^{f}±2.07

Values (mean of duplicates ±SD) in same row with different letters are significantly different (P<0.05).

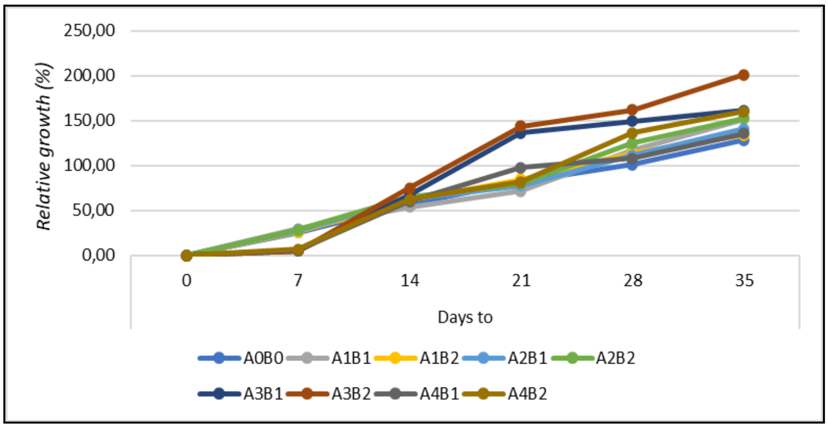

Figure 4. Relative growth of *Eucheuma denticulatum* in each treatment.

Carrageenan yield. The yield of carrageenan showed significant differences between treatments (p<0.05). The yield of carrageenan ranged from 0.40 to 1.11%. The carrageenan yield in each treatment can be seen in Table 5. The amount of carrageenan yield of *E. denticulatum* is presented in Figure 5.

Table 5

Carrageenan yield of *Eucheuma denticulatum* in all treatments

Treatment	*Average carrageenan yield (%) ± SD*
No LOF (A0B0)	$0.40_{a}\pm0.01$
A1B1	$0.48_{c}\pm0.02$
A1B2	$0.80_{f}\pm0.02$
A2B1	$0.69_{e}\pm0.01$
A2B2	$0.44_{b}\pm0.01$
A3B1	$1.09_{g}\pm0.01$
A3B2	$1.11_{g}\pm0.02$
A4B1	$0.60_{d}\pm0.01$
A4B2	$0.61_{d}\pm0.02$

Values (mean of duplicates ±SD) in same row with different letters are significantly different (P<0.05).

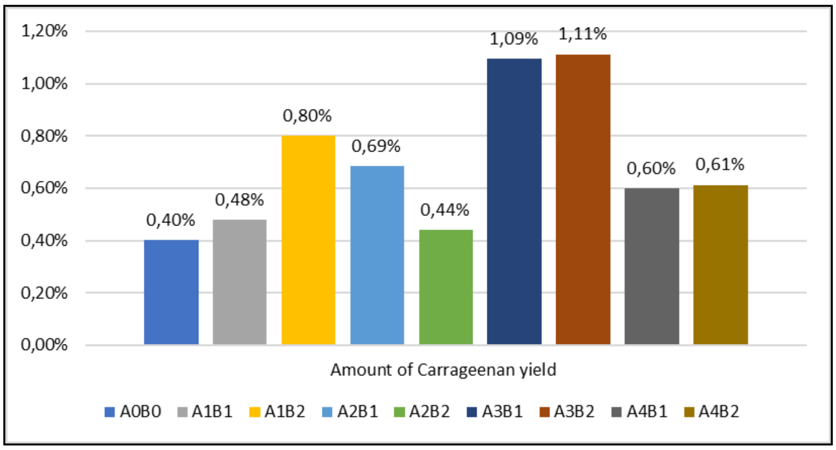

Figure 5. Amount of carrageenan yield of *Eucheuma denticulatum* in each treatment.

The results of the measurement of water quality parameters at the research site indicated quite suitable conditions for the growth of *E. denticulatum* seaweed. The water quality parameter is a supporting factor for seaweed growth. The results of observations of water quality parameters are presented in Table 6.

Table 6

Water quality parameters

Parameter	*Unit*	*Value*	*Ideal value*	*Source*
Depth	m	0.3-0.5	0.3-0.6	Irawan et al (2019)
Current speed	$m\ s^{-1}$	0.5-0.6	0.2-0.4	Indonesian National Standard (2010)
Nitrate	$mg\ L^{-1}$	0.001-0.025	>0.04	Indonesian National Standard (2011)
Orthophosphate	$mg\ L^{-1}$	0.001	>0.1	Indonesian National Standard (2011)
Temperature	^{0}C	25-31	26-32	Indonesian National Standard (2011)
pH		8.0-8.5	7.0-8.5	Indonesian National Standard (2011)
Salinity	‰	30-34	28-34	Indonesian National Standard (2011)

Discussion. The growth of *E. denticulatum* which was observed for 35 days showed an increase in growth from day 1 to day 17 and experienced the highest growth on day 24, because at that time the seaweed cells were still young, which allowed an active vegetative growth, accelerating the daily growth rate.Meanwhile, on the 35th day, the seaweed experienced a decrease in the growth rate of each treatment because it was suspected that at that time the seaweed had reached the maximum growth rate limit.

The highest daily growth rate in this study was 3.21±0.13% day^{-1}, which is greater than the results of the study of Sarira et al (2018), 2.78% day^{-1}, of Yusup et al (2017), 1.7-2.91% day^{-1} and of Yuliana et al (2013), 2.73% day^{-1}. In the use of LOF, attention shoud focus on the right dose and soak time. According to Pratiwi & Fadilah (2017), the impact of using organic fertilizer on seaweed growth is quite high if applied at the right dose. According to Anggadiredja et al (2006), a good seaweed growth has a growth rate of more than 2% day^{-1}. The average daily growth rate of *E. denticulatum* in the A3B2 treatment was considered good because, according to Ariyati et al (2016), thedaily growth of seaweed was 2.03-2.36% day^{-1}, which is fairly good. The increase in seaweed growth was found in the 3rd week because at that time the seaweed had passed a period of adaptation to its environment.

The A3B2 treatment and the A3B1 treatment were not significantly different, so the A3B1 treatment was considered the best because although using the same dose, due to a shorter time of soaking the seaweed. Treatment with a LOF dose of 7 mL L^{-1} of seawater gave better results compared to other doses, because in this treatment the seaweed could optimally absorb the macro and micronutrients contained in the fertilizer, to the seaweed needs for growing.

The absolute growth of seaweed ranges from 32.90±0.45 to 51.26±1.05 g. This value is greater than the study of Yuliana et al (2017), where *E. denticulatum* is soaked for 24 hours in LOF, with the best absolute growth of 21.59%. The results of the analysis of variance stated that there was a significant difference ($p<0.05$) between the LOF dose treatment and soaking time impacts on the absolute growth of *E. denticulatum*. The highest absolute growth of *E. denticulatum* occurred at the dose of A3B2 treatment, due to a higher nutrient absorption. Nutrients in this study were fulfilled by LOF containing growth hormones such as gibberellins, auxins, and cytokinins, which accelerate the process of growth and development of seaweed seedlings. The availability of the required amount of nutrients must be balanced so that it can stimulate the thallus growth and accelerate the formation of new shoots (Ginting et al 2015; Aliyas et al 2019).

The relative growth in the A3B2 treatment experienced a higher and more stable increase compared to all treatments; the lowest relative growth was found in the control treatment. The results of variance calculation showed that the relative growth of *E. denticulatum* was significantly influenced by differences in the treatment dose of LOF and soaking time ($p<0.05$). Treatment dose LOF 7 mL L^{-1} of seawater with a soaking time of 30 minutes gave an increase in the growth of *E. denticulatum* which was higher on the 7^{th} and 14^{th} days, decreased on the 21^{st} day, and increased again on the 28^{th} day until the end of maintenance. LOF with hormone content applied to *E. denticulatum* has assisted the enzymatic process in converting starch into sugar which is then used as an energy source, so growth takes place quickly. According to Usumagi & Polanunu (2019), the role of growth hormone contained in the LOF will accelerate the process of growth and development of seaweed seedlings. The hormone auxin accelerates the formation and elongation of the thallus, the hormone gibberellin helps the enzymatic process to convert starch into sugar as an energy source for growth, and the hormone cytokinin stimulates the process of cell division and the formation of new shoots.

The results of variance for the yield of *E. denticulatum* carrageenan showed a significant difference between treatments ($p<0.05$). However, two treatments were not significantly different, namely treatments A3B2, with a yield of 1.11% and A3B1, with a yield of 1.09%. Based on the results of observations, the value of carrageenan extract for all treatments was very low, in contrast to the research of Asmi et al (2013), where it was 19.37%. The low carrageenan extract in all treatments was due to the small thallus of the *E. denticulatum*, not too rubbery and less elastic. *E. denticulatum* is a source of iota carrageenan, which is capable of forming a smooth gel (Necas & Bartosikova 2013; Siregar et al 2016).

The low content of carrageenan obtained during the study could also be caused by the lack of absorption of nutrients because the experiment is performed in a location with strong currents, at the beginning of the rainy season. Strong waves make it difficult for seaweed to absorb nutrients that are useful for the growth and formation of carrageenan (Aslan 1998). The seaweed carrageenan content is usually high in summer, due to high photosynthetic rates and low nitrogen content, whereas in spring carrageenan levels are low (Sangkia et al 2018). The yield produced in this study did not meet the standard requirements for carrageenan yield set by the Ministry of Trade of the Republic of

Indonesia, which is a minimum of 25% (Syamsuar 2006). This is because *E. denticulatum* has 2 phases of its life cycle, namely the vegetative phase and the generative phase. During the vegetative phase, the energy is distributed for the growth and formation of carrageenan, followed by a generative phase where the energy for the carrageenan production is reduced at the advantage of the growth process. The carrageenan content decreases while growth continues until it reaches a maximum point (Sulistijo 1996; Safia et al 2019).

The depth requirement for seaweed cultivation using the offshore method is not less than 0.3–0.6 m, at the lowest low tide, because at that time *E. denticulatum* gets sufficient sunlight for the photosynthesis process (Anggadiredja et al 2010). The current velocity is not suitable for the growth of *E. denticulatum* because the location of seaweed rearing is close to breakwater corals so it is in strong wave conditions. A good current velocity for seaweed cultivation activities is 0.2-0.4 m s^{-1}; out of that range, it he seaweed cultivation location is not optimal for seaweed growth (SNI 2011; Parenrengi et al 2016). Concentrations of nitrate and orthophosphate are not ideal for *E. denticulatum* cultivation because their values are <0.2 mg L^{-1}. The low concentration of nitrate at the study site occurred because the samples for nitrate testing were taken at the surface layer (0.5 m deep), where the nutrients in that layer were mostly utilized by phytoplankton (Suniada 2012).

A heavy rain that fell for several days during the study was another factor of the lower temperature. Water temperature affects the rate of photosynthesis and can damage enzymes and cell membranes that are unstable to low and high temperatures. At low temperatures, protein and fat membranes can be damaged as a result of the formation of crystals in cells, thus affecting seaweed life, such as survival, growth and development, reproduction, photosynthesis, and respiration (SNI 2011). The pH value obtained at the research site is 8–8.5. The optimal range of pH values for stable conditions and higher growth of seaweed is 6.5–8.5 (Mudeng et al 2014). The salinity of the waters at the study site was rather low, due to the heavy rains that fell for several days during the study, ranging from 30–34‰, while thestandard for *E. denticulatum* cultivation refer to a range of 32–35 ‰ (SNI 2011).

Conclusions. The study showed the growth data of *E. denticulatum* treated with liquid organic fertilizer, as a way to improve the quality of seaweed seedlings in Bolok Village, Kupang Regency. The best daily growth rate and relative growth were found at a dose of 7 mL L^{-1} of seawater treatment, with a soaking time of 30 minutes, while the highest absolute growth and the best carrageenan yield were found at a dose of 7 mL L^{-1} of seawater treatment, with an immersion time of 15 minutes.

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Conflict of interest. The authors declare no conflict of interest.

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