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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2022; 8(2): 69-74 Araştırma Makalesi/Research Article

A Preliminary Study on the Effects of Inorganic Nutrient Enrichment on the Growth and Survival Rates of Green seaweed *Caulerpa racemosa*

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Abstract

Caulerpa racemosa is one of the edible green seaweeds abundant in the Philippine waters. This seaweed is commonly collected from the wild and sold fresh in the local marketplace throughout the country. Due to harvesting pressures and increasing demand, the expansion of its culture techniques needs to be further explored. In this study, the effects of inorganic nutrient enrichment on the growth and survival rates of green seaweed *C. racemosa* were determined under laboratory conditions for 21 days. Three different concentrations of inorganic fertilizer (ammonium phosphate): 10 ppm (T1), 20 ppm (T2), 30 ppm (T3), and Control (T4, seawater only) were used in the study with 3 replicates. Sampling was done every week. Results revealed that the specific growth rates (SGR) of nutrient-enriched *C. racemosa* in T1, T2, T3, and T4 groups were calculated as 1.41±1.09 % day^{-1}, 0.76±0.76 % day^{-1}, 1.64±2.01 % day^{-1}, and -0.44±0.48 % day^{-1}, respectively. Although no significant difference (p>0.05) was observed among the treatments, 30 ppm (T3) concentration obtained the highest SGR and mean weight throughout the study period. All treatments survived (100%) after 21 days. Our preliminary results indicated that inorganic nutrient enrichment might have positive effects on the growth of green seaweed *C. racemosa*. However, further studies are needed on the effects of inorganic fertilizers as nutrient enrichment for *C. racemosa* to come up with a robust conclusion.

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INTRODUCTION

Seaweeds or macroalgae are one of the top commodities in the world aquaculture (Azis et al., 2019). Among the important seaweeds is the genus of *Caulerpa*, which is commonly known as sea grape because of its grape-like or circular shapes of ramuli containing a high nutritional value that is usually consumed directly or made of various kinds of processed products (Azis et al., 2019; Fakhrulddin et al., 2021). *Caulerpa* species belongs to green seaweed, which gradually increasing its demand in certain places owing to its rich bioactive compounds and secondary metabolites (De Souza et al., 2009; Yangthong et al., 2009; Matanjun et al., 2009; Lin et al., 2012; Santos et al., 2015; Pangestuti and Kim, 2015; Santos et al., 2015; Rabia, 2016). Various species of *Caulerpa* ranges its crude protein level from 3.6 to 7.5 % dry weight (Paul and de Nys, 2008). *C. lentillifera* contained a comparatively high in polyunsaturated fatty acid (PUFA) at more than 5 % of dry weight basis and it has also omega-3 fatty acids like for example linolenic acid (Matanjun et al., 2009; Kumari et al., 2010; Saito et al., 2010). Other pigments and PUFAs in *Caulerpa* play an important role in antioxidant activity (Murata et al., 1999; Bocanegra et al., 2009). Moreover, minerals are the major component of *Caulerpa* species (Paul and de Nys, 2008), including micronutrients like iron and zinc and also the essential trace elements (e.g., chromium, molybdenum, nickel, cobalt, selenium, and vanadium) (Peña-Rodriguez et al., 2011). Therefore, seaweed like *Caulerpa* could be a good source of minerals and trace elements as suggested for daily intake for human (Indergaard and Minsaas, 1991; Ortega-Calvo et al., 1993; Rupérez, 2002; Dawczynski et al., 2007; Paul et al., 2014).

The Tawi-Tawi's local market in the southern Philippines and in other regions as well vastly obtained their sold seaweeds, such as *Caulerpa* species, from the natural environment (Tahiluddin et al., 2022a), and its market demand is continuously increasing. Farming of *Caulerpa* is another measure to lessen its pressure in the wild stock (Gennaro & Piazzi, 2014; Asmida et al., 2017; Susilowati, 2019). Farming of *Caulerpa* species is popular in the Indo-Pacific region (de Gaillande et al., 2017). The cultivation of *Caulerpa* species (*C. racemosa* and *C. lentillifera*) in the Philippines. was started early 1990s and is still widely practiced nationwide (Estrada et al., 2021). *C. racemosa*, in particular, is commonly cultured in man-made ponds in the intertidal mangrove zone (Horstmann, 1983). With the aim of improving the production of *Caulerpa* from aquaculture, there is a need to explore other aspects, such as the incorporation of inorganic nutrient enrichment.

Inorganic nutrient enrichment with different nutrients or fertilizers to boost the seaweeds in terms of production and farmer profits have been reported by various researchers (Luhan et al., 2015; Tahiluddin, 2018; Sahir et a., 2019; Illud et al., 2020; Robles, 2020; Tahiluddin & Terzi, 2021; Tahiluddin et al., 2021b; Tahiluddin et al., 2022b; Sarri et al., 2022). The seaweed farmers in Tawi-Tawi, Philippines, are growing red seaweed *Kappaphycus* species nutrient-enriched with inorganic fertilizers (Tahiluddin et al., 2022b; Tahiluddin et al., 2021a, 2021b and 2021c). Among the utilized inorganic fertilizers is ammonium phosphate [$(NH_4)_3PO_4$], which showed a significant increase in growth and nitrogen assimilation in the cultured seaweed *Kappaphycus* species (Tahiluddin et al., 2021b, 2022b). There are few studies that applied nutrient enrichment to grow *Caulerpa* species, either by using organic fertilizers like cow dung (Rabia, 2016) and vermicompost (Susilowati, 2019) or inorganic fertilizer like Conway's and Von Stotch (Fakhrulddin et al., 2021). The influence of ammonium phosphate on *Caulerpa* species has not yet been studied. Thus, this study aimed to evaluate the effects of nutrient enrichment using ammonium phosphate on the growth and survival rates of green seaweed *C. racemosa*.

MATERIALS AND METHODS

Study site and duration

The experiment was conducted in the Phycology Laboratory of Mindanao State University Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Multi-species Hatchery, College of Fisheries, Sanga-Sanga, Bongao, Tawi-Tawi, Philippines for 21 days from April to May of 2019.

Source and acclimatization of *Caulerpa racemosa*

The naturally growing *C. racemosa* at the MSU-TCTO floating cage, Bongao Channel, Tawi-Tawi, Philippines (Figure 1) was collected manually, including its roots, ramuli, and rhizoid. The seaweed was cleaned by removing the adhering materials such as other seaweeds, debris, and sand, then it was rinsed with sterilized seawater. Clean seaweed was acclimatized for one week in a tank and cultured in a plastic bottle container with sand and sterilized seawater, then covered with PE transparent cellophane. A fluorescent lamp (Firefly, T8) was used as a light (75 µmol photon $m^{-2} s^{-1}$), and 12:12 hours of light and dark were applied. The temperature and salinity of the water for the whole culture duration were 26.5-27 ºC and 34-35 ppt, respectively.

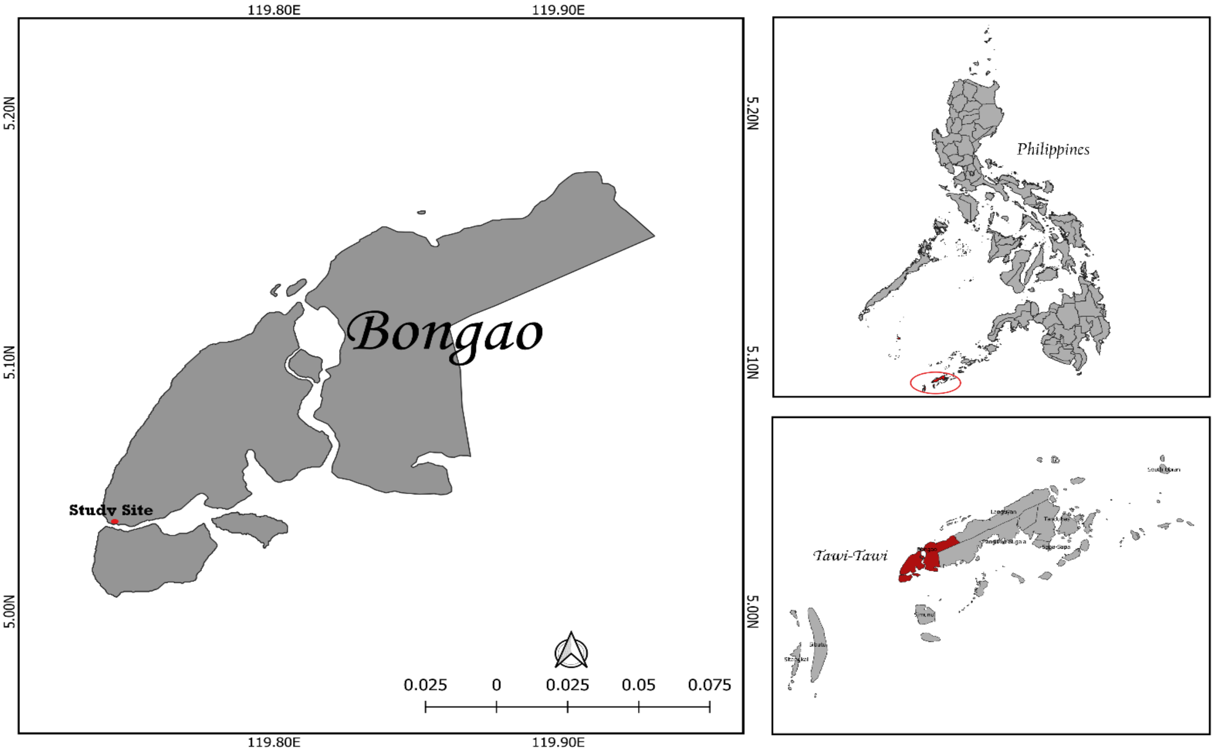

Figure 1. Collection site of *Caulerpa racemosa*

Culture of *Caulerpa racemosa*

Ammonium phosphate fertilizer (16-20-0) which contains nitrogen, phosphorus, and potassium or NPK, was used in culture media. Fertilizer was pounded until the size was fine enough to be dissolved in the seawater. The solutions were prepared according to the added nutrients in the sterilized seawater of the culture media, which served as the treatments of the study, namely: Treatment 1 (T1) – 10 ppm, Treatment 2 (T2) – 20 ppm, and Treatment 3 (T3) – 30 ppm, and Control – only sterilized seawater (T4). Each treatment was in triplicate, and a total of 12 containers were used. Clean sand was provided in each 1200 mL bottle. The seaweed was planted in the sand by submerging the seaweed's root to the bottom. The light was provided, and the temperature and salinity were maintained as mentioned earlier. The water in the containers was changed 100% weekly with fresh, sterilized seawater mixed with the respective concentrations of nutrient.

Growth sampling

The sampling of seaweed was done every 7 days of the culture period. Seaweed was patted with tissue paper to remove the excess water. All seaweed in the culture containers was weighed using a top loading weighing scale. The growth (μ) expressed as a specific growth rate (SGR) was calculated following the formula of Luhan et al. (2015).

$$\mu = \frac{\ln(Wf) - \ln(Wi)}{DOC} x\ 100$$

Where:

ln = Natural log
Wf = Final weight
Wi = Initial weight
DOC = Days of culture

Data analysis

The data on the growth and survival rates were analyzed in IBM SPSS software version 25. Determination of the significant differences of the treatments were computed using One-way Analysis of Variance (ANOVA) and Post-hoc (Duncan) was used to rank the means. To compare each treatment across culture periods, t-test was used. The data were expressed as mean ± SE (standard error). The used level of significance was $p<0.05$.

RESULTS

The specific growth rate (SGR) of *Caulerpa racemosa* in laboratory-cultured enriched with different concentrations of ammonium phosphate for 21 days is shown in Figure 2. One-way ANOVA revealed that nutrient enrichment with ammonium phosphate on the *C. racemosa* did not significantly influence its SGR.

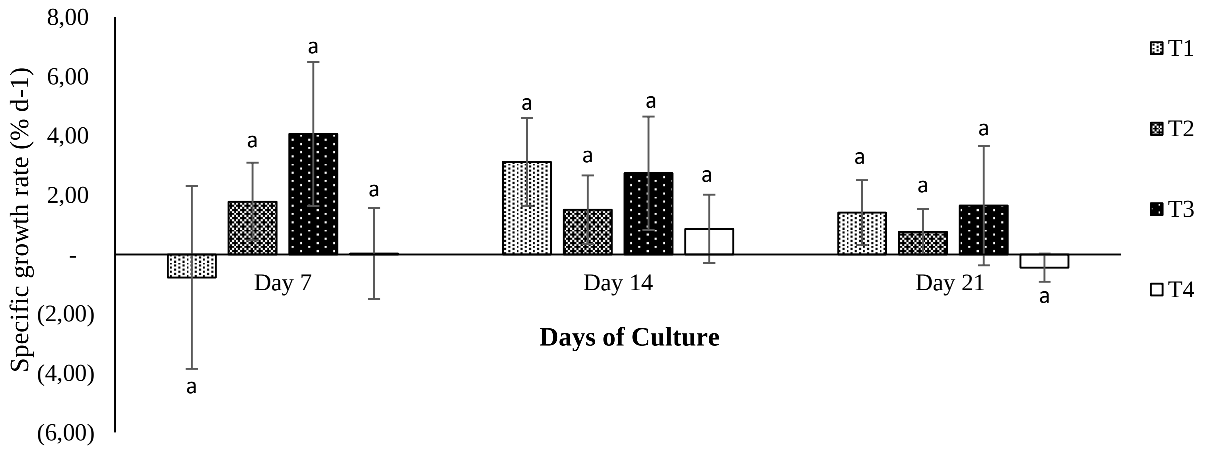

Figure 2. Mean specific growth rate of *C. racemosa* in every sampling. T1=10 ppm, T2=20 ppm, T3=30 ppm, and T4= Control-SSW only. Bar with the same letters are not significantly different ($p>0.05$). Error bars in SEM (standard error mean), n=12.

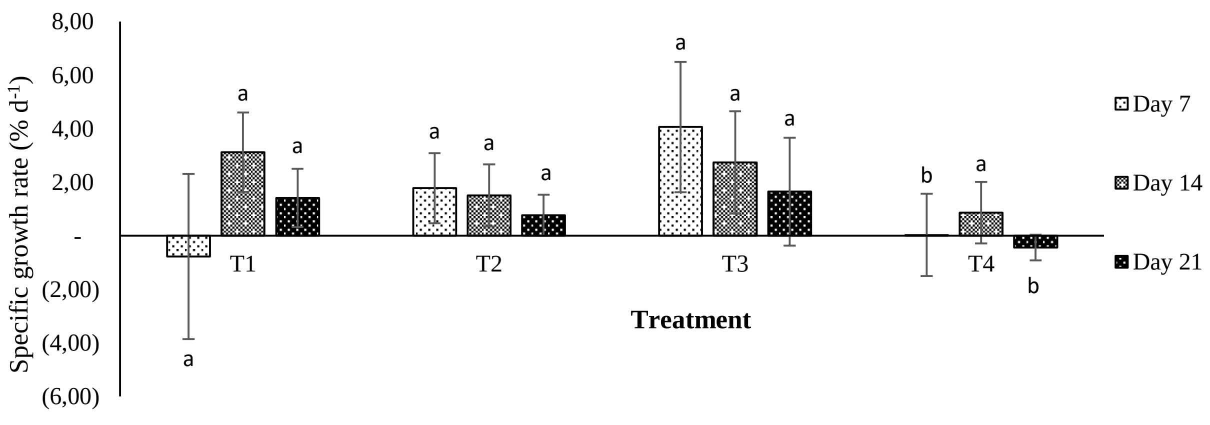

Figure 3. Change of mean specific growth rate of *C. racemosa* in every sampling. T1=10 ppm, T2=20 ppm, T3=30 ppm, and T4= Control- SSW only. Bar with different letters are significantly different ($p<0.05$). Error bars in SEM (standard error mean), n=12.

During the 7 days of the experiment, T3 (4.06±2.43 % day^{-1}) obtained the highest SGR, followed by T2 (1.78±1.31 % day^{-1}), then Control (0.03±1.54 % day^{-1}) and the lowest was T1 (-0.78±3.08 % day^{-1}). On day 14, T1 (3.12±1.48 % day^{-1}) had the highest SGR succeeded with T3 (2.74±1.91 % day^{-1}) followed by T2 (1.50±1.16 % day^{-1}), and the least was Control (0.86±1.15 % day^{-1}). On day 21, T3 (1.64±2.01 % day^{-1}) received the highest SGR, while T1, T2, and Control had SGRs of 1.41±1.09 % day^{-1}, 0.76±0.76 % day^{-1}, and-0.44±0.48 % day^{-1}. Although there were no significant differences among treatments from day 7 to 21, it can be seen in Figure 2 that the T3 (30 ppm) consistently gained the highest SGR. In terms of change in SGR during samplings, T1, T2, and T3 did not show a significant change in SGR from day 7 to 21. However, in a Control group, its SGR significantly increased (p<0.05) from day 7 to 14 but significantly dropped (p<0.05) from day 14 to 21. All the seaweed in all treatments survived (100%) throughout the culture period, although there was a whitening of some parts of seaweed's roots, ramuli, and rhizoids.

DISCUSSION

Although there was no significant difference, higher growth of laboratory-cultured *C. racemosa* was observed when enriched with 30 ppm of ammonium phosphate fertilizer, and without fertilizer, the growth declined after 21 days. The availability of nutrients accessible to the seaweeds enhanced its nitrogen content, a nutrient crucial for seaweed growth, and a previous study indicated that with a higher concentration of nutrients, stored nitrogen was also higher (Tahiluddin et al., 2021b), resulting in better growth performance (Tahiluddin et al., 2022b). This indicates that as a higher concentration of ammonium phosphate is made readily available to *C. racemosa*, the seaweed takes advantage of this nutrient to fuel its physiological needs; hence, the growth was higher in higher concentrations. Rabia (2016) reported that *C. lentillifera* farmed using the sowing method in fishpond performed better growth rate as the nutrient content in substrates was high in concentrations, improving the seaweed productivity and growth compared to the tray method unattached on the substrate of the pond. It is related to the nutrient availability in the culture area or container, which is very significant as fertilizer applied will enhance the plant growth and biomass produced (Fakhrulddin et al., 2021). A positive result is congruent to the study of Zuldin et al. (2019) on *C. macrodisca* with a positive growth rate of 5.13±0.06 % g day^{-1} cultured in a tank. In addition, Paul et al. (2014) obtained results on the mean weight of 0.1 kg $week^{-1}$ *C. racemosa* and 1.5 kg $week^{-1}$ for *C. lentillifera* cultivated using the tray culture method in a 6-week culture period. An almost similar study with used of nitrate nutrient (1mM NO_3-N) to improve the specific growth rate with 0.97 % day^{-1} of the red seaweed *Kappaphycus alvarezii* cultured in the laboratory (Sahoo & Ohno, 2003). According to the result of Luhan et al. (2015), the *K. alvarezii* enriched using 10 ppm of sodium nitrate improved the growth with an SGR of 2.34 % day^{-1} similar result to the findings of Sarri et al. (2022), where nutrient enrichment with 8.82 g L^{-1} urea fertilizer in *K. striatus* obtained good growth with 3.90 % day^{-1} SGR after 45 days of cultured in the farm.

The *C. racemosa* laboratory-cultured with different concentrations of ammonium phosphate in the present study has no effect on its survival rate. The concentrations of fertilizer are still under the tolerable level of *C. racemosa*. The application of fertilizer or nutrients in the culture water is very important in order to achieve target production (Luhan et al., 2015). The benefits can provide by the nutrients depending on the type of fertilizers and the concentrations (Sarri et al., 2022).

CONCLUSION

Our preliminary results on the effects of inorganic nutrient enrichment on *C. racemosa* cultured under laboratory conditions showed that 30 ppm of ammonium phosphate fertilizer obtained better growth performance after 21 days, albeit no significant difference. This suggests that nutrient enrichment can enhance the production of *Caulerpa* species. Further studies need to be conducted in terms of different concentrations, longer culture periods, different culture environments, and effects on the quality of *Caulerpa* on inorganic nutrient enrichment.

COMPLIANCE WITH ETHICAL STANDARDS

a) Authors' Contributions

A. B. T.: Designed the study.

R.J.F. R. & A. B. T.: Performed the experiment work.

R.J.F.R & A.B.T.: Drafted the paper.

b) Conflict of Interest

The authors declare that there is no conflict of interest.

c) Statement on the Welfare of Animals

Ethical approval: For this type of study, formal consent is not required.

d) Statement of Human Rights

This study does not involve human participants.

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