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Growth rate and carrageenan yield of *Kappaphycus alvarezii* in a land-based setup using artificial seawater

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ABSTRACT

Kappaphycus alvarezii is among the most significant tropical red algae commercially cultivated for its economic significance due to the kappa-carrageenan. It is used for multiple products and applications such as gelling, thickening, and stabilizing water-binding agents, and texturing a variety of dairy-based and instant products. With the rising demands and associated farm problems, seaweed farms need alternative cultivation techniques to boost seed stock quality. The percent growth rate and carrageenan yield of a land-based cultivated *K. alvarezii* collected from Calatagan, Batangas were determined for 30 days under optimum salinity (30-31 ppt) and temperature (29-31°C). One 5 g seedling of *K. alvarezii* was placed in each of the twelve aquariums (12 x 6 x 8 in) having 5.6 L of four different media (seawater, Blue Treasure salt, Coral Pro Salt and rock salt). Results showed that the higher percent growth rate in seawater and Blue Treasure salt did not differ significantly (4.58 ± 0.51 % day^{-1} and 3.94 ± 0.39 %day^{-1}, respectively). In addition, the carrageenan yield in Blue Treasure salt (37.75 ± 3.03%) was significantly higher than seawater (18.06 ± 1.23%) but both meet the industrially required carrageenan yield. On the other hand, lower percent growth rate was observed in Coral Pro Salt while seedlings disintegrate in rock salt after a week. The study suggests that Blue Treasure salt can be an alternative medium for growing *K. alvarezii* in land-based cultivation under optimum salinity and temperature.

Keywords: *Kappaphycus alvarezii*, growth rate, carrageenan yield, artificial seawater mix

1. Introduction

Kappaphycus alvarezii is among the most significant tropical red algae with rigid but fleshy morphology, and is commercially cultivated for its economic significance due to the kappa-carrageenan produced from the biomass that boosts immense market demands [1,2]. *K. alvarezii* has become the most vital reservoir of k-carrageenan, a fundamental resource for multiple products and applications such as for gelling and thickening, stabilizing, water-binding agent, and texturing variety of dairy-based and instant products [3,4]. It is vastly cultivated in Southeast Asian countries, specifically in the Philippines, which provides 55% out of the 96.5% of the total production of k-carrageenan [5].

K. alvarezii, which best grows at salinity of 30-31 ppt and temperature of 29-31ºC, has a minimum optimal percent growth rate of above 3.5 [1,6]. For the past decades, seaweed farming has been globally introduced to many countries as a sustainable alternative livelihood and later on become a major aquaculture industry. Industrially, carrageenan yield ranges from 15-64% in the Philippines and around 38% globally [3,7,8]. For almost 25 years, the global demand for carrageenan has increased annually by 5%, leading to the establishment of factories and abundant cultivation of related red seaweeds in the Philippines. These seaweeds include the species of *K. alvarezii* and other varieties [9].

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Seaweed farming in the Philippines accounted for 69% of total aquaculture production due to its significant market importance and demand for carrageenan [10], wherein Calatagan, Batangas is among the pilot farming sites since the 1970s. However, poor seed stock quality prompts a decline in production due to consistent threats. Diseases such as epiphyte infestations and "ice-ice" diseases, which typically affect *Kappaphycus* genera, have become a constant challenge for seaweed farms [11].

The cultivation of *K. alvarezii* in seagrass ecosystems can maintain optimal conditions for growth, including salinity, temperature, light intensity, pH, current velocity, and free CO_2 [12]. The off-bottom method could result in higher growth rates, biomass outputs, and carrageenan contents due to the high nutrient levels, clear water, and ideal growing conditions in the cultivation environment. However, these requirements can be limited by epiphyte infestations and herbivore grazing. To address these challenges, we investigated some alternative cultivation techniques for *K. alvarezii.* The study evaluated the percentage growth rate and carrageenan yield of *K. alvarezii* grown on land-based cultivation in different media under optimal salinity and temperature conditions. These alternative techniques could help reduce farm-related concerns associated with *K. alvarezii* cultivation.

2. Materials and methods

2.1. Gathering and preparation of seaweed material and seawater

Seedlings of *K. alvarezii* were obtained from ELFARCO Seaweed Farm in Calatagan, Batangas. The seedlings were transported to the experimental site in Calamba City, Laguna, which took approximately 3 h. To prevent any stress to the seedlings, they were wrapped with newspapers soaked in seawater and placed inside a cooling container. After reaching the experimental site, the seedlings were allowed to acclimatize for 24 h using the source seawater. They were then cut into their experimental weight and given another 24 h to acclimate. Seawater from the sampling site was collected weekly and used to replace the experimental set-up every 6 days.

2.2. Preparation of different media

Four different media (rock salt, Coral Pro Salt, Blue Treasure salt, and seawater) were used as treatments in the experiment. To create a salinity of 30-31 ppt, 270 g of rock salt, 238 g of Coral Pro Salt, and 250 g of Blue Treasure salt were dissolved in 5.6 L of distilled water. As for the seawater treatment, the source seawater collected from the site was used, and distilled water was added to achieve the required salinity. The composition of the different media is presented in Table 1.

Table 1. Composition of the different seawater media at 31 ppt (based on the product specifications).

Composition (g/L)	Natural seawater	Blue Treasure salt	Coral Pro salt	Rock salt
Rubidium	0.000002	0.00010-0.00012	-	-
Lithium	0.000012	0.00012-0.00014	-	-
Bromine	0.0007	0.02-0.04	-	-
Fluorine	0.00006	0.0007-0.0010	-	-
Boron	0.00040	0.004-0.005	-	-
Strontium	0.00013	0.008-0.009	-	0.0135
Iron	0.00000003-0.0000003	0.00005-0.0002	-	0.0424
Chlorine	0.47	15.32-15.77	-	162.62
Sulphate	0.03	2-2.26	-	1.0098
Sodium	0.40	8.24-8.60	-	103.923
Magnesium	0.04	1.24-1.28	1.20-1.27	0.1404
Potassium	0.009	0.32-0.36	0.35-0.38	0.0448
Calcium	0.009	0.38-0.40	0.40-0.43	0.1172
Phosphate	0.00000003-0.000003	-	≤0.00003	-
Nitrate	0.0000009-0.00004	-	≤0.0003	-
Aluminum	0.0002	-	-	0.0251
Barium	0.0000003	-	-	0.0014
Manganese	0.00000002-0.0000002	-	-	0.0011
Carbon	0.0020	-	-	-
Silicon	0.0000006-0.00012	-	-	-
Iodine	0.0000003	-	-	-
Copper	0.00000002-0.0000002	-	-	-
Arsenic	0.0000002-0.0000003	-	-	-

2.3. Cultivation set-up

An experiment was conducted using a completely randomized design in land-based cultivation of *K. alvarezii*. The aquariums had a dimension of 6 in x 8 in x 12 in and four different media were used (rock salt, Coral Pro Salt, Blue Treasure salt, and seawater) under optimal salinity of 30-31 ppt and temperature of 29-31°C. Each aquarium contained one seedling of *K. alvarezii*, weighing 5 grams. A total of twelve aquariums with four different media having three replicates were utilized and monitored in a covered area for 30 days under natural light conditions.

The salinity in the aquariums was measured three times a day using a hand-held refractometer (01502B Handheld 0-100% Salinity Refractometer with ATC, WANT Balance Instrument Co., Ltd., Changzhou, Jiangsu, China). The water levels were also checked and maintained daily. If the salinity level increased, 500 to 1000 mL of distilled water was added as a countermeasure. The temperature was kept accurate and monitored using a heater with a thermostat (JIX HF-50W Aquarium Heater, FOSHAN YUANSI IMP&EXP CO., LTD, Guangdong, China) and a digital thermometer (RESUN DT-01 Digital Aquarium Thermometer, Shenzhen Xing Risheng Industrial Co., Shenzhen, China).

The light intensity was measured twice using a light meter (Light Sensor logger LI-1500, LI-COR, Lincoln, Nebraska, USA), at around 1100 and 1400. In the experimental setup, the light readings ranged from 49.29 ± 12.24 to 295.79 ± 562.60 µmol photons $m^{-2}s^{-1}$. These readings are comparable to the range of minimum photosynthesis, which is 60-150 µmol photons $m^{-2}s^{-1}$ for shade algae, and maximum photosynthesis, which is around 500 µmol photons $m^{-2}s^{-1}$ for sun algae, with an irradiance level of around 2500 µmol photons $m^{-2}s^{-1}$ [13].

Furthermore, three heavy-duty aerators (RESUN ACO-012 Electromagnetic Air Pump, Shenzhen Xing Risheng Industrial Co., ShenZhen, China) were used to allow proper water circulation allowing efficient absorption of nutrients by the seedlings.

2.4. Measurement of percent growth rate (%day^{-1})

The weight of *K. alvarezii* seedlings was measured every 6 days for 1 month using a standard weighing scale (PL-209 Digital Scale, JunGuan Equipment Trading Co., Shanghai, China). The percentage growth rate was calculated using the formula [14]: $(W_t - W_0) / W_0$ x 100, where W_0 is the initial wet weight of *K. alvarezii* in *g*, W_t is the final wet weight of *K. alvarezii* in *g*, and *t* is the number of days of culture.

$$\%day^{-1} = [(\frac{W_t}{W_o})^{\frac{1}{t}} - 1]x\ 100\% \quad (1)$$

2.5. Measurement of carrageenan yield

After the 30-day cultivation, seedling samples were sent to the Lipa Quality Control Center (LQCC) for the extraction of the refined carrageenan utilizing the extraction method [15] where the samples were treated in 500 mL of 1 M KOH for 2 h at 80°C in water bath followed by extraction in 500 mL deionized water at 80°C for 5 h, then the mixture was homogenized and pressure filtered while still hot. The hot filtered solution was allowed to flow in three volumes of isopropanol, stirred and the precipitate was eventually collected through centrifugation, and further dried in an oven at 60°C. The carrageenan yield was determined using the formula below [16].

$$Carrageenan\ yield = \frac{Carrageenan\ dry\ weight\ (g)}{Seaweed\ dry\ weight\ (g)} \times 100\% \qquad (2)$$

2.6. Statistical treatment

One-way Analysis of Variance (ANOVA) was used to determine the significant difference on the growth rates and carrageenan yield of *K. alvarezii* cultivated in different media. Data were tested for normality and log transformed if data was not uniform. Tukey's HSD was used as post-hoc analysis. Analyses were done in R software (version 3.5.2, R Core Team, Vienna, Austria).

3. Results and discussion

3.1. Percent growth rates of *K. alvarezii* cultivated in different media

Over a 30-day cultivation period, *K. alvarezii* is capable of thriving in a land-based setup subjected to different media. Figure 1 shows the percent growth rates of *K. alvarezii* obtained from the four different media (Coral Pro Salt - 2.29 ± 0.62 %day^{-1}; Blue Treasure salt - 3.94 ± 0.39 %day^{-1}; seawater - 4.58 ± 0.51%day^{-1}). Our result was comparable to rates reported for *K. alvarezii* such as 1.46 ± 0.06 %day^{-1} [17], 1.96 ± 0.08 % day^{-1} [18], and 3.76 ± 0.54 %day^{-1} [6], indicating media used in the present study has potential for possible land-based cultivation. Seawater treatment having 4.58 ± 0.51 %day^{-1} yielded the highest record percent growth rate followed by the Blue Treasure salt treatment and Coral Pro Salt treatment with 3.94 ± 0.39 %day^{-1} and 2.29 ± 0.62%day^{-1}, respectively. However, seedlings in rock salt media disintegrate after a week in cultivation. The percent growth rate in Blue Treasure salt and seawater did not differ significantly but significantly higher in comparison with the percent growth rate in Coral Pro Salt.

Recorded growth rates are comparably higher than those of existing studies using land-based seaweed cultivation [18, 19]. Only Blue Treasure salt and seawater treatments met the generally accepted percent growth rate in industrial demands at 3.5%day^{-1} [19]. However, the percent growth rate under the Coral Pro Salt treatment (2.29±0.62%day^{-1}) in the present study exhibited higher than [18] and [19], which are 1.46 ± 0.06%day^{-1} and 1.96 ± 0.08%day^{-1}, respectively. Therefore, we suggest the use of artificial seawater mixes in place of natural seawater for land-based cultivation. It does not only provide the necessary nutrients for the growth of *K. alvarezii*, but also minimize the risk of epiphyte infestations and ice-ice disease, which can reduce seaweed biomass production.

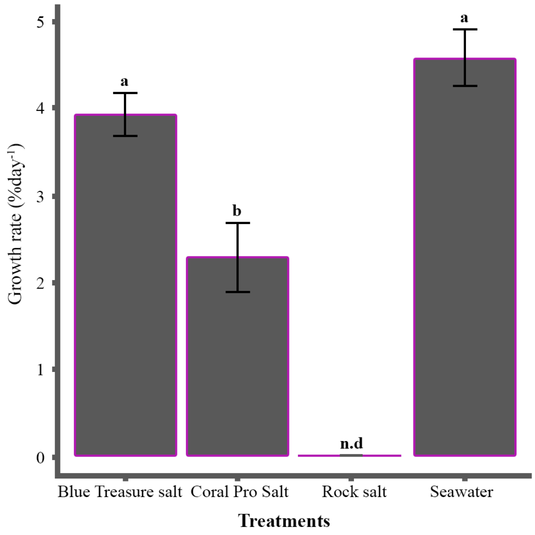

Figure 1. The statistical analysis of the percent growth rate of *K. alvarezii* in different treatments (n.d. - no data ANOVA at p<0.05, vertical bars with the same letter are not significantly different; the vertical bars and lines are the means and standard deviation from three thalli respectively).

3.2. Total carrageenan yield of *K. alvarezii* cultivated in different media

Figure 2 shows that carrageenan yields were significantly higher in Blue Treasure salt (37.75±3.03%) than seawater (18.06±1.23%). Our result is in parallel with [20] (34.94±3.26; 37.60±1.34; 43.37±1.67%), [19] (51.48±0.38 to 54.25±1.11%), and [21] (22.62%). No data for rock salt as seedlings were disintegrated after a week of cultivation. On the other hand, no data for Coral Pro Salt due to possible operational error.

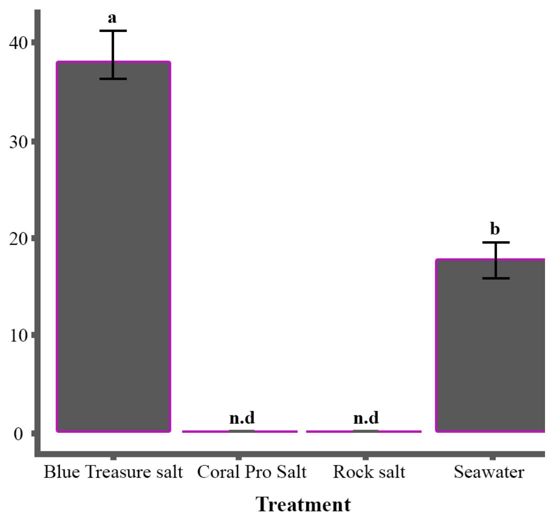

Figure 2. The statistical analysis of the carrageenan yield of *K. alvarezii* in different treatments (n.d. - no data, ANOVA at p<0.05, vertical bars with the same letter are not significantly different; the vertical bars and lines are the means and standard deviation from three thalli respectively).

The carrageenan yield obtained from the cultivated *K. alvarezii* under the Blue Treasure salt treatment meets the industrial requirements for carrageenan yield, which is approximately 38%. Although the carrageenan yield under seawater treatment showed a lower yield, it still falls within the accepted Philippine standard of 15-64%. It has been reported that the percentage carrageenan yield is influenced by various factors such as extraction methods, ecological factors such as light, temperature, and water content. These common influencing factors may affect the yield upon drying [22].

4. Conclusions

The present study showed that *K. alvarezii* cultivated using seawater and Blue Treasure salt yielded the highest percent growth rate with 4.58 ± 0.51 %day^{-1} and 3.94 ± 0.39 %day^{-1}, respectively. In addition, the carrageenan yield cultivated using Blue Treasure salt was significantly higher (37.75%) than seawater (18.06%) but both are within the standard. Thus, Blue Treasure salt can be used as an alternative medium for cultivating *K. alvarezii* if seawater is not available or limited for land-based cultivation.

The use of Blue Treasure salt instead of seawater for land-based cultivation of *K. alvarezii* seedlings is recommended. Artificial seawater can also be considered as it does not have any adverse effects on the growth rate or carrageenan yield. It is important to analyze and improve the components of artificial seawater to meet the demands of seaweed cultivation in tanks. Additionally, carrageenan extraction methods warrant further investigation to minimize extraction errors and identify the most suitable process to optimize carrageenan yield. The physicochemical parameters such as dissolved oxygen, pH, and others should be monitored regularly.

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