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Cultivation of Seaweed *Kappaphycus alvarezii* with Various Substrates Different on Laboratory Scale

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ABSTRACT

Kappaphycus alvarezii is an important red algae and can be used as one of the primary raw materials in fisheries. It is widely cultivated because of its relatively cheap production technology, and post-harvest handling is simple and easy. This algae has excellent economic value for carrageenan producers. Carrageenan is used as a food ingredient, cosmetics, and medicine. The success of cultivating *K. alvarezii* seaweed can be achieved if a suitable environment for its growth supports it. One of the environmental aspects that influence the growth of *K. alvarezii* seaweed is the bottom substrate of the waters. This research aims to analyze the best substrate for cultivating green *K.alvarezii* seaweed on a laboratory scale. This research used an experimental method with a Completely Randomized Design (CRD) consisting of 4 treatments and three repetitions, resulting in 12 experimental units. The treatments tested were different substrates: a coral substrate, a sand substrate, a volcanic rock substrate, and a coral sand substrate. The results of this study showed that the average survival rate in various substrate treatments ranged from 7.16% to 39%, final weight ranged from 1.43 g to 7.8 g, specific loss rate ranged from -3.157%/day to -5.124%/day, carrageenan yield ranged from 6.8% to 18.4%, and thallus tissue showed that all treatments still showed the presence of cortex and medullary tissue with varying shapes and structures.

Keywords: Carrageenan, *Kappaphycus alvarezii*, Seaweed, Substrate, Tissue.

1. INTRODUCTION

Kappaphycus alvarezii is a leading commodity in the Indonesian fisheries and marine sector because this alga produces carrageenan with high economic value. According to the Direktorat Jendral Perikanan Budidaya (2021), seaweed is a commodity that contributes significantly to the value of national fishery exports. Therefore, the Ministry of Maritime Affairs and Fisheries targets national seaweed production to reach 10.25 million tons in 2021 (Sarira and Pong-Masak, 2019). Nusa Tenggara Barat (NTB) is a region or center for cultivating *K.alvarezii* seaweed. People, especially in coastal areas, widely cultivate this type of seaweed because the production technology is accessible and production costs are relatively small. Besides being an industrial raw material, seaweed can be consumed directly as food (Arzani et al., 2020).

The success of *K.alvarezii* seaweed cultivation activities is largely determined by choosing the correct location. Environmental parameters that determine the appropriate location for cultivating *K. alvarezii* are physical environmental conditions, which include current speed, temperature, depth, brightness, substrate, and chemical environment, namely salinity, pH, CO_2, dissolved oxygen, nitrate, and phosphate, as well as biology which includes pests and diseases. Sujatmiko and Angkasa (2017) state that ecological conditions, including physical, chemical, biological, and environmental, mainly determine suitable land cultivation. Generally, people cultivate in waters with coral, sand, or rock substrates.

According to Erwansyah (2021), the growth of *K.alvarezii* seaweed is also influenced by the environmental conditions of the waters of the cultivation location. The cultivation location must have environmental conditions similar to its natural habitat. The seaweed *K.alvarezii* lives attached to aquatic substrates in the form of coral or rocks and likes continuous water movement. Several previous studies have been carried out on cultivating *K.alvarezii* seaweed on a laboratory scale. However, the resulting growth is not optimal, and there are obstacles to adapting to environmental conditions such as their natural habitat. Jailani et al. (2015) stated that cultivation techniques are among the most crucial things that cause the failure of seaweed production. Initial seed weight, daily handling, pest and disease prevention are aspects of seaweed cultivation that cultivation practitioners have not mastered optimally.

This research aims to analyze the best substrate for cultivating green *K.alvarezii* seaweed on a laboratory scale. The successful cultivation of *K.alvarezii* seaweed can be achieved by optimizing supporting factors. One of the supporting factors in seaweed cultivation is the substrate. Therefore, it is necessary to conduct research on cultivating green *K.alvarezii* seaweed with various substrates on a laboratory scale.

This study analyzes the best substrate for cultivating green *K.alvarezii* seaweed on a laboratory scale.

2. RESEARCH METHOD

Time and Place

This research was carried out from December 2022 to August 2023 at the Fish Production and Reproduction Laboratory, Aquaculture Study Program, Mataram University.

Method

This research used an experimental method with a Completely Randomized Design (CRD) consisting of 4 treatments and three repetitions, resulting in 12 experimental units. The treatments tested were:

P1: Coral substrate
P2: Coral sand substrate
P3: Volcanic rock substrate
P4: Sand substrate

Parameters

Survival Rate

The survival rate of seaweed was calculated using data at the beginning and end of the study. The survival rate of seaweed, according to Yustiani et al. in Yudiastuti et al. (2017), is calculated using the following formula:

$$SR = Wt / Wo \times 100\%$$

Information
SR : Survival Rate (%)
Wt : Final weight of seaweed (g)
Wo : Initial weight of seaweed (g)

Final Weight

The final weight of seaweed was measured at the end of the rearing period, namely on the 30th day.

Specific Growth Rate

The specific decline rate of seaweed can be calculated using the formula Muchlisin et al. (2017) as follows:

$$LPS = \frac{LnWt - LnWo}{Wo} \times 100\%$$

Information
LPS = specific decline rate (%/day)
Wo = Initial weight of seaweed (g)
Wt = Final weight of seaweed (g)
t = Maintenance Time (days)

Carrageenan

The *K.alvarezii* sample was drained from the container, washed thoroughly using water, and weighed on an analytical balance weighing 20 g. The samples were dried in the sun to reduce the water content for 24 hours. The dried samples were cut into small pieces to facilitate a blender's grinding process. Next, 75 ml of 96% alcohol is added and blended until smooth. Next, the extract is filtered using a filter cloth and dried. Calculation of the percentage of carrageenan uses the following formula Majid et al. (2018):

$$Kr = Wc / Wm \times 100\%$$

Information:
Kr : Carrageenan content
Wc : Weight of carrageenan (g)
Wm : Dry weight of seaweed (g)

Water Quality Measurement

The water quality observed is temperature, salinity, pH, DO, phosphate, and nitrate.

***K. alvarezii* seaweed thallus tissue**

Thallus tissue was observed by taking samples and making slices of the thallus from *K.alvarezii* seaweed as thin as possible. Then, place the thallus slices on a covered glass to observe under a microscope.

Data Analysis

Data on survival rate, final weight, specific growth rate, and carrageenan obtained were analyzed using Microsoft Excel and Analysis of variance (ANOVA) at a confidence level of 95% with the SPSS program to determine the effect of the treatment in the study. Significantly different results were further tested using the Duncan Test. Water quality data was analyzed descriptively.

3. RESULT AND DISCUSSION

Survival

The results of this study show that the average survival rate of *K.alvarezii* seaweed cultivated in the laboratory on different substrates ranges from 7.16% to 39% (Figure 1). The analysis of variance shows that cultivating K. alvarezii seaweed with various substrates has a significantly different effect ($p<0.05$) on the survival rate of *K.alvarezii* seaweed on a laboratory scale.

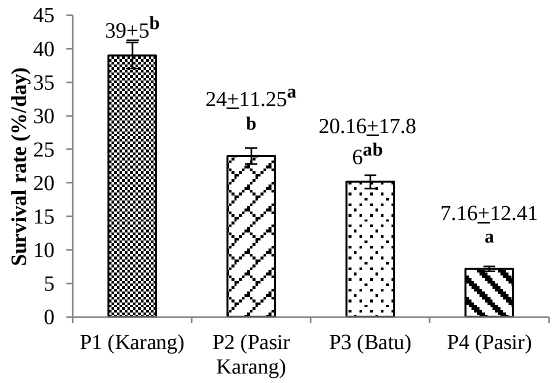


Figure 1. Survival rate of green *K. alvarezii*

Duncan's test results showed that the coral substrate treatment (P1) provided the highest level of survival, namely 39%, and was significantly different from the sand substrate treatment (P4) at 7.16% but not significantly different from the coral sand substrate treatment (P2) at 24% and volcanic rock substrate treatment (P3) was 20.16%.

Survival rate is the percentage of seaweed seeds that survive until the end of the cultivation period. The results of this study indicate that differences in the substrate provided can influence the survival rate of green *K.alvarezii* seaweed. Based on the research results, the highest average survival rate was obtained in the coral substrate treatment (P1). This happens because, in its natural habitat, *K.alvarezii* grows in waters that have coral reefs. This is in line with the statement by Khotijah et al. (2020) that *K.alvarezii* seaweed grows attached to coral substrates using a holdfast attachment. The ability to attach to coral substrates means that *K. alvarezii* can defend itself against the action of pounding waves. The coral substrate also provides additional nutrition to *K. alvarezii* seaweed to survive.

Absolute Weight

The results of this study show that the average final weight of *K.alvarezii* seaweed cultivated in the laboratory with different substrates ranges from 1.43% to 7.8% (Figure 2). The variance analysis results show that cultivating *K.alvarezii* seaweed with different substrates has significant effects ($p<0.05$) on the final weight of *K. alvarezii* seaweed on a laboratory scale.

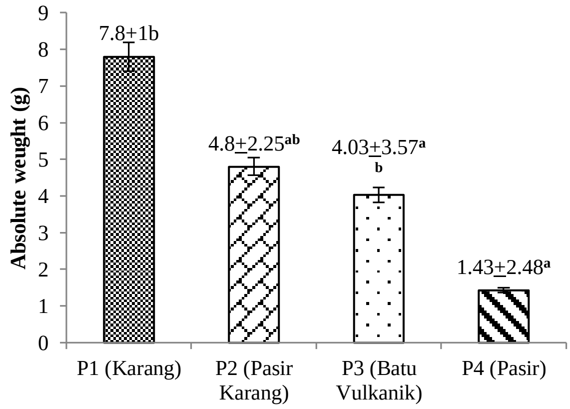


Figure 2. Absolute weight of green *K.alvarezii*

Duncan's test results showed that the coral substrate treatment (P1) gave the highest final weight, namely 7.8 g, and was significantly different from the sand substrate treatment (P4) at 1.43 g but was not significantly different from the coral sand substrate treatment (P2) at 4.8 g and the coral sand substrate treatment (P2), volcanic rock substrate (P3) of 4.03 g.

Based on the research results, the average final weight growth of *K. alvarezii* in each treatment showed differences, where the best results were found in the coral substrate treatment (P1) because *K. alvarezii's* natural habitat is that it lives on coral substrates. Destalino (2013) states that the main habitat of *K.alvarezii* is flat coral reef areas, requiring sunlight for photosynthesis. Therefore, this type generally grows well in areas permanently submerged in water and attached to essential substrates in the form of dead coral, live coral, and mollusk shells. Aini et al. in Irfan et al. (2022) that the nitrate and phosphate content in coral skeletons can directly support the growth of seaweed attached to the coral skeleton.

The lowest final weight growth results were found in the sand substrate treatment (P4).

The primary substrate is a factor that must be considered in seaweed cultivation. Rohman et al. (2018) stated that substrate is very important as a nutrient for seaweed. However, it is also a habitat for other animals and plants, which can affect seaweed plants due to competition in getting nutrients, sunlight, and living space.

Specific Rate

The results of this study show that the average specific decline rate for green *K.alvarezii* seaweed cultivated in the laboratory with different substrates ranges from -3.157%/day to -5.124%/day (Figure 3). The analysis of variance shows that cultivating *K.alvarezii* seaweed with various substrates has no significantly different effect (p>0.05) on the specific reduction rate of green *K.alvarezii* seaweed on a laboratory scale.

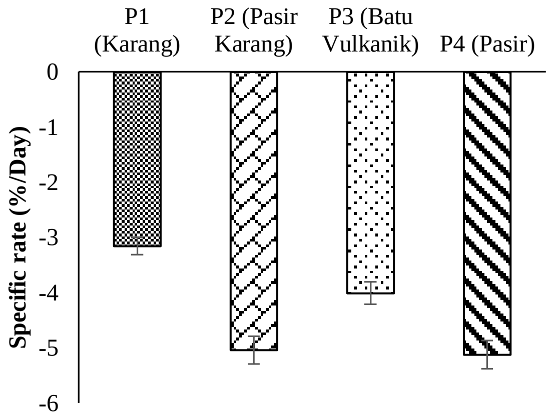


Figure 3. Specific rate of Green *K. alvarezii*

The research results show that green *K.alvar*ezii seaweed cultivated with different substrates on a laboratory scale experiences varying weight loss. Based on the research results, the highest specific reduction rate was found in the sand substrate treatment (P4). The weight loss of *K.alvarezii* occurred after entering the second week. This is because *K.alvarezii* cannot adapt well. After all, the adaptation process in *K.alvarezii* seaweed requires much energy to survive. This is in line with the statement by Gultom et al. (2019) that seaweed cannot adapt well, causing seaweed to experience stress due to changes in environmental conditions. This existence causes seaweed growth to become low and hampers its growth process.

Yusnaini et al. in Cokrowati et al. (2019) stated that seaweed undergoes an adaptation process and then experiences a rapid growth phase. Then, there is a decrease in cell growth ability, which causes the seaweed's growth ability to slow. The ability of seaweed to absorb nutrients also affects its growth, which results in bleaching of the *K.alvarezii* seaweed thallus so that the thallus cannot absorb nutrients from the substrate optimally.

Carrageenan

The results of this study show that the average specific growth rate of *K. alvarezii* seaweed cultivated in the laboratory on different substrates ranges from 6.8% to 18.4%.

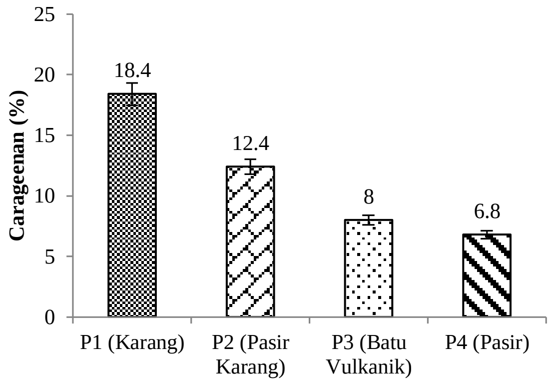


Figure 4. Carageenan of green *K.alvarezii*

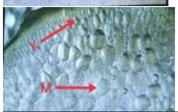
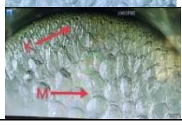
Carrageenan is a polysaccharide extracted from the seaweed *K. alvarezii*. Carrageenan is found in the cell walls of seaweed thallus. According to Samsuari (2006), carrageenan is found in the cell walls of seaweed or its intracellular matrix, and carrageenan is a significant component of the dry weight of seaweed compared to other components. *K. alvarezii* produces kappa carrageenan, which dissolves in hot water and forms cells in water.

Based on research results, the yield value of carrageenan produced by *K.alvarezii* seaweed with different treatments ranged from 6.8-18.4%. The carrageenan yield obtained in this study was relatively low. This is because the seaweed in all treatments cannot adapt to their respective environments, so the process of Nutrient absorption in the talus does not go well. This is in line with the statement by Pakniany et al. (2023) that obstruction of nutrient absorption is a factor that causes carrageenan production not to be optimal. It is known that seaweed needs nutrients for its growth, including carrageenan formation. The low yield of yeast yield is also influenced by the harvest age, which is 30 days. Asikin and Kusumaningrum (2019) stated that yeast's high and low yield values are influenced by the harvest age of seaweed, where the polysaccharide compounds contained in it are different at each harvest age. Apart from that, extraction time and temperature also play an important role in determining the yield value of a yeast product. Apart from that, where the sample is taken also influences the yield value. According to Kumayanjati and Dwimayasanti (2018), the environmental conditions where seaweed grows can determine the yield value of the carrageenan produced.

Thallus Tissue of *K. alvarezii* Seaweed

The results of observations of *K.alvarezii* cell structure at the beginning and end of the study are presented in Table 1.

Table 1. Thalus tissue of *K.alvarezii*

No.	Treatment	Cell Structure (days-30)	Information
1	First growth		K : Kortikal M : Medular
2	P1		K : Kortikal M : Medular
3	P2		K : Kortikal M : Medular
4	P3		K : Kortikal M : Medular
5.	P4		K : Kortikal M: Medular

Based on the results of visual observations of *K. alvarezii* seaweed thallus tissue slices, it can be seen that tissue slices from the initial *K. alvarezii* seaweed seedlings show that the cells in the cortex are elliptical, small in size, and appear dense. Meanwhile, in the medulla, the cells appear larger but not as dense as in the cortex. This part of the cortex is part of the newly formed young cells. This is in line with the statement of Darmawati (2012), who stated that, in general, it shows that the cortical cells (K) are smaller in size with an elongated shape with thick and dense cell walls in the surface layer of the thallus. These cortical cells decrease linearly and develop into medullary (M) cells that are larger and rounder but less dense than cortical cells.

After 30 days of maintenance with different substrate treatments, the results of observations of *K. alvarezii* seaweed tissue slices on coral substrate (P1), rock substrate (P3), and sand substrate (P4) showed that the cell shape was relatively the same as the tissue slices. In early seeds, the cell shape tends to be elliptical, dense, and small in the cortex, and towards the middle, namely in the medulla, the size becomes more significant. Achmad (2016) stated that in healthy *K.alvarezii* seaweed cells, the distance between the cells still appears tight.

A different thing can be seen in the treatment of the coral sand substrate (P2), where the structure of the cortex and medulla cells looks irregular, the distance between the cells is very loose, and the cells even appear to be starting to disappear. This is in line with the statement of Quer'e et al. (2015) that very severe *K. alvarezii* seaweed tissue is shown by deterioration and tissue death, characterized by epithelial cells starting to disappear or not appearing solid. This is thought to be influenced by the substrate used in treatment (P2) in the form of a combination of sand and coral substrates, so the *K.alvarezii* seaweed is challenging to adapt to in the process of attachment to the substrate. This is in line with the statement by Hayashi et al. (2007) that different environmental conditions of seaweed greatly determine the speed of seaweed in meeting nutrient needs for thallus growth.

Thallus growth is an increase in cell size or a change in the state of several cells to form organs with different structures and functions.

Water Quality

The results of water quality measurements during the research were still considered optimal for cultivating green *K.alvarezii* seaweed. The results of water quality measurements during the research are presented in Table 2.

Table 2. Water quality

Parameter	P1	P2	P3	P4	Feasibility
Temperature (°C)	28.1-30	28.1-29.2	28.1-29.2	28.1-29.4	26-30 (Khotijah et al., 2020)
Salinity (ppt)	28-30	28-29	28-29	28-29	28-35 (Atmanisa et al., 2020)
pH	7.43-7.50	7.43-7.50	7.35-7.42	72-7.26	6.0-9.0 (Risnawati et al., 2018)
DO (mg/L)	5.1-6.8	4.3-4.3	4.3-4.4	4.3-4.4	4.5-9.8 (Risnawati et al., 2018)
Light intensity (lux)	635	625	625	625	500-1000 (Sitorus et al., 2020)
Phosphate (mg/L)	0.01-<0.02	0.01-<0.02	0.01-<0.02	0.01-<0.02	0.2-1.04 (Anggadiredja et al., 2008)
Nitrate (mg/L)	3.3-3.5	3.4-3.5	3.2-3.4	3.4-3.5	0,9-3.50 (Asni, 2015)

Water quality parameters are one of the important factors in cultivating *K. alvarezii* seaweed. According to Basir et al. (2017), measuring water quality is important for the sustainability of cultivation. The results of temperature measurements during the research ranged between 28.1-30^{0}C in all treatments. This shows that the cultivation media still has a good range of temperature values for the growth of *K. alvarezii*. According to Atmanisa et al. (2020), seaweed can grow well in the temperature range of 26–30^{0}C. According to Syahrir (2020), high-temperature increases can cause seaweed thallus to become pale, yellowish, unhealthy, wither, and susceptible to disease. Temperature has a direct influence on the life of seaweed, especially in the process of photosynthesis. A very high level of fluctuation will stress the seaweed, thus affecting its growth rate.

The results of salinity measurements during the research ranged from 28-30 ppt. The salinity in all treatments in this study was still optimal for the growth of *K.alvarezii* seaweed. According to Atmanisa et al. (2020), the appropriate salt content for *K.alvarezii* seaweed is 28–35 ppt.

The degree of acidity in seaweed cultivation research media ranges from 7.12-7.50. It can be said that the conditions with this pH value are optimal for the feasibility of seaweed cultivation. According to Nur et al. (2016), a pH range of less than 6,5 will suppress the growth rate, and even the acidity level can be deadly, and there will be no reproduction rate. pH value of 6.5 – 9 is the optimal range in water. Risnawati et al. (2018) stated that the optimal acidity value for seaweed growth ranges from 6.0 to 9.0. Very acidic or alkaline water will endanger the life of organisms because it will cause metabolic and respiration disorders.

The results of DO measurements during the study ranged from 4.3-6.8 mg/L. This value indicates optimum conditions to support the growth of *K. alvarezii*. This aligns with Risnawati et al. (2018) statement that the dissolved oxygen (DO) value that meets the requirements for the life and growth of *K.alvarezii* is 4.5 – 9.8 mg/L.

The results of light intensity measurements in the research obtained a value of 625 lux. Light intensity affects the photosynthesis process because it influences the growth process of the seaweed *K. alvarezii*. According to Sitorus et al. (2020), the light intensity value that supports seaweed growth ranges from 500 to 1000 lux.

Nitrate is an essential nutrient for the seaweed growth process. Nitrate levels obtained during the study ranged from 3.2-3.5 mg/L. Risnawati et al. (2018) state that nitrate levels above 0.2 mg/L cause eutrophication (enrichment) and stimulate the growth of algae and aquatic plants. According to Asni (2015), good algae growth requires a nitrate range of 0.9-3.50 mg/L, further stated by Atmanisa et al. (2020) that the nitrate requirements of each algae vary greatly. Suppose the nitrate level is below 0.1 mg/L or above 45 mg/L. In that case, nitrate is a limiting factor, meaning that at this level, nitrate is toxic and can cause eutrophication, stimulating rapid phytoplankton growth. Pramesti (2013) states that nitrate plays a role as a building block or primary ingredient for protein and the formation of chlorophyll. Plants that experience a lack of nitrates experience a malfunction in the photosynthesis process in their bodies, which will affect their growth process.

The research results show that the phosphate content ranges between <0.01-<0.02 mg/L. According to Anggadiredja et al. (2008), the phosphate content suitable for seaweed cultivation is around 0.02-1.04 mg/L. Seaweed needs phosphate for its growth process, and it is an essential nutrient in growth because it is a nutrient for seaweed. The phosphate content affects the fertility level of the waters. The phosphate absorbed by seaweed is generally in the form of orthophosphate.

4. CONCLUSION

The use of coral substrate, stone substrate, and a combination of sand and coral substrate in cultivating green *K.alvarezii* seaweed on a laboratory scale provides the same ability to maintain survival, final weight, and specific reduction rate but cannot provide good carrageenan value. The structure of the cortex and medulla cells in the coral substrate, volcanic rock substrate, and sand substrate still showed good condition.

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