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The Effect of Different Substrates on Laboratory Scale Cultivation of *Kappaphycus alvarezii*

Pengaruh Perbedaan Substrat pada Budidaya *Kappaphycus alvarezii* Skala Laboratorium

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ABSTRACT

Seaweed is a leading commodity in the Indonesian fisheries sector. Indonesia is the second largest seaweed-producing country in the world after China. *K.alvarezii* is a type of seaweed that produces kappa carrageenan which can be used as a raw material in industry, making *K.alvarezii* seaweed very popular for cultivation. Cultivation of *K.alvarezii* seaweed has developed widely in Indonesian waters. The success of cultivating *K.alvarezii* seaweed can be achieved if it is supported by a suitable environment for its growth. One of the environmental aspects that influences the growth of *K.alvarezii* seaweed is the bottom substrate of the waters. This research aims to analyze the best substrate for the survival rate of brown *K.alvarezii* seaweed cultivated on a laboratory scale. This research used an experimental method using a Completely Randomized Design (CRD) consisting of 4 treatments and each treatment was carried out 3 times to obtain 12 experimental units. The treatments tested were different substrates consisting of sand, coral, volcanic rock, and coral sand. The results of the research show that different types of substrate have a real influence on the survival rate of brown *K.alvarezii* seaweed cultivated on a laboratory scale. Coral substrate gave the best results with a survival rate of 100%, final weight of 20 g, and carrageenan yield value of 26%.

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

ABSTRAK

Rumput laut merupakan komoditas unggulan sektor perikanan Indonesia. Indonesia merupakan negara penghasil rumput laut terbesar kedua di dunia setelah Tiongkok. *Kappaphyces alvarezii* merupakan salah satu jenis rumput laut penghasil kappa karagenan yang dapat digunakan sebagai bahan baku industri, sehingga rumput laut *K.alvarezii* sangat populer untuk dibudidayakan. Budidaya rumput laut *K.alvarezii* telah berkembang luas di perairan Indonesia. Keberhasilan budidaya rumput laut *K.alvarezii* dapat tercapai jika didukung oleh lingkungan yang sesuai untuk pertumbuhannya. Salah satu aspek lingkungan yang mempengaruhi pertumbuhan rumput laut *K. alvarezii* adalah substrat dasar perairan. Penelitian ini bertujuan untuk menganalisis substrat terbaik bagi tingkat kelangsungan hidup rumput laut coklat *K.alvarezii* yang dibudidayakan dalam skala laboratorium. Penelitian ini menggunakan metode eksperimen dengan menggunakan Rancangan Acak Lengkap (RAL) yang terdiri dari 4 perlakuan dan masing-masing perlakuan dilakukan sebanyak 3 kali sehingga diperoleh 12 satuan percobaan. Perlakuan yang diuji adalah substrat berbeda yang terdiri dari pasir, karang, batuan vulkanik, dan pasir karang. Hasil penelitian menunjukkan bahwa jenis substrat yang berbeda memberikan pengaruh nyata terhadap tingkat kelangsungan hidup rumput laut coklat *K.alvarezii* yang dibudidayakan dalam skala laboratorium. Substrat karang memberikan hasil terbaik dengan tingkat kelangsungan hidup 100%, berat akhir 20 g, dan nilai rendemen karagenan 26%.

Kata Kunci: Rumput Laut, *Kappaphycus alvarezii*, Substrat, Karagenan, Jaringan Talus

INTRODUCTION

Seaweed is a leading commodity in the Indonesian fisheries sector. FAO (2022) that Indonesia is the second largest seaweed-producing country in the world after China. Indonesia exported seaweed to reach 231,829.70 tonnes with a value of USD 397.16 million in 2022. This amount increased 12.44% compared to the previous year of 206,185.10 tonnes with a value of USD 222.61 million (BPS, 2022). Seaweed consists of various types; one type of seaweed that is quite popular and has high economic value is *Kappaphycus alvarezii.*

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K. alvarezii is a type of seaweed that produces kappa carrageenan which can be used as a raw material in industry (Indriyani et al., 2019). Carrageenan is used by the food industry as a thickener, stabilizer, and gel-former and is also used by non-food industries such as cosmetics, paint, and textiles and by the pharmaceutical industry (Arzani et al., 2020). *K.alvarezii* has relatively high levels of carrageenan, namely 62-68% of its dry weight (Wibowo et al., 2020). Apart from that, *K.alvarezii* also has several advantages, namely cultivation technology that is easy to carry out, relatively small capital expenditure, and short harvest time, making the *K.alvarezii* seaweed species very popular for cultivation.

Cultivation of *K. alvarezii* seaweed has developed widely in Indonesian waters. However, successful cultivation of *K. alvarezii* can be achieved if it is supported by a suitable environment for its growth. One environmental aspect that is important to pay attention to is the type of bottomwater substrate. Seaweed is a marine plant that is classified as a benthic macroalgae which mostly lives attached to the bottom of the waters. According to Ardiyanto et al. (2020), the basic substrate as a place for seaweed to live is divided into two types, namely soft substrate which includes mud, sand, or sand mixed with mud, and hard substrate which includes dead coral, live coral and rocks. Various types of substrates have different characteristics which of course influence the growth of *K.alvarezii* seaweed. Irfan et al. (2021) that the use of coral substrates gets the best results in cultivating seaweed *Gelidium* sp. laboratory scale, then in research by Genara et al. (2022) that the use of sand as a substrate obtained the best results on a laboratory scale cultivation of *Caulerpa racemosa*. Therefore, this research aims to analyze the best substrate for the survival rate of brown *K. alvarezii* seaweed cultivated on a laboratory scale.

MATERIALS AND METHOD

Time and place

This research was carried out for 30 days starting from July 7 to August 7, 2023, at the Production and Reproduction Laboratory of the Aquaculture Study Program, Faculty of Agriculture, University of Mataram.

Method

This research used an experimental method using a completely randomized design (CRD) consisting of 4 treatments: 1) *K. alvarezii* seaweed cultivated on a sand substrate, 2) *K. alvarezii* seaweed cultivated on a coral substrate, 3) *K. alvarezii* is cultivated on a volcanic rock substrate, 4) *K. alvarezii* seaweed is cultivated on a coral sand substrate

Cultivation preparation

The cultivation container is a transparent plastic jar with a capacity of 5 L, 12 of them, washed using water. White LED lights are attached to the shelf where the jars are placed to get the specified amount of light intensity, and then each jar is given aeration.

Substrate preparation

The substrate used in the research consisted of 4 types, namely white sand substrate, dead coral substrate, river volcanic rock substrate, and dead coral sand substrate. Before use, the substrate is first cleaned and dried in the sun for one day. Next, the substrate was weighed for each treatment according to the determined amount. After the substrate is put into the container, 4 L of seawater is added.

Seed preparation

The brown *K.alvarezii* seeds used were tissue culture seeds obtained from seaweed farmers in Gerupuk Village, Central Lombok. The seeds are first acclimatized for 3 days, and then the seeds are selected to take young talus and healthy talus conditions. Seedlings were weighed with an initial weight of 20 g for each treatment.

Planting

Planting seeds on coral and volcanic rock substrates, the seeds are planted by tying them using neat rope, the largest talus is tied to one of the corals or rocks so that it does not come off easily and is attached well. Meanwhile, planting seeds in sand and coral sand substrates are planted by immersing the tip of the talus in the sand.

Water quality maintenance and monitoring

Maintenance of *K.alvarezii* seaweed is carried out for 30 days, cleaning the talus area and observing the condition of the talus. Every day, water is added to ensure that the water volume does not decrease due to evaporation. Siphoning is also done if there is a lot of dirt in the water. Replacement of 50% of the total water is carried out if the water condition is cloudy. Water quality measurements are carried out twice during the maintenance period. The first measurement is carried out after planting the seeds, then the second measurement is carried out before the seaweed is harvested.

Harvesting

Harvesting of *K.alvarezii* is carried out on the 30th day of cultivation by taking and draining the seaweed from a jar, then the harvested seaweed is weighed.

Carrageenan analysis

Seaweed samples from each treatment were dried for 2-3 days, then after the seaweed was dry, the carrageenan analysis process was carried out. Carrageenan yield calculated by this formula (Ainsworth and Blanshard, 1980; Majid et al., 2018) :

$$\text{Carrageenan yield} = \frac{\text{Dry weight of carageenan}}{\text{Dry weight of } K.\ alvarezii} \times 100\%$$

Thallus tissue observation

Thallus tissue observations were carried out twice, at the beginning of maintenance and the end of maintenance (harvest). The *K.alvarezii* thallus was sliced thinly using a razor blade and placed in a glass slide. The slices were observed using a microscope and photographed

Survival rate

The survival rate of seaweed was calculated using the initial data and final data of the study. Seaweed survival according to Yustiani et al. (2013); Yudiastuti et al. (2017) is calculated using the following formula:

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

Information:

SR : Survival Rate (%)

Wo : Weight-0 (g)

Wt : Weight-t (g), t=30 days

Data analysis

The data obtained was tested using Analysis of variance (ANOVA) at a confidence level of 95% and a significant value of 5% using the SPSS program to determine the effect of the treatment on the research. Meanwhile, for the analysis of carrageenan, slices of talus tissue and water quality were analyzed descriptively.

RESULT AND DISCUSSION

Survival rate

The results of this study showed that the final weight of *K. alvarezii* seaweed cultivated for 30 days with different substrates on a laboratory scale ranged from 22.6% to 100%. The highest survival rate was found in the coral substrate treatment (P2), namely, 100%, and the lowest rate was in the rock substrate treatment (P3), 22.6%. The results of Anova statistical analysis showed that the different substrates in laboratory-scale *K.alvarezii* seaweed cultivation had significant differences ($p>0.05$) in survival rates.

The factor that influences the survival rate of seaweed is the adaptability of the seaweed. The occurrence of bleaching on the thallus indicates that the seaweed has not been able to adapt to the new environmental conditions. The cells at the tip of the thallus are still small and are susceptible to stress and bleaching, causing disruption in nutrient absorption and a decrease in growth in the *K. alvarezii* seaweed. This is in line with the statement (Pande et al., 2021) stating that the adaptation process in the talus causes the talus to not be able to adapt well to new water conditions, changing color to pale white, brittle and even breaking at the tip of the talus which causes thallus weight is reduced. This is also supported by the statement of Aquilino et al. (2009); Togatorop et al. (2017) stated that the adaptation process of seaweed inhibits its growth rate because some of the energy is used to stay alive due to a reduction in energy output and an increase in energy output so that the growth rate decreases. Nishara et al. (2013) *in* Togatorop et al. (2017) also stated that the reduction in energy in seaweed occurs due to changes in physiological habits including photosynthesis, respiration, and metabolic processes. Survival rate indicates the physiological ability to adapt to the environment.

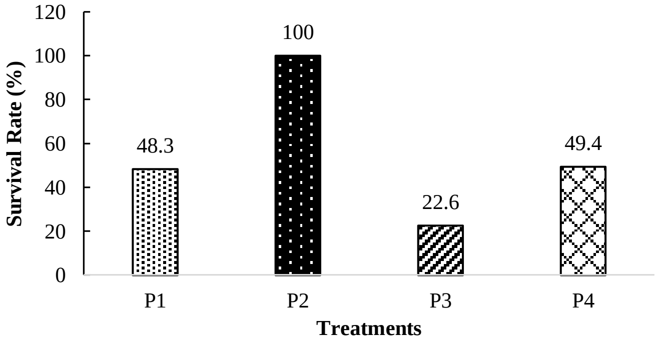


Figure 1. Survival rate of *K. alvarezii*

Final Weight

The final weight of *K.alvarezii* seaweed cultivated for 30 days with different substrates on a laboratory scale ranged from 4.52 g to 20 g. The highest final weight was in the coral substrate treatment (P2), namely 20 g, and the lowest final weight was in the rock substrate treatment (P3), 4.52 g. The results of Anova statistical analysis showed that the substrate differences in laboratory scale *K.alvarezii* seaweed cultivation were significantly different ($p > 0.05$) from the final weight.

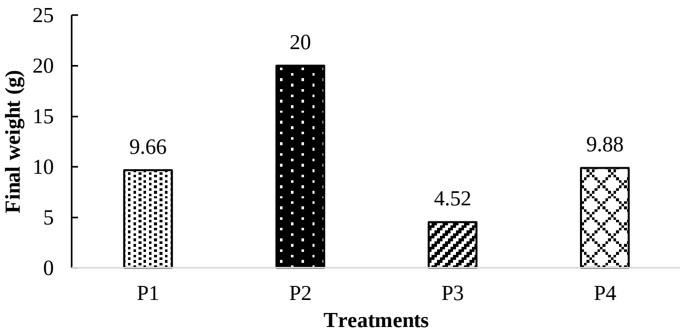


Figure 2. Final weight of *K. alvarezii*

The use of coral substrate provides the highest final weight value because, in its natural habitat, *K.alvarezii* grows in waters containing coral reefs. This is in line with Erwansyah (2021) which states that *K.alvarezii* has a habitat in coastal waters that have coral reefs. Basuki (2008); Irfan et al. (2021) also stated that seaweed is often found in seawaters with coral substrates because the coral substrate is stable so that the seaweed can stick firmly to the substrate so that it is not easily blown away during big waves. Coral substrate can also provide additional nutrients that can influence the growth of seaweed. This is in line with the statement of Aini et al. (2013); Irfan et al. (2021) that the nitrate and phosphate content in coral skeletons can directly influence the growth of seaweed attached to the coral skeleton. Qin, (2018) stated that seaweed can live on various aquatic substrates such as coral, coral rubble, and coral sand, but better nutrient availability can stimulate increased seaweed growth.

Carrageenan yield

Based on the results of the carrageenan analysis, the yield of carrageenan from each treatment was obtained, where the highest value was found in the coral substrate treatment (P2), namely 26%, and the lowest value was found in the rock substrate treatment (P2) 13%.

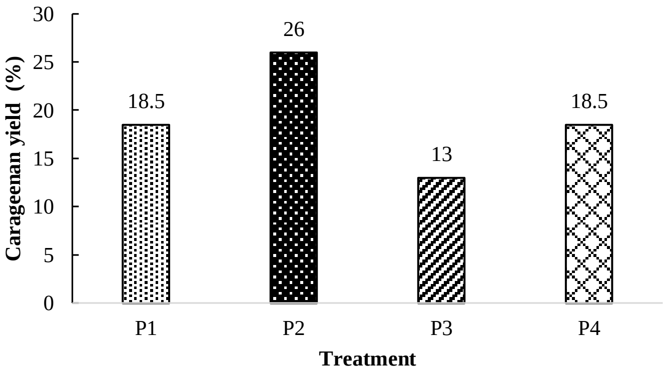
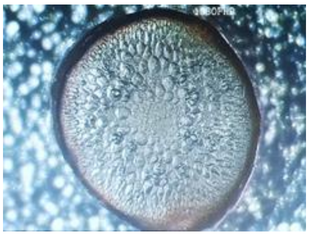


Figure 3. Final weight of *K. alvarezii*

Tissue of thallus

Based on the results of visual observations of *K.alvarezii* seaweed thallus tissue slices using a microscope, the initial tissue slices of *K.alvare*zii seaweed show that the cells on the outside close to the skin tissue are elliptical in shape, small in size, and look dense.

Table 1. Tissue of *K. alvarezii*

Treatments / Day-	Figure	Description
Control / day-0		The shape of the cells tends to be oval, the size of the cells near the skin tissue is smaller, and the distance between the cells appears tight, whereas as you move towards the center of the thallus the cell size appears larger.
P1 / day-30		The shape of the cells tends to be round, and the size of the cells appears larger, but the cells on the outside close to the skin tissue are not as dense as at the beginning.
P2 / day-30		The cell shape is relatively the same as the initial tissue slice, the cells on the outside close to the skin tissue are oval in shape, small in size, and look dense and as they move towards the center the size becomes larger.
P3 / day-30		The cell shape is rounder, and the cell size appears larger but the cells appear to have a lot of space between cells.
P4 / day-30		The cell shape tends to be round, and the cell size appears larger, but the cells on the outside close to the skin tissue are not as dense as at the beginning.

Meanwhile, in the middle of the thallus, the cells appear larger but not as dense as the cells near the skin tissue cells. These small cells are young, newly formed cells. This is in line with Darmawati's (2012) statement *in* Putri et al. (2021) which states that the histology of healthy *K. alvarezii* seaweed tissue has small, oval-shaped cells located on the surface of the thallus (cortical), and will get bigger in the middle part of the talus (medullary). This is also supported by the statement by Maulani et al. (2018) that the tissue of the brown *K.alvarezii* seaweed variety with healthy cell shapes shows that the cell walls are tight and not loose.

Observations of thallus tissue slices were carried out again after rearing with different substrates for 30 days. It was found that the results of observations of seaweed tissue slices maintained on the coral substrate (P2) showed cell shapes that were relatively the same as the initial tissue slices, the cell shapes tended to be round, oval, and dense. Where as you move towards the middle the size becomes larger. Furthermore, the results of observations on the sand substrate (P1) and coral sand substrate (P4) showed that the cell shape was round and oval, and the cell size tended to be larger but not as dense as P2 (coral substrate). Meanwhile, the results of observations on the volcanic rock substrate (P3) showed that the cell shape was more rounded, the cell size was larger, but there was a lot of space between the cells. This is in line with the statement of Hayashi et al. (2007); Darmawati (2012) stated that different environmental conditions in seaweed greatly determine the growth rate of the thallus which influences the increase in cell size or changes in the state of several cells to form organs that have different structures and functions.

Water quality

Water quality is important in the survival of *K. alvarezii*. The parameters measured are pH temperature, dissolved oxygen, nitrate, and phosphate salinity.

Table 2. Result of water quality measurement

Parameters	Value	Ideal	References
Temperature (^oC)	28,5 – 28,9	27 - 30	(Khotijah et al., 2020)
pH	7,47 – 7,76	6,8 – 9,6	(Hardan et al., 2020)
Dissolved oxigen (mg/L)	4,2 – 4,8	≥4	(Atmanisa et al., 2020)
Salinity (ppt)	30	28 - 34	(Atmanisa et al., 2020)
Nitrate (mg/L)	< 10	0,9 – 3,5	(Yulius, 2019)
Phosphate (mg/L)	< 1	0,5 - 1	(Astriana et al., 2019)

The results of temperature measurements in the media during the research were 28.5-28.9 C. The optimal temperature for the growth of *K.alvarezii* is around 27-30 C (Khotijah et al., 2020). Julius (2019); Lutfiati et al., (2022) stated that a temperature of 25–31^oC is a suitable temperature range for *K.alvarezii* to grow. The temperature obtained in this study is considered suitable for seaweed cultivation. Temperature greatly influences the ability of seaweed to carry out photosynthesis and indirectly influences the solubility of oxygen. According to Pauwah et al. (2020), temperature plays an important role in the growth of *K.alvarezii* because it is related to the processes of photosynthesis, metabolism, and respiration of seaweed, the metabolic rate increases due to increasing water temperature. Water temperatures that are too high will cause the seaweed talus to become pale and unhealthy, while low temperatures will cause seaweed growth to slow.

The results of pH measurements in the media ranged from 7.47–7.76. According to Hardan et al. (2020) who stated that the optimal pH value for seaweed cultivation ranges from 6.8–9.6. Atmanisa et al. (2020) stated that in seaweed cultivation activities, the pH that is good for growth is in the range of 7 – 9. So the pH value obtained in this study is considered good for the growth of *K.alvarezii*. Lutfiati *et al.* (2022) that pH affects the growth and biological activity of seaweed. Metabolic and respiration processes will be disrupted when the pH levels in the water change, which conditions endanger the survival of seaweed. Changes in pH can cause seaweed to experience disruption in the cell walls in transferring substances needed by cells from the external environment. This will stress the seaweed and cause bleaching of the talus (Loban and Harison, 2004; Cahyani et al., 2021).

Dissolved Oxygen during the research ranged between 4.2-4.8 mg/L. Atmanisa et al. (2020) that the dissolved oxygen needed for cultivating *K.alvarezii* to grow is 2-4 mg/L, but seaweed will grow well in a dissolved oxygen range of more than 4 mg/L. Seaweed uses dissolved oxygen for respiration. In the respiration process, cells need oxygen for glucose to become the energy needed by seaweed for growth (Tarmizi and Diniarti, 2022)

The results of salinity measurements in the *K. alvarezii* cultivation media were 30 ppt, which is a good value to support the growth of *K.alvarezii*. Atmanisa et al. (2020) that salinity between 28 – 34 ppt is considered good for supporting *K.alvarezii* cultivation activities, while the optimal salinity value in seaweed cultivation activities is 33 ppt. According to Yuliyana (2018), salinity plays a major role in thallus growth, color, and morphogenic development because it is directly related to osmoregulation that occurs in cells. Arisandi et al. (2011) stated that salinity is a chemical factor that influences the physical properties of water. An increase in salinity causes a difference in the osmotic pressure of seaweed and the osmotic pressure in the water, thus inhibiting seaweed from absorbing water and elements that take place in the osmosis process.

The nitrate measurement results obtained a value of <10 mg/L. According to Yulius et al. (2019), the sufficient nitrate content for *K.alvarezii* is 0.9 – 3.5 mg/L. It can be said that the nitrate levels obtained during the research were optimal for seaweed growth. Seaweed requires sufficient nitrate levels to form organic compounds. Lutfiati et al. (2022) that the nitrate element in waters is needed by seaweed to support seaweed growth such as the production, and formation of carbon hydrates, proteins, and fats and the production of food reserves in the form of organic compounds.

The results of phosphate measurements in the *K. alvarezii* rearing media were found to be less than 1 mg/l. This phosphate condition can still be tolerated by *K. alvarezii*. According to Astriana et al. (2019), the safe value for the phosphate concentration needed for seaweed growth is 0.5–1 mg/L. It can be said that the phosphate levels obtained during the research were optimal for seaweed growth. Phosphate in waters is needed as a nutrient (macronutrient) which is needed in sufficient quantities for growth, especially in the process of producing energy for cells. Phosphate has a role in the formation of meristem tissue, stimulates cell division, repairs damaged tissue, and is a constituent of protoplasm (Zainuddin, 2023).

CONCLUSION

Based on the research results, it can be concluded that coral substrates provide the best results for the survival rate of the *Kappaphycus alvarezii* brown morphotype.

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