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 main raw materials in fisheries, it is widely cultivated because its production technology is relatively cheap, and post-harvest handling is relatively simple and easy. This algae has great economic value for carrageenan producers. Carrageenan has uses as a food ingredient, cosmetics and medicine. 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. The aim of this research is 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 3 repetitions, resulting in a total of 12 experimental units. The treatments tested were different substrates consisting of a coral substrate, sand substrate, volcanic rock substrate and 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: Tissue, *K. alvarezii*, carrageenan, seaweed, substrate.

1. INTRODUCTION

K. alvarezii is a leading commodity in the Indonesian fisheries and marine sector, because this type of algae produces carrageenan which has high economic value. According to the Directorate General of Aquaculture (2021), seaweed is a commodity that has a large contribution 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). West Nusa Tenggara (NTB) is one of the regions that is a place or center for the cultivation of *K. alvarezii* seaweed. This type of seaweed is widely cultivated by people, especially in coastal areas because the production technology is easy and production costs are relatively small.' Apart from being an industrial raw material, seaweed can also be food that is consumed directly (Falih, 2021).

The success of *K. alvarezii* seaweed cultivation activities is largely determined by choosing the right 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, CO2, dissolved oxygen, nitrate and phosphate, as well as biology which includes pests and diseases. Sujatmiko (2017), states that suitable cultivation land is mainly determined by ecological conditions which include physical, chemical and biological environmental conditions. 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 on the cultivation of *K. alvarezii* seaweed on a laboratory scale have been carried out, however The resulting growth is not optimal and there are obstacles to adapting to environmental conditions such as their natural habitat. Jailani (2015) stated that the problem of cultivation techniques is one of the most crucial things that causes the failure of seaweed production. Initial seed weight, daily handling, pest and disease prevention are aspects of seaweed cultivation that have not been mastered optimally by cultivation practitioners.

Successful cultivation of *K. alvarezii* seaweed can be achieved by optimizing supporting factors in seaweed cultivation. One of the supporting factors in seaweed cultivation is the substrate. Therefore, it is necessary to carry out research related to the cultivation of green *K. alvarezii* seaweed with various different substrates on a laboratory scale.

MATERIALS AND METHODS

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.

Tools and materials

The tools used in this research were aeration, stationery, blender, Dissolved Oxygen meter, camera, lux meter, microscope, pH meter, refractometer, phosphate test kit, nitrate test kit, scales, and 5 liters jars. The materials used are seawater, volcanic rock, coral, sand, green *K. alvarezii* seaweed.

Research methods

This research used an experimental method with a Completely Randomized Design (CRD) consisting of 4 treatments and 3 repetitions, resulting in a total of 12 experimental units. The treatments tested were:

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

Research Parameters

The parameters analyzed in this study were survival rate, final weight, carrageenan analysis and *K. alvarezii* cell structure.

1. 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.,* (2013) in Yudiastuti *et al.,* (2017) is calculated using the following formula:

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

Information
SR. : Survival Rate / Survival (%)
$W\ t$: Final weight of seaweed (g)
$W\ o$: Initial weight of seaweed (g)

2. Final Weight

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

3. Specific Growth Rate

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

LPS= $\underline{(\text{ Ln Wt-Ln Wt })}$ x 100%

t

Information

LPS = specific decline rate (%/day)

$W\ o$ = Initial weight of seaweed (g)

$W\ t$ = Final weight of seaweed (g)

t = Maintenance Time (days)

4. Carrageenan

The *K. alvarezii* sample was drained from the container and washed thoroughly using water then weighed on an analytical balance with a weight of 20 g. The samples were dried by drying them in the sun to reduce the water content for 24 hours. The dried samples were cut into small pieces to facilitate the grinding process using a blender. Next, 75 ml of 96% alcohol is added and then blended until smooth. Next, the extract is filtered using a filter cloth and carried out drying. 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)

5. Water Quality Measurement

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

6. *K. alvarezii* seaweed thallus tissue

Observation of thallus tissue was carried out by taking samples and making as thin slices of the thallus from *K. alvarezii* seaweed as possible. Then place the thallus slices on a cover 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.

RESULTS AND DISCUSSION

Survival

The results of this study show that the average survival rate of *K. alvarezii* seaweed cultivated in the laboratory on various different substrates ranges from 7,16% to 39% (Figure 1). The results of analysis of variance show that cultivating *K. alvarezii* seaweed with various different substrates has a significantly different effect ($p<0.05$) on the survival rate of *K. alvarezii* seaweed on a laboratory scale.

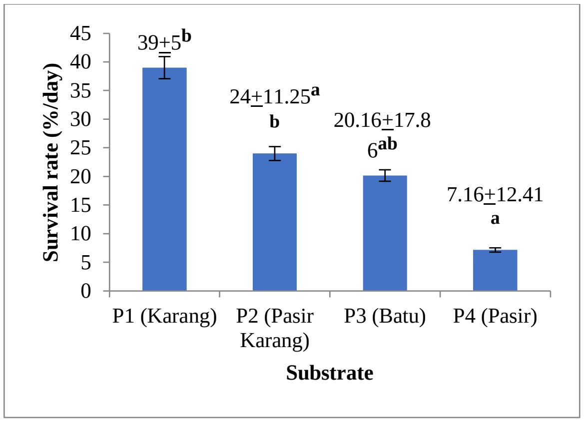


Figure 1. Survival rate of green *K. alvarezii*

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 (2020), that *K. alvarezii* seaweed grows attached to coral substrates using an attachment called a holdfast. The ability to attach to coral substrates means that *K. alvarezii* is able to defend itself against the action of pounding waves. Coral substrate also provides additional nutrition to *K. alvarezii* seaweed so that it can survive.

Absolute Weight

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

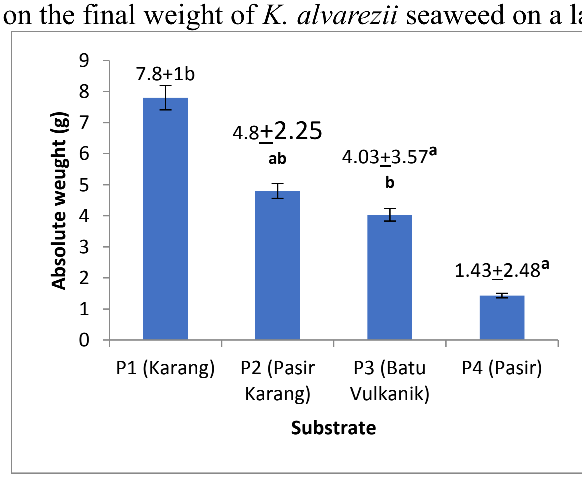


Figure 2. Absolute weight of green *K. alvarezii*

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 basically *K. alvarezii* natural habitat is that it lives on coral substrates. . This is in accordance with the statement by Destalino (2013), that the main habitat of *K. alvarezii* is that it lives in coral reef flat areas and requires sunlight for photosynthesis. Therefore, this type generally grows well in areas that are always submerged in water and attached to basic substrates in the form of dead coral, live coral and mollusc shells. Aini *et al.,* (2013) in Irfan *et al.,* (2021), 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 basic substrate is a factor that needs to be considered in seaweed cultivation. (Rohman *et al.*, 2018) stated that substrate is very important as a nutrient for seaweed, but besides that 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 various different substrates ranges from -3.157%/day to -5.124%/day (Figure 4.1.3). The results of analysis of variance show that cultivating *K. alvarezii* seaweed with various different substrates has no significantly different effect ($p>0.05$) on the specific reduction rate of green *K. alvarezii* seaweed on a laboratory scale.

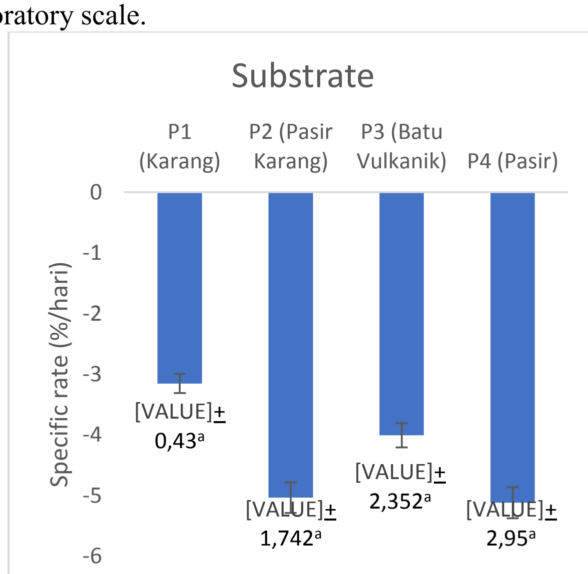


Figure 3. Specific rate of Green *K. alvarezii.*

Based on the results of research that has been carried out, it shows 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, because the adaptation process in *K. alvarezii* seaweed requires a lot of energy to survive. This is in line with the statement by Gultom et al., (2019) that seaweed that cannot adapt well causes seaweed to experience stress due to changes in environmental conditions. This existence causes growth seaweed becomes low and its growth process is hampered. Yusnaini *et al.,* (2000) in Cokrowati *et al.,* (2019) stated that seaweed that has undergone an adaptation process then experiences a rapid growth phase and then there is a decrease in cell growth ability which causes the seaweed's growth ability to become 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 various different substrates ranges from 6,8% to 18,4%.

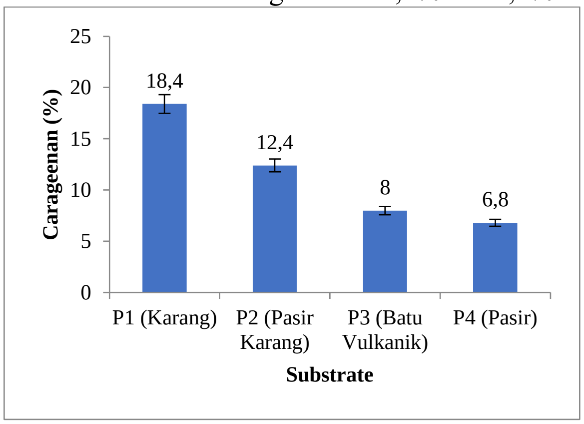


Figure 4. Carageenan of green *K. alvarezii*

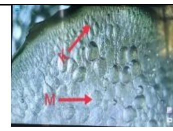
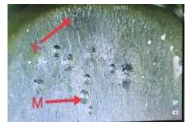
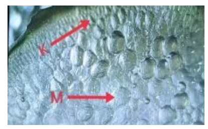
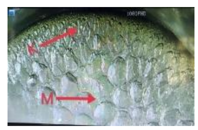
Carrageenan is a polysaccharide extracted from the seaweed *K. alvarezii*. Carrageenan is found in the cell walls of seaweed thallus. According to Samsuari (2006), carrageenan is found in the cell walls of seaweed or its intracellular matrix and carrageenan is a large component of the dry weight of seaweed compared to other components. *K. alvarezii* produces a type of 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 due to the seaweed in all treatments being unable to adapt to their respective environments, so the process 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 to not be optimal. It is known that seaweed really needs nutrients for its growth, including the formation of carrageenan. The low yield of yeast yield is also influenced by the harvest age, which is 30 days. Asikin *et al.,* (2019), stated that the high and low yield values of yeast 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, the place where the sample is taken also influences the yield value. According to Kumayanjati (2018) that the environmental conditions where seaweed grows can determine the yield value of the carrageenan produced.

Thalus 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.

No.	**Treatment**	**Cell Structure Hari Ke-30**	**Informa tion**
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 which are larger and rounder, but less dense compared to cortical cells.

After 30 days of maintenance with various 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 larger. 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 Quere *et al.,* (2015), that in very severe *K. alvarezii* seaweed tissue, it is shown by deterioration and tissue death which is 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 that the *K. alvarezii* seaweed is a little difficult to adapt to in the process 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. Thalus growth is an increase in cell size or a change in the state of a number of cells to form organs that have 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.

Parameter	P1	P2	P3	P4	Kelayakan
Suhu (^{o}C)	28.1-30	28.1-29.2	28.1-29.2	28.1-29.4	26-30 Rahma (2020)
Salinitas (ppt)	28-30	28-29	28-29	28-29	28-35 Rahma (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 (2018)
Intensitas Cahaya (lux)	635	625	625	625	500-1000 Sitorus *et al*., (2020)
Fosfat (mg/L)	<0.01-<0.02	<0.01-<0.02	<0.01-<0.02	<0.01-<0.02	0,2-1,04 Anggadiredja (2008)
Nitrat (mg/L)	3.3-3.5	3.4-3.5	3.2-3.4	3.4-3.5	0,9-3.50 Arni (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 in the sustainability of cultivation. The results of temperature measurements during the research were in all treatments ranging between 28,1–30^{0}C. This shows that the cultivation media still has a good range of temperature values for growth of *K. alvarezi*i. According to Rahmah (2020) that 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 and yellowish, unhealthy, wither and very 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 carried out 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 Rahmah (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) that 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 waters. Risnawati *et al.,* (2018), stated that the optimal acidity value for seaweed growth ranges from 6.0-9.0. Water that is very acidic or alkaline 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 is in line with Risnawati's (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 500-1000 lux.

Nitrate is an important nutrient for the seaweed growth process. Nitrate levels obtained during the study ranged from 3,2-3,5 mg/L. Risnawati *et al.,* (2018) that nitrate levels above 0.2 mg/L causes eutrophication (enrichment) and will 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. If the nitrate level is below 0.1 mg/L or above 45 mg/L, then nitrate is a limiting factor, meaning that at this level nitrate is toxic and can cause eutrophication which can stimulate rapid phytoplankton growth. Pramesti (2013) states that nitrate plays a role as a building block or basic ingredient for protein and the formation of chlorophyll. Plants that experience a lack of nitrate result in the photosynthesis process in their bodies not running optimally, 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 really needs phosphate for its growth process and is an important 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.

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 in maintaining 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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