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The effect of substrate type composition on the growth of the seaweed *Gracilaria verrucosa* in controlled containers

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ABSTRACT

Naturally, seaweed *Gracilaria* lives on various aquatic substrates including sandy, mud, coral and coral fragments, but scientific information that reveals the extent to which substrates influence the growth of this type of seaweed in cultivation has not been widely studied. The aim of this research is to determine the type of substrate that influences the growth of seaweed *G. verrucosa*, and to determine the best substrate for seaweed *G. verrucosa*. Collecting samples of seaweed *G. verrucosa* in the waters of Kalumata Ternate for 5 days. The cultivation process in a controlled container was carried out at the Unkhair Integrated Laboratory UPT, Sasa Village for 45 days. The overall research implementation time is 50 days from May – July 2022. The results of the research showed that the composition of the substrate type had a very significant effect on the absolute weight growth and relative growth of the seaweed *G. verrucosa*. The type of coral fragment substrate had the best influence on absolute weight growth with an average value of 57.33 grams, followed by sandy substrate at 37 grams, and the lowest was muddy substrate at 30.66 grams. The coral fragment substrate also had the best influence on relative growth at around 8.991%, and the lowest was found on the muddy substrate at around 7.593%.

INTRODUCTION

Seaweed *Gracilaria verrucosa* is a type of red algae (Rhodophyta) that grows in the tropics and subtropical shallow ocean waters. Types of seaweed This sea has the potential to be developed for export because it contains high agar agar and useful for various purposes. This type of seaweed is also is a leading commodity fisheries that have economic value important (**Ruslaini, 2016**).

Several genera of seaweed that have commercial benefits include *Kappahycus* and *Gracilaria*, because they can be cultivated vegetatively. One type of seaweed that has been widely cultivated is *Gracilaria verrucosa*. This type of seaweed has benefits because it produces agar compounds (**Fanni *et al.*, (2021).** Agar contains gelatinous hydrocolloid compounds which are commonly used as thickening agents in the food industry (**Mundira & Sinurat, 2011**). The type of seaweed *Gracilaria* is able to adapt widely and develops well in seawater and brackish water, making it suitable for cultivation in these waters (**Anton, 2017**).

Naturally, seaweed *Gracilaria* lives on various aquatic substrates including sandy, mud, coral and coral fragments (**Alifatri, 2012**), but scientific information that reveals the extent to which substrates influence the growth of this type of seaweed in cultivation has not been widely studied.

The natural existence of this type of seaweed in waters is very potential and its presence is quite abundant, however efforts to develop its cultivation have never been carried out in waters, especially the waters of Ternate Island and North Maluku in general. To obtain initial information regarding this type of seaweed commodity, it is necessary to carry out initial research to obtain data and information on the existence of this type of seaweed in an effort to develop its cultivation in a sustainable manner. The objectives of this study are to find out the type of substrate that affects the growth of seaweed *G. verrucosa* and to find out the best substrate on seaweed *G. verrucosa*.

The hypotheses of this study are Ho: The composition of the substrate type had no effect on the growth of seaweed *G. verrucosa* in controlled containers, H_1: The composition of the substrate type affects on the growth of seaweed *G. verrucosa* in controlled containers. The rules for decision-making are F count < F table; Ho accepted, reject H_1, and F count > F table; Ho rejected, accepted H_1

MATERIALS AND METHODS

Collecting samples of seaweed *G. verrucosa* in the waters of Kalumata Ternate for 5 days. The cultivation process in a controlled container was carried out at the Unkhair Integrated Laboratory UPT, Sasa Village for 45 days.The overall research implementation time is 50 days from May – July 2023.

The collection of seaweed seeds is carried out naturally in Kalumata waters, at a depth of around 0.5 - 1 meter. The seeds obtained are then put into the storage container provided. The seeds are then taken to the laboratory for weighing.

After seaweed seeds were found, cleaning was carried out. Next, weigh the initial weight of the thallus, which is 50 grams, which is then used for the planting process. The initial weight of the thallus was measured using a digital scale (0.01 gram).

The thallus that has been weighed is then tied to each span rope that is placed into each container according to the treatment. The length of the span rope is 50 cm which is tied to the thallus at 4 planting points with a distance of 15 cm between each planting point.

The process of maintaining seaweed *G.verucosa* is carried out for 45 days. The container used for maintenance is a fiber tub with a container length of 90 cm, width of

30 cm for 9 (nine) units. During the maintenance process, seaweed is given 20 ml of liquid NPK fertilizer with the aim of fertilizing the plants during maintenance.

The research used 3 different substrate treatments with 3 replications, namely: Treatment A: Sandy substrate, Treatment B: Muddy substrate, Treatment C: Coral fragment substrate. This determination is based on the fact that the seaweed type *G. verrucosa* usually occupies sandy, muddy and coral fragment substrate habitats (**Murni, 2016**).

The parameters observed were growth which was measured once a week. The growth observed in each treatment was recorded. To observe the condition of aquatic environmental parameters, it is observed once a week. The environmental parameters observed include: temperature, pH, salinity and dissolved oxygen. Temperature, pH, salinity and dissolved oxygen were measured using a thermometer, pH meter, DO meter and hand refractometer.

To analyze the growth data of the seaweed *G. verucosa*, a predetermined formula was used, namely absolute weight growth and relative growth rate.

To calculate the growth rate (relative growth rate) the formula from **Dharmawaty *et al.* (2016)** as follows:

$$LPN = \text{Ln}\left(\frac{Wt - Wo}{t}\right) \times 100\%$$

Where:
LPN = Growth rate (%)
Wo = *G. verucosa* wet weight at the start of the study (grams)
Wt = *G. verucosa* weight at the end of the study (grams)
t = maintenance time (45 days)

To calculate absolute weight growth using the formula from **Irfan & Subur (2022)** as follows:

$$W = Wt - Wo$$

Where :
W = Growth (Growth in absolute weight) (grams)
Wt = Final weight (grams)
Wo = Initial weight (grams)

RESULTS

1. Relative growth

The data in Table 1 presents the relative growth of the seaweed *G. verrucosa*.

Table 1. Relative growth (%) of seaweed G. verrucosa

Test	**Treatment**		
	A	**B**	**C**
1	8.024	7.9	8.78
2	7.963	7.324	9.023
3	8.083	7.557	9.171
Total	24.07	22.781	26.974
Average	8.023	7.593	8.991

Table 1 shows that the highest average relative growth of seaweed G. verrucosa was in treatment C (coral fragment substrate), followed by treatment A (sandy substrate), while the lowest was in treatment B (muddy substrate). Of the three treatments, each experienced an increase in relative growth, however treatment C had the highest relative growth rate, namely 8.991%, followed by treatment A with an average of 8.023% and treatment B had the lowest relative growth of 7.593%. To determine the effect of each treatment on the relative growth of the seaweed *G. verrucosa*, it is shown in the Table 2.

Table 2. Results of fingerprint analysis of various relative growth rates of seaweed *G. verrucosa*

Source of diversity	**Degree of freedom**	**Sum of square**	**Middle square**	**F count**	**F table**	
					0.05	**0.01**
Treatment	2	3.075	1.537	36.595**	4.46	8.65
Error	6	0.253	0.253			
Total	8					

Information: ** = very significant

The results of the analysis of variance in Table 2 show that the composition of the substrate type has a very real influence on the relative growth of the seaweed G. verrucosa, because F count > F table 0.05 and 0.01, so the effect is very significant on the relative growth of the seaweed *G. verrucosa*. There was a very real effect so a further LSD test was carried out as shown in Table 3.

Table 3. LSD test results effect of substrate type composition on the relative growth of seaweed *G. verrucosa*.

Treatment	**Average**	**Difference**			**LSD test**	
					0.05	**0.01**
C	8.991	-			0.385	0.561
A	8.023	0.968 *	-			
B	7.593	1.398 *	0.43tn	-		

Information : * = real different; tn = not significantly different

From the results of the LSD test (Table 3), it shows that treatment C has a significantly different effect on treatments A and B. Treatments A to B are not significantly different, so treatment C (coral fragment substrate) gives the best results on the relative growth of seaweed *G. verrucosa*.

2. Absolute weight growth

Absolute weight growth is the reduction of the average final weight to the average initial weight during the maintenance period of seaweed *G. verrucosa*. The average absolute weight growth of seaweed *G. verrucosa* is presented in Table 4.

Table 4. Analysis results of average absolute weight growth (grams) of seaweed *G. verrucosa* in each treatment

Test	Treatment		
	A	**B**	**C**
1	37	35	52
2	36	27	58
3	38	30	62
Total	111	92	172
Average	37	30.66	57.33

Data in Table 4 shows that the highest average absolute weight growth of seaweed G. verrucosa was in treatment C (coral fragment substrate), followed by treatment A (sandy substrate), while the lowest was in treatment B (muddy substrate). Of the three treatments, each experienced an increase in growth, but treatment C had the highest absolute weight growth of 57.33 grams, followed by treatment A with an average weight of 37 grams and treatment B which had the lowest growth of 30.66 grams. To determine the effect of each treatment on the absolute weight growth of seaweed *G. verrucosa*, an analysis of variance was carried out, the results of which can be seen in Table 5.

Table 5. Results of analysis of fingerprint analysis of absolute weight growth of seaweed *G. verrucosa*

Source of diversity	Degree of freedom	Sum of square	Middle square	F count	F table	
					0.05	**0.01**
Treatment	2	1.164.666	582,333	40.943**	4.46	8.65
Error	6	85.34	14.223			
Total	8					

Information: ** = very significant

Table 5 shows the calculated F value (40.943) > the F table value of 0.05 or 0.01. This means that the treatments tried had a very real influence on the absolute weight growth of seaweed *G. verrucosa* or in other words, the composition treatment of different types of substrates has very significant differences in the absolute weight growth of seaweed *G. verrucosa*. There was a very real difference in influence, so it was continued with a further BNT test, the results of which are presented in Table 6.

Table 6. LSD Test Results effect of substrate type composition on absolute weight growth of seaweed *G. verrucosa*

Treatment	Average	Difference			LSD Test	
					0.05	0.01
C	57,33	-			7.100	10.330
A	37	20.33 **	-			
B	30.66	26.67 **	6.34tn	-		

Information: ** = very significant , tn = not significantly different

The results of the LSD test (Table 6) show that treatment C has a very significant effect on treatment A and treatment B. The effect from treatments A to B is not significant, so that treatment C (coral fragment substrate) gives the best results on the relative growth of seaweed *G. verrucosa*.

3. Water quality

Water quality parameters measured during the study are presented in the Table 7 below.

Table 7. Water quality during research

Water quality	Range value
Temperature (°C)	27.6 - 27.9
pH	7.25 - 7.27
DO (ppm)	6.2 - 6.6
Salinity (‰)	25 - 27

DISCUSSION

Coral fragment substrates provide the highest relative growth due to the high supply of nutrients in the substrate which can be given to the seaweed *G. verrucosa* thereby spurring an increase in the relative growth rate of 8.991%, while on sandy and muddy substrates a relative growth rate of 8.991% is obtained. 8.023 % and 7.593 %. **Anton (2017)** stated that seaweed *G. verrucosa* can live on various aquatic substrates such as coral, coral rubble, rocky and muddy sand, but the better availability of nutrients available on these various substrates can stimulate increased growth of the seaweed that is maintained.

Coral fragment substrate is the best substrate that can spur and increase the growth of seaweed *G. verrucosa*, because the substrate is a suitable habitat for seaweed *G. verrucosa*, the coral fragment substrate is also able to provide good nutrient availability to support the growth of seaweed *G. verrucosa*. This is in line with Anton (2017) opinion that coral reefs are a shelter and a place to find food for various marine organisms including seaweed.

The difference in the average absolute weight growth value of seaweed *G. verrucosa* in this study was due to differences in the composition of the type of substrate used. The coral fragment substrate provided the highest absolute weight growth value compared to other substrates. Coral debris substrate is a suitable substrate for the life of the seaweed *G. verrucosa*. Coral fragment substrate is also able to provide a better nutrient supply so that it can support the growth of seaweed *G. verrucosa*. On the coral fragment substrate, it also does not cause turbidity in the research container, thus allowing the penetration of incoming sunlight without experiencing obstacles needed by seaweed to carry out the photosynthesis process so that it can support its growth. The seaweed *G.verrucosa* is very dependent on sunlight for its photosynthesis process (**Sediadi, 2002**).The increase in cell division activities, so that the cells widen and become longer, is due to photosynthesis activities, which causes seaweed to develop and grow (**Murni, 2016**).

Reef substrate type, coral fragments are the habitat of various types of Gracillaria including *G. verrucosa*. The types of substrate that are suitable for the growth and development of *G. verrucosa* are coral substrate and coral fragments, because both substrates have optimal water clarity for the growth of *G. verrucosa* (**Anton, 2017**). This statement is in accordance with what was stated by **Nontji (1993)**, the life of seaweed is limited or only a little on sandy and muddy substrates, which is due to the fact that there are few hard substrates available for seaweed to attach to. The chemical composition of the substrate has no effect on the life of seaweed, but is only a habitat for seaweed to attach itself to at the bottom of the waters.

Seaweed sticks to hard substrates. Water with hard substrates such as coral and dead coral, is characterized as good water with good clarity. This is an important factor for the continuity of photosynthetic activity of seaweed. Sea waves cannot move hard basic substrates such as coral rocks which are a suitable living place for seaweed as an aquatic plant (**Atmajaya, 1999**).

One of the water quality parameters that affects the growth of seaweed is temperature (**Ruslaini, 2016**). Temperature has an important role for the life and growth of seaweed. Water temperature can affect several physiological functions of seaweed such as photosynthesis, respiration, growth, and reproduction (**Pongmasak & Sarira, 2018**).

The water temperature obtained during the research ranged from 27.6 – 27.9°C. According to **Ruslaini (2016)**, the appropriate temperature parameters needed for the growth and development of *G. verrucosa* seaweed are around 25-30°C. The water temperature obtained during the study was suitable for the growth of seaweed *G. verrucosa.*

pH is a limiting factor for the life and existence of a plant. Although seawater has a relatively stable pH value, it can be affected by photosynthesis activities, temperature, and industrial waste. The optimal aquatic pH for seaweed cultivation is generally 7.3 – 8.2 (**Pongmasak & Sarira, 2015**).

Cultivated biota has an adaptation to pH values. The measured pH is around 7.25-7.27. The pH for the growth of seaweed *G. verrucosa* is 7.0 – 7.2 (**Anton, 2017**). Compared with the pH obtained, it is still in good condition for seaweed *G. verrucosa* which lives normally.

The DO content obtained during the research was 6.2-6.6 ppm, making it suitable for the survival and growth of the seaweed *G. verrucosa*. The main role of DO is to support the growth process, metabolic activity, reproduction and fertility of seaweed (**Lobban & Harrison, 1997**). The best growth of seaweed is at a DO content of around 3-8 ppm and greater than 4 ppm (**Pongmasak & Sarira , 2018**).

The measured DO is around 3-5 ppm. Compared with the DO obtained, it is still in good condition for seaweed *G. verrucosa* which lives normally. Each marine organism has a different tolerance range to salinity including.*G.verrucosa* , so salinity is one of the important factors that affect the survival and growth of organisms. According to **Pongmasak & Sarira (2018)**, the salinity range of seaweed growth can thrive in tropical areas that have aquatic salinity of 32-34 ppt. However, if the fluctuation is outside the ideal range, it will cause low growth and rapid aging of seaweed. To get salinity with this range, the cultivation location should not be close to river mouths or other freshwater sources.

Changes in the morphology and physiology of seaweed are caused by changes in the movement of ions and water molecules in the seaweed cell membrane. Salinity plays a role in supporting the growth of the seaweed that is maintained. (**Wong & Chang, 2000**). *G. verrucosa* requires a salinity of 15-30 ppt with optimal salinity ranging from 20-35 ppt (**Anton, 2017**). The salinity measurement obtained was 25-27 ‰., so it is suitable for the seaweed *G. verrucosa*.

CONCLUSION

The composition of the substrate type has a very significant effect on the relative growth rate and absolute weight growth of the seaweed *G. verrucosa*. The coral fragment substrate type gave the best influence on relative growth of around 8.991%, and the lowest was on muddy substrates around 7.593% and gave the best influence on absolute weight growth with an average value of 57.33 grams, followed by sandy substrates at 37 grams, and the lowest on a muddy substrate was 30.66 grams.

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