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Effect of Culture Combination on Growth and Carrageenan Content of *Kappaphycus alvarezii* Green and Red Variety

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Abstract. Indonesia as second of the biggest *Kappaphycus alvarezii* producer in the world, which were cultured using a different variety called green and red variety of *K. alvarezii*. The aims of this study is to determine the effect of culture combination method on growth and yield of carrageenan extracted a red and green variety of *K. alvarezii*, which were used the randomized block design was conducted in. The result demonstrated among red and green did not show significantly ($p>0.05$) in growth at two weeks of culture periods. However, beyond 3-5 weeks of culture periods, *K. alvarezii* green variety showed significantly different ($p<0.05$), which mix culture method showed better growth than the red variety. Interestingly, *K. alvarezii* green variety demonstrated higher carrageenan content when mixed with red variety in one raft during the culture period, which was presented correlation with growth parameter. Furthermore, combination culture method could be suggested for seaweed farmers that face problems in occupying mono variety *K. alvarezii* seed during seaweed culture.

1. Introduction

Indonesia contains a large marine economic potential, which is one of the marine economic potentials is seaweed cultivation. *K. alvarezii* is one of the huge farmed algae globally, which is used extracted for the carrageenan industry[1]. Seaweed cultivation in Indonesia has great potential to produce products with high export value. Started from 2007, Indonesia was able to reach the first place as a major producer of seaweed [2]. There are 3 types of seaweed commonly cultivated in Indonesia, the popular one and huge of number culture is *K. alvarezii*. which is mostly cultivated by seaweed farmers in Indonesia.

K. alvarezii seaweed farmers in Indonesia recognize two varieties that are commonly cultivated, namely the red variety and the green variety[3]. Based on field observations, these two varieties are cultivated by farmers in different planting methods. The first methods were planting separately were in one floating raft using one variety. This method is done by farmers in Tanjung Village, Saronggi District, Sumenep Regency, East Java, Indonesia. The second method was in one raft two varieties are cultivated in a mixed-method, either alternating between ties in one rope or alternating between ropes. This mixed planting method is carried out by seaweed farmers in Pekandangan Village, Bluto District, Sumenep Regency, East Java, Indonesia.

The method of planting in these two areas is not certain what effect it gives to seaweed, therefore to determine the growth and carrageenan content resulting from the combination of planting methods for *K. alvarezii* red and green varieties, a research was conducted on how to combine better planting methods, whether red varieties and green varieties are cultivated separately or mixed in the cultivation process.



2. Materials and methods

2.1 Materials

The equipment used includes the main raft measuring 10 x 5 m² as a cultivation tool for 2 rafts. The main raft was divided into 24 plots of experimental unit rafts measuring 1 x 1.25 m². The distance between the bulkheads of the experimental unit raft is 1 m. The 10 mm PE anchor rope is 3 m, the 4 mm PE rope is 90 m, the PE rope is 2 mm in diameter by 6 kg and 4 anchors (from cement sacks or cement cast), supporting equipment such as baskets, knives, saws, scales and machetes. To measure the quality of water, a thermometer, a mini disc, a universal pH indicator paper, a flow bulb, and a hydrometer (measuring the salinity of water) is used. 32 kg of red variety of seaweed seeds of *Kappaphycus alvarezii* and 32 kg of green varieties came from seaweed cultivators from Tanjung Village.

2.2 Research Design

This research uses experimental methods. The experimental design used was a randomized block design, with four treatments and six replications. The treatments imposed in the study were: Green variety seaweed as mono culture (R₁), Red variety seaweed as mono culture (R₂), Green varieties of seaweed and red varieties planted mix alternating between ties in one rope ris (R₃), and green varieties of seaweed and red varieties planted mix alternating between rises (R₄).

The experimental unit raft measuring 1 x 1.25 m consists of 4 rope ropes. In 1 rope there are 4 bunches of seaweed weighing 100 gr.

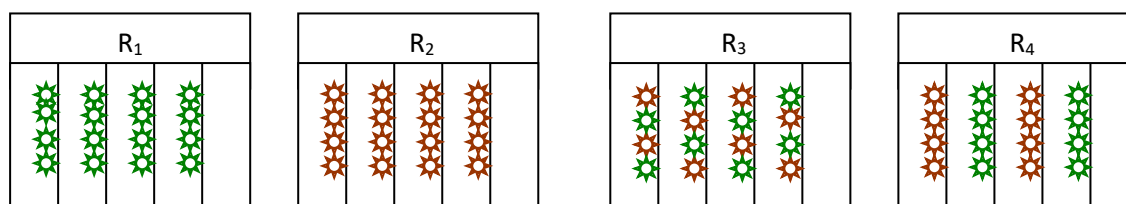


Figure 1. Research design for *K. alvarezii* culture, where Green variety seaweed as mono culture (R₁), Red variety seaweed as mono culture (R₂), Green varieties of seaweed and red varieties planted mix alternating between ties in one rope ris (R₃), and green varieties of seaweed and red varieties planted mix alternating between rises (R₄).

2.3 Culture methods

The main sealed raft measuring 1 x 1.25 m² consists of four ropes followed culture method by [4]. Seaweed seeds for each treatment were tied to the PE rope at 12 points 25 cm apart from the other bonding points. Treatment R₁ and R₂ were tied uniformly according to the treatment. On R₃ there are two different seeds which are tied alternately in one riser. Meanwhile, R₄ two different seeds were tied to the rope alternately. The binding of the riser to the raft is only carried out when the raft is ready to be brought to the research area, namely when the seawater begins to tide. While waiting for high tide, seaweed seeds are stored in the shade that is not exposed to sunlight.

2.4 Growth of *K. alvarezii*

Growth of *K. alvarezii* was determined by the weight of the sample every 7-days for 40 days of culture. The sample was untied and measure using digital measurement then were tied into the main raft.

2.5 Carrageenan extraction

Carrageenan content was determined using an extraction method followed by [5]. Carrageenan content was expressed as a percentage (%).

2.6 Data Analysis

Analysis of variance (ANOVA) using the SPSS version 23 was conducted to evaluate the weight of growth and carrageenan content ($\alpha=0.05$) and was continued analysis using Duncan's multiple range test to determine significant differences.

3 Results and Discussion

3.1 Growth weight

Culture of *K. alvarezii* using the different method was showed increasing weight start from second weeks to end of culture process. Interestingly, in this study, *K. alvarezii* red variety did not show significantly different ($p>0.05$) when culture mix or separately with green variety. However, *K. alvarezii* green variety was performed significantly different in growth weight ($p<0.05$) started from the third weeks of culture, which was R_3 showed the highest weight than other methods (Table 1).

Table 1. Weight of growth *K. alvarezii* green and red varieties^{1,2}.

Variety	Treatment	Seaweed Weight (g)± SD				
		1 st Week	2 nd Week	3 rd Week	4 th week	5 th week
Red	R_2	113.0±11.47 ^a	173.5±30.33 ^a	184.8±23.58 ^a	229.4±28.46 ^a	238.5±28.10 ^a
	R_3	124.0±17.29 ^a	162.3±5.75 ^a	204.2±24.69 ^a	238.6±36.87 ^a	245.9±30.82 ^a
	R_4	130.8±21.02 ^a	152.3±18.90 ^a	185.8±21.61 ^a	207.3±32.45 ^a	207.3±33.90 ^a
Green	R_1	117.6±7.46 ^a	147±7.77 ^a	158.5±14.77 ^b	179.9±22.50 ^b	189.0±21.08 ^b
	R_3	114.5±11.97 ^a	153±4.00 ^a	181.7±17.07 ^a	211.8±16.99 ^a	225.6±22.87 ^a
	R_4	116.5±12.56 ^a	158±27.66 ^a	164.8±19.57 ^{ab}	197.9±17.21 ^{ab}	203.0±17.18 ^{ab}

¹Values within a column with different superscript showed significantly different ($p<0.005$).

² Green variety seaweed as mono culture (R_1), Red variety seaweed as mono culture (R_2), Green varieties of seaweed and red varieties planted mix alternating between ties in one rope ris (R_3), and green varieties of seaweed and red varieties planted mix alternating between rises (R_4).

Seaweed growth is categorized into somatic growth and physiological growth. Somatic growth is growth based on the addition of weight and length of the thallus[6]. The growth of *K. alvarezii* seaweed is a weight gain. The highest weight of green varieties was found in treatment R_3 , then followed by treatment R_4 , and the lowest in treatment R_1 . This shows that if the green variety of seaweed is cultivated with red varieties alternating between ties in the rope, the green variety will get a better weight. This cultivation method is better than the green varieties cultivated with red varieties alternating between ropes and without red varieties.

The growth of seaweed is influenced by internal and external factors. Internal factors that influence include type, part of the thallus and age, while external factors that influence include the physical and chemical conditions of the waters then competition between seaweed in one culture system [6,7]. The difference in the carrageenan content of green varieties in the treatment was following the differences in the weight of the green varieties. Carrageenan is a primary metabolic produced from seaweed, so that if the growth increases, the carrageenan produced also increases. If seaweed is cultivated under the same conditions of light intensity and water movement, it will cause the same carrageenan content[8]. The conditions of the same water intensity and movement at the research location caused no differences in the carrageenan content of the chocolate varieties in each treatment. In the study, it is suspected that there are other factors besides maintenance time, light intensity, and water movement, which is thought to be due to the existence of two different varieties that interact to cause differences.

The interaction of the two different varieties is thought to have occurred between the cultivated varieties. Competition between organisms can be in the form of living space or the use of food sources such as light [9]. Supported by the opinion of [10], that this competition can cause one of the dominant organisms to prevent other organisms from obtaining food, living space, light as a source of food and growth.

Growth is also affected by the absorption of sufficient light intensity. Apart from light, the process of photosynthesis also involves the absorption of carbon dioxide (CO_2) and a little oxygen (O_2). So

due to competition for light sources and nutrients in the same living space, there is a competition between green varieties and red varieties.

Green varieties are spurred in utilizing living space, food sources and light so that they experience increased growth and carrageenan production. The more interactions between red varieties and green varieties (R_3) the higher the weight and the higher the carrageenan content of green varieties.

3.2 Carrageenan content

Carrageenan content was demonstrated significant differences among *K. alvarezii* green variety after 40-days of culture but *K. alvarezii* red variety did not show significantly different by different culture methods. Carrageenan content each *K. alvarezii* variety were summarized in Table 2.

Table 2. Carrageenan content of *K. alvarezii* green and red Variety^{1,2}

Treatment	Carrageenan Content (%)	
	Green Variety	Red Variety
R_1	17.51 ± 8.53^b	-
R_2	-	20.55 ± 5.26^a
R_3	27.31 ± 8.91^a	19.56 ± 9.49^a
R_4	19.07 ± 4.42^{ab}	16.44 ± 4.49^a

¹Values within a column with different superscript showed significantly different ($p < 0.005$).

² Green variety seaweed as mono culture (R_1), Red variety seaweed as mono culture (R_2), Green varieties of seaweed and red varieties planted mix alternating between ties in one rope (R_3), and green varieties of seaweed and red varieties planted mix alternating between rope (R_4).

This study demonstrated that green varieties of seaweed and red varieties planted mix alternating between ties in one rope showed carrageenan content based on [5], which were demonstrated carrageenan content between 26-27%.

4. Conclusion

The combination of planting methods has no effect on the growth and carrageenan content of *K. alvarezii* varieties red. The combination of planting methods affected growth from week 3 to week 5 and the carrageenan content of *K. alvarezii* green varieties.

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