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RESEARCH PAPER

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Effect of red, blue and white lights on the growth of red alga, *Gracilaria salicornia* (C. Agard) dawson of in vitro

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Abstract

This study showed that mean growth of *G. Salicornia* collected from North Sulawesi waters varied and appeared different among treatments. Those exposed to the red, blue and white lights had mean growth of 1.0658, 0.9695 and 1.0788, respectively. The one way analysis of variance revealed that there was no significant difference among light treatments in mean growth of *G. salicornia* ($P \geq 0.05$). Mean relative growth rate was 7.34% for red light, 4.8609 % for blue light, and 7.2748 % for white light, respectively. Two-way ANOVA also showed no significant difference in the relative growth rate of *Gracilaria salicornia* from the three light treatments ($P \geq 0.05$). Nevertheless, there was significant difference in growth rate based on observation time ($P < 0.05$), in which Tukey test indicated that period 1 differred from period 2, 3, and 4. Light and observation time interactions did not show significantly different effect ($P \geq 0.05$) on the algal growth. So Algae can living free on light different, grow and survival. But it must be to environment condition free from parasite and other epifit.

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Introduction

Algae, in international business world, are known as "seaweeds". Alga is a lower level plant possessing no proper structure, such as root, stem and leaf, but consisting merely of thallus. Algae belong to non-vascular plants, can photosynthesize due to having chlorophyl-a and simple reproductive structure (Dawes, 1981). Marine algae have a lot of benefits, can be directly eaten by human and utilized as raw materials in various industries, such as food, textile, pharmaceutics, cosmetics, and etc. (Winarno, 1990; Abdl El.Baky *et al.,* 2008; Gamal, 2009), because of its phycocoloid substance content, such as agar, alginate and carrageenan. *Gracilaria* is one of red algae retaining important economic value since it contains agar. Its content is high enough ranging between 16 % - 45% (Zatnika, 1993; Kumampung *et al.,* 2006). Algae are also very potential for highly bioactive secondary metabolite production for new pharmaceutical agents (Gamal, 2009), anti-bacterial, anti-cancer, anti-obesity, anti-fungi, and anti-viral agents (Zandy *et al.,* 2007; Pangestuti and Kim, 2011: Kusmiati, *et al.,* 2010).

There are numerous benefits of alga, *Gracilaria salicornia*, so that its utilization needs to be optimally done, since it will require a lot of these plants. For its utilization, dependence on the natural population could cause the algal community imbalance, in which the diversity of red algae will decline and other species could dominate the waters. Hence, this alga, G. *Salicornia,* needs to be cultivated, and thus, algal seeds are highly necessitated. The seeds can come from nature or artificial germination. Seed supply through artificial germination could be carried out in vitro for generative or vegetative seeds. The former is done by culturing the algal spores, and the latter through in vitro culture by taking a small part of thallus. In vitro culture needs to consider the environmental factors and even the culture media. Environmental condition can affect the algal seed growth, and light color can influence the algal growth condition. Tsekos *et al.* (2002) reported that red and blue light radiations affected the morphometric, while blue color light influences the metabolisms, respiration, carbohydrate degradation, and pigment synthesis. This study was intended to know the effect of red, white, and blue light on the growth of vegetative seeds of algae, and how far the effect of the light color treatment.

Material and methods

Alga sampling and media preparation

Algae, *Gracilaria salicornia,* were collected from natural waters in North Sulawesi at low tide, cleansed from epiphytes, and brought to the tissue culture laboratory of Faculty of Agriculture, Brawijaya University, Malang. Before cultured in vitro, the algae were acclimated for 2 days. The culture media was made of seawater filtered and sterilized in an autoclave, then added 2 ml of PES solution and 1.5 ml of germanium, and stirred up to being homogenous.

In vitro culture

After acclimatization, the thallus was cleaned, cut about 3-4 cm long, and weighed. The weight of algal thallus ranged between 0.7000-1.1000 gr, and put into PES media-containing culture flask and inserted in the culture cupboard facilitated with fluorescent light of red, white and blue colors, each of which was 40 watts. During the culture, lighting period was set as 12 hours light and 12 hours dark. The algae, *Gracilaria salicornia,* were cultured for 5 weeks and weighed every week using a 0.0001 mg--Ohaus digital balance. Each treatment had 3 replications. The algal growth was observed and visualized. All equipments used were kept clean and steril using an autoclave heated at 121^{0}C for 60 minutes. The media quality was also kept in suitable condition for algal growth.

Data analysis

Growth analysis used daily growth data to calculated relative growth rate using the formula of Evans (1972), as follows:

$$RGR = \frac{Ln\ (W_2 / W_1)}{T_2\text{-}T_1} \times 100$$

where :

RGR = relative growth rate (%)

Ln = naperian logaritm

W_1 and W_2 = total weight at initial and end period (gr)

T_1 and T_2 = initial and final (week)

Data of mean weekly weight were then analyzed by IBM SPSS software, version-21 (Complete Randomized Design), and relative growth rate using Group Randomized Design.

Results

Mean growth of alga, Gracilaria salicornia

Algae, *Gracilaria salicornia* from natural seawater of North Sulawesi were invitrolized for 5 weeks, and weekly weight measurement data were presented in Table 1. Growth of the red algae treated with red light was 0.9707, 0.9727, 0.9908, 1.0643, and 1.3306 at the first, second, third, fourth, and fifth week, respectively, with entire mean growth of 1.0658. For blue light treatment, it was 0.8783, 0.8108, 0.9967, 1.0601, and 1.1018 at the first, second, third, fourth, and fifth week, respectively, with entire mean growth of 0.9695. For white light treatment, it was 0.9773, 0.8984, 0.9857, 1.1774, and 1.3551 at the first, second, third, fourth, and fifth week, respectively, with mean growth of 1.0788.

Table 1. Weight measurements of *in vitro* alga, *Gracilaria salicornia* per week.

WEIGHT INCREMENT (gr) OF ALGA, *Gracilaria salicornia*						
LIGHT		WEEK				
		1	2	3	4	5
Red	1	0.771	0.7711	0.7722	0.8250	1.0610
	2	1.178	1.1732	1.2244	1.3544	1.8630
	3	0.963	0.9737	0.9758	1.0135	1.0677
Blue	1	0.759	0.6800	0.7020	0.7439	0.7790
	2	1.066	0.9968	1.4518	1.5075	1.5587
	3	0.810	0.7556	0.8362	0.9290	0.9672
White	1	0.770	0.7594	0.7730	0.7800	0.7895
	2	1.232	1.0926	1.2022	1.3850	1.5514
	3	0.930	0.8433	0.9818	1.3672	1.7244

Table 2. a) Descriptive variable of weight increment of the alga, *Gracilaria salicornia* b) Anova.

Descriptives

Pertumbuhan berat

	N	Mean	Std. Dev	Std.Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound	Min	Max
Red	15	1.0658	.28233	.07290	.9095	1.2221	.77	1.86
Blue	15	.9695	.29961	.07736	.8036	1.1354	.68	1.56
White	15	1.0788	.31700	0.8185	.9032	1.2543	.76	1.72
Total	45	1.0380	.29720	0.4430	.9487	1.1273	.68	1.86

a)

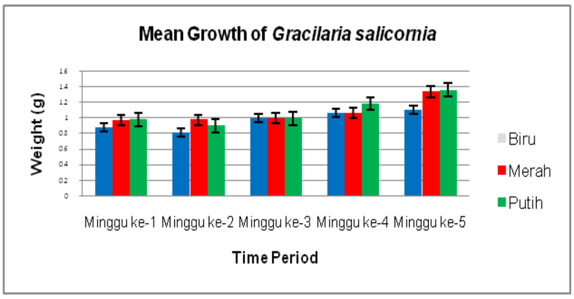
Anova

Weight Growth

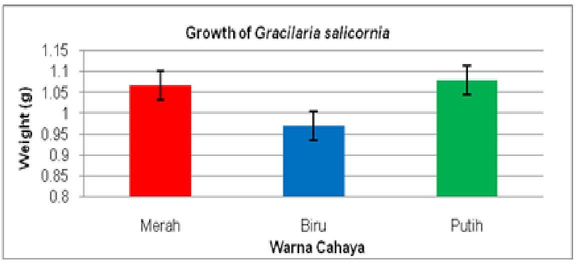
	Sum of Square	df	Mean Square	F	Sig.
Between Groups	.107	2	.594	.594	.557
Within Group	3.780	42			
Total	3.886	44			

b)

From the graph of mean growth of the alga, *Gracilaria salicornia,* it is apparent that there is different effect between the treatments (Fig. 1a &b).



a)



b)

Fig. 1. a) Graph of mean growth of alga, *Gracilaria salicornia, per week.* b) Mean growth during the study (vertical line indicates standard deviation).

One-way ANOVA showed that there was no significant difference in growth rate of the algae treated with different light colors ($P \geq 0.05$) meaning that red, white, and blue lights did not give significantly different effect on the algal growth.

Relative Growth Rate of the Algae, *Gracilaria salicornia*. Relative *Growth* rate (RGR) of the red alga, *Gracilaria salicornia,* is presented in Table 3.

Table 3. Relative Growth Rate of *Gracilaria salicornia* use the formula of Evans (1972).

LIGHT	PERIOD			
	1	2	3	4
	0.012969	0.142552	6.61398	25.15838
Red	-0.4083	4.271587	10.09076	31.88295
	1.104984	0.21544	3.790732	5.209712
	-10.9909	3.184061	5.797321	4.610443
Blue	-6.71185	37.60093	3.764848	3.339949
	-6.95221	10.13557	10.52409	4.029656
	-1.38619	1.77504	0.901487	1.210591
White	-12.0079	9.559303	14.15469	11.34576
	-9.78618	15.20649	33.11325	23.21143

Based on Fig.2, it is apparent that there is difference in relative growth rate among treaments and time periods. In the first week, there was no weight increment, but even thallus weight decline (Table 3). In the next weeks (week 2, 3, and 4), growth occurred with thallus weight increment indication.

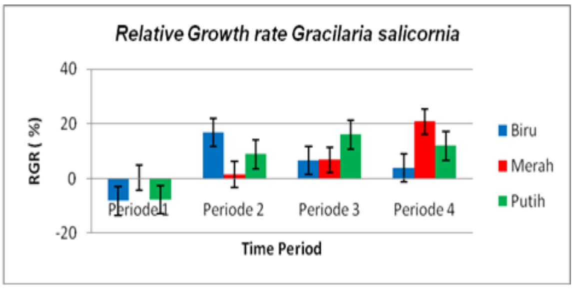


Fig 2. Relative growth rate of the red alga, *Gracilaria salicornia.*

Algal relative growth rate showed that in red light treatment, the average RGR (%) of *Gracilaria salicornia* was 0.23655%, 1.543193%, 6.831825%, and 20.75% in the first, second, third, and fourth period, respectively. As a whole, mean RGR (%) at this light exposure factor was 7.34%. In blue light factor, the mean RGR (%) was -8.218 %, 16.9735 %, 6.695421 %, and 3.993 % in the first, second, third, and fourth period, respectively, with entire mean RGR of 4.8609 %. In white light factor, the mean RGR (%) was -7.7267%, 8.8469%, 16.056%, and 11.92259% in the first, second, third, and fourth period, respectively, with entire mean RGR of 7.2748 %. As a whole, the mean RGR (%) was -5.236 %, 9.121218 %, 9.861241 %, and 12.222 % in the first, second, third, and fourth period, respectively (Table 4).

Table 4. a) Descriptive Statistcs, relative growth rate of *Gracilaria salicornia.* b) Anova.

Descriptive Statistics Dependent Variable : RGW				
Light	**Period**	**Mean**	**Std. Deviation**	**N**
Red	Period 1	0.236550	0.7810251	3
	Period 2	1.543193	2.3631397	3
	Period 3	6.831825	3.1556597	3
	Period 4	20.750347	13.8722205	3
	Total	7.340479	10.4865936	12
Blue	Period 1	-8.218318	2.4041303	3
	Period 2	16.973520	18.1988572	3
	Period 3	6.695421	3.4679649	3
	Period 4	3.993349	.6360246	3
	Total	4.860993	12.3012118	12
White	Period 1	-7.726246	5.6023201	3
	Period 2	8.846943	6.7439992	3
	Period 3	16.056477	16.1898740	3
	Period 4	11.922594	11.0117569	3
	Total	7.274817	13.1397738	12
Total	Period 1	-5.236171	5.1319123	9
	Period 2	9.121218	11.8427705	9
	Period 3	9.861241	9.6237737	9
	Period 4	12.222097	11.4553631	9
	Total	6.492096	11.7367288	12

a)

Test of Between. Subject Effects Dependent Variable: RGW					
Source	**Type III Sum Of Square**	**df**	**Mean Square**	**F**	**Sig.**
Model	4302.103[a]	12	358.509	4.225	.001
Light	47.915	2	23.957	.282	.756
Period	1697.837	3	565.946	6.670	.002
Light*Period	1039.048	6	173.175	2.041	.099
Error	2036.478	24	84.853		
Total	6338.581	36			

a. R Squared = .679 (Adjusted R Squared =518)

b)

ANOVA shows (Table 5) that there is no significantly different effect of red, blue, and white light treatments on the relative growth rate of the alga, *Gracilaria salicornia* (P≥0.05) (Fig. 3).

Table 5. Tukey test to see differences between the time periods.

Multiple Comparisons

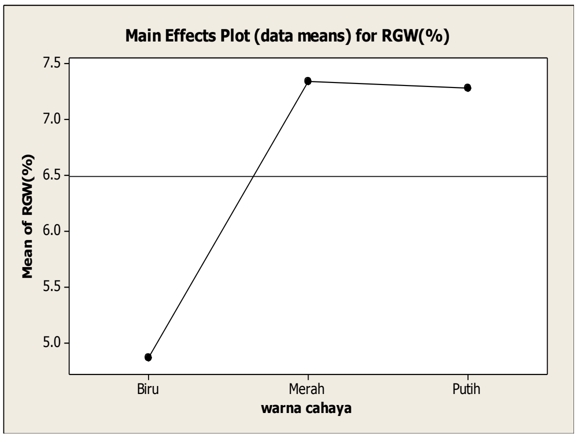
Dependent Variable : RGW
Tukey HSD

(1)Periode	(J) Periode	Mean Difference (I-J)	Std Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Periode 1	Periode 2	-14.357390*	4.3423816	.015	-26.336322	-2.378457
	Periode 3	-15.097412*	4.3423816	.010	-27.076345	-3.118479
	Periode 4	-17.458268*	4.3423816	.003	-20.437201	-5.479336
Periode 2	Periode 1	14.357390*	4.3423816	.015	2.378458	26.336322
	Periode 3	-.740022	4.3423816	.998	-12.718955	11.238910
	Periode 4	-3.100878	4.3423816	.947	-15.079811	8.878054
Periode 3	Periode 1	15.097412*	4.3423816	.010	3.118479	27.076345
	Periode 2	.740022	4.3423816	.998	-11.238910	12.718955
	Periode 4	-2.360856	4.3423816	.947	-14.339789	9.618077
Periode 4	Periode 1	17.458268*	4.3423816	.003	5.479336	29.437201
	Periode 2	3.100878	4.3423816	.891	-8.87054	15.079811
	Periode 3	2.360856	4.3423816	.947	-9.618077	14.339789

Based on observed means.

The error term is mean Square (Error)=84.853

*The mean diffrence is significant at the .05 level.



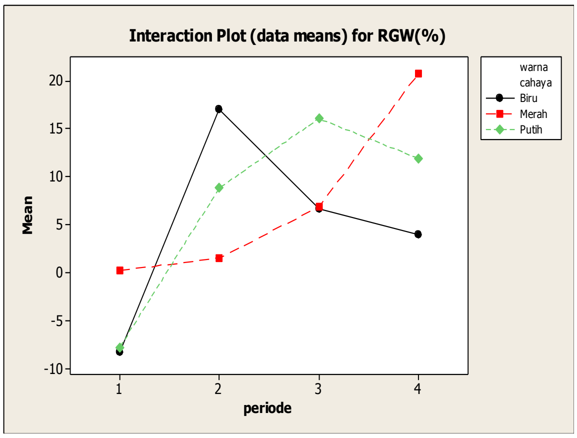
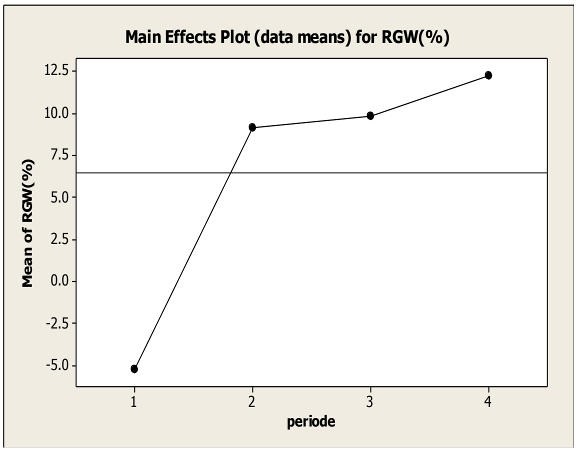


Fig. 3. Graph of relative growth rate of alga, *Gracilaria salicornia, under light treatment.*



Nevertheless, there is highly significantly different effect of time period on the relative growth rate of the algae (P<0.05) (Fig.4). Since there was significant different effect on the growth rate among time periods, Tukey test was used to see differences between the time periods (Table 5) This study found that period-1 had significantly different effect on the relative growth rate from that of period 2, 3 and 4 (P<0.05) with significance value of period-1 to 4 was 0.015, 0.010 and 0.03, respectively. The relative growth rate of period-2 was not significantly different from that of period-3 and period-4 (P≥0.05) (Table 5) Period-3 and 4 did not have different effect on the relative growth rate of the alga as well.

Moreover, ANOVA showed that light color and time period interactions show no significantly different effect on the relative growth rate ($P \geq 0.05$).

Discussion

Plant growth including algae occurs continuously throughout their life, depending upon meristem availability, assimilation output, hormone, other growth substances and supporting environment (Gardner *et al.*, 1991). Hormones affecting the growth is auxin, giberelin dan sitokinin, stimulating the algal growth. Growth could occur due to cell division in the meristem part, and thus, it occurs at the edge of thallus (apex), where the thallus will grow longer or the bud appears. One of the environmental factors affecting the algal growth is light, the visible electromagnetic energy of about 380–750 nm. Light is also called photon, a particle package.

This study found that *G. salicornia* can live and grow in PES culture media under light treatment. Fig. 5 shows difference in mean growth of the alga, *Gracilaria salicornia,* in spite of thallus weight decline in the second week. The initial thallus weight of *in vitro* alga, *G salicornia,* ranged from ± 0.7000 to 11000 gr. Mean thallus weight decline occurred from 0.8783 to 0.8108 gr under the blue light treatment and 0.9773 to 0.8984 gr under the white light treatment. Except that there was thallus weight increment under red light treatment. No thallus growth in the second week could result from that the algae were still in adaptation to the culture media. This condition will limit the photosynthetic rate affecting their growth. According to Herber *et al.* (2010) and Gao *et al.* (2011), stress could occur in algae from dessication causing the photosynthetic rate be restricted without electron flow interruption between photosystem I and II. In the third and fourth weeks, thallus weight increment occurred. In the fifth week, the algae cultured under the white and red light treatment had better growth than those cultured under the blue light. It could result from that the initial weight of the algae kept under the blue light treatment was lower than both other treatments. Also, with the same number of watts, color could influence the light intensity, and blue color had the smallest light intensity.

Despite apparently different mean growth under the light treatment, ANOVA showed that there was no different effect of the three light treatments ($P \geq 0.05$) on the growth rate. It means that the red, white, and blue light treatments do not give different effect on the mean growth rate. Light intensity highly affects the algal growth. Rosman *et al.* (2004) uttered that longer lighting period exposed to the plant could give the good vegetative or generative growth. They also added that lighting period influenced the methol synthetic process in higher level plant, mentha. Thus, enough lighting process is capable of synthetizing the growth hormones in the red alga, *Gracilaria selicornia,* stimulating the thallus growth. Auxin is one of the plant hormones taking part in growth and plant response to the environmental changes (Tromas & Perrot-Rechenmann, 2010). In *G. salicornia* treated with blue light, when cells were observed under TEM, there were numerous chloroplasts, functioning in photosynthetic process in which light is taken by the pigment through the chloroplast. The pigment highly affecting the photosynthetic process is chlorophyl. Red algae have chlorophyl-a and d. Chlorophyl is known as primary pigment, especially chlorophyl-a, due to its direct role in photosynthesis. It utilizes the sunlight for photosynthetic process, and then photosynthesis causes the growth. Thallus growth occurs through repeated mitotic cell divisions so that sufficient number of carbohydrates could be formed for the development and thallus growth. The carbohydrates containing hard cellulose will give a stiff form of the thallus making the thallus slightly erect. The photosynthetic reaction equation is as follows :

$$6\ O_2 + 6\ H_2O \text{ --------------}\rightarrow C_6H_{12}O_6 + 6\ O_2$$

The UV B light influenced the occurrence of floridean starch, cell wall thickness, reduced the intracellular space and phycoprotein reduction in red algae, *Hypne musciformis,* cultured *in vitro* (Schmidt *et al.,* 2012). In addition, UV sunlight radiation had an optimal range for algal spore development (Poldan *et al.*, 2004), and excessive UV radiation could affect the spore morphology and development.

Photosynthesis could still occur in *G. salicornia* treated with blue light despite very low light intensity. *G. saliconia* could still live and grow because the chloroplast production is more than 1 as one of the physiological and morphological strategies to overcome the effect of extreme environment, in this case low light intensity. Similar evidence was also found that physiological and morphological strategy of the organisms are done to adapt to from long exposure period extreme envirnmental conditions (Nybakken, 1992). *Porphyra sp* cultured in controlled environment showed the effect of red and green light radiation on the morphometrics (Tsekos *et al., 2*002).

Growth can also besupported by the quality of culture media, in which good and suitable environmental conditions, such as temperature and salinity, will support the algal growth. Salinity range of 30-35‰ is good and optimal for algal growth (Utojo *et al.,* 2007; Utojo, 2007 and Mustafa *et al.,* 2007). According to Karsten and West (1996), the best salinity for algal growth was 30‰. Unfavorable water salinity will disturb the osmotic pressure in alga cells, since salinity is directly proportional to osmotic pressure. If the salinity of the culture media is high the osmotic pressure in algal cells will be high, too. The algae living in high water salinity the osmotic pressure will be higher than those in lower water salinity. The ability to regulate the osmotic pressure could be different from one alga to the other depending upon their adaptability. Euryhaline algae will be able to adapt to the environmental condition with high salinity fluctuation, while stenohaline algae can only sustain in small range of salinity level. In this study, the salinity of the culture media was set at the range salinity of 30-35 to support the algal growth.

Water temperature of living media highly influences the algal life as well, such as survivorship and growth, respiration and photosynthesis. Good temperature for algal survival and growth varies with species, habitat types and geographic position. They could be eurythermal or stenothermal. With geographic position, algae can live at 0-10°C in the polar zone, 10-15°C in temperate zone, 10-20°C in subtropical area, and 15-30°C in the tropical area (Anonimous, 2011). Algae occurring in the area exposed to high environmental temperature for long period of time will affect their occurrence due to dessication. Moreover, Poldan *et al*, (2004) stated that there is effect of ultraviolet radiation on growth, morphological shape of the tissue and pigment composition from long exposure period. In this study, the temperature of culture media was in the good range for algal life and growth, 28-30°C.

Conclusion

There was no significant difference in mean growth rate and relative growth rate of *Gracilaria salicornia* under the light treatments. In observation period, Tukey test showed that week-1 was significantly different from week-2, 3 and 4, while week-2, 3, and 4 were not different in relative growth rate. Light and observation period interactions did not also have significantly different growth rate. Nevertheless, all algae, G. *salicornia,* could live and grow. Living media quality during the study, temperature and salinity, enabled to support the algae, *G. salicornia,* to live and grow. Algae can living free on light different. It can grow and survival but the environmental condition must be free from parasit and other epifit.

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