The utilization of seaweed-based liquid organic fertilizer to stimulate *Gracilaria verrucosa* growth and quality

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Abstract

The aim of this research is to analyze potency and generate organic fertilizer with noneconomic seaweed as material which is bioecologically environment-friendly to stimulate *Gracilaria verrucosa* production. By utilizing inexpensive and bioecologically beneficial material, it is expected to increase the growth, production, and repair the quality of *Gracilaria verrucosa*. The specific target of this research is to analyze the effectivity of an-organic fertilizers (urea) with seaweed-based liquid organic fertilizer. The hormone growth of generated urea and seaweed liquid fertilizers is analyzed, including auxin, gibberellin, cytokinin, as well as nutrient (macro and micro). Moreover, the fertilizer is tested on *Gracilaria verrucosa*. Several noneconomic seaweeds (*Codium* sp., *Ulva* sp., *Padina* sp., *Amphiroa* sp.) are found in waters area of Sulawesi Tengah. Based on laboratory testing result, it shows that organic fertilizer which has good potency by looking at N, P, and K contents is organic fertilizer *Ulva* sp., with content of N (0.45 mg/L), P (7.67 mg/L), and K (11.06 mg/L). The result of the research shows that there are apparent differences between the tested treatment ($p < 0.05$) on *Gracilaria* sp., growth, and the highest testing on giving liquid fertilizer *Ulva* sp. (71.0 g) with dose 400 mL/15 L water. The result of *Gracilaria verrucosa* quality by using liquid fertilizer *Ulva* sp. is 40.89%, urea fertilizer 38.62%, and without fertilizer 32.57%. The hormone content of *Ulva* sp. growth is IAA (0.3961 ppm), gibberellin (36.9595 ppm), and kinetin (3.3718 ppm), and urea fertilizer contains IAA (0.4063 ppm), gibberellin (30.2047 ppm), and kinetin (0.0717 ppm).

Keywords Agar value · Fertilizer · Growth hormone · Production · *Ulva* sp.

Mathematics Subject Classification 62J12 · 62G99

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Introduction

Gracilaria seaweed is famous because of its economic benefit in agar production, human food, pet food, medicine, biofuel, and fertilizer (Buchholz et al. 2012). Seaweed has been applied widely as fertilizer (seaweed fertilizer) for horticulture and legume plants because it has many mineral contents which cannot be found in other fertilizers. Besides, it contains important minerals which are needed by plants, and seaweed also contains growth stimulating a hormone that stimulates growth proven by plants growth and the amount of crop (Fornes et al. 2002; Sivasankari et al. 2006; Padhi and Swaim 2006; Prithiviraj 2009).

Generally, seaweed contains essential minerals (iron, iodine, aluminum, manganese, calcium, dissolved nitrogen, phosphorus, sulfur, chlorine, silicon, rubidium, strontium, barium, titanium, cobalt, boron, copper, potassium, and other elements). Nasmia (2014) reports that the result of

mineral content analysis in *Caulerpa racemosa* seaweed contains ammonia 0.03 ppm, nitrate 0.1 ppm, magnesium 0.40–0.52%, and calcium 0.09–0.19%. Meanwhile, Villares et al. (2007) explain that *Ulva lactuca* contains higher N, K, Mg, and Fe elements and contains microelement such as Cu, Mn, Zn, B, Al, Ni, Cr, Cd, and Pb.

Seaweed liquid fertilizer fermentation generates organic anion which may bind metals such as Al, Fe, and Ca. Therefore, ions will be free from the metals binding and then phosphate provided in the soil. Giving organic fertilizer may repair the soil structure and make the soil granule become big and able to retain water so that aeration inside the soil may be fluent and increase root development (Foth 1994). Furthermore, as added by Mukherjee and Patel (2020) seaweed can increase plant growth, seedling growth, and both root hair and secondary root development and can increase nutrient incorporation, fruit regulation, and pest and disease resistance and improve stress management (dryness, salinity, and temperature).

There is no information yet about seaweed fertilizer usage for water plants. Nevertheless, polyculture of two kinds of seaweed in which *Gracilaria* sp. with *Caulerpa* sp. conducted in Takalar, South Sulawesi, shows the increasing in higher *Gracilaria* sp. production than monoculture *Gracilaria* sp. that is conducted at the same time (Syamsuddin et al. 2016). *Caulerpa racemosa* existence is assumed which may increase *G. verrucosa* growth, since it contains mineral compounds and bioactive compound which is flavonoid that can dissolve in water (Harborne 1987; Syamsuddin et al. 2016). Robinson (1995) reports that flavonoid in plants functions to help increasing growth, photosynthesis, and having activities as anti-bacteria and anti-virus. In addition, Montano and dan Topas (1990) explain that noneconomic *Caulerpa racemosa* seaweed extract contains plants growth stimulator substance such as auxin and gibberellin. Based on that matter, in this research an experiment of noneconomic seaweed potency as environment-friendly organic fertilizer in cultivation media in order to stimulate *Gracilaria verrucosa* growth is conducted. Growth controller substance (growth hormone) provided at *Ulva* sp. is analyzed, and the effectiveness between giving seaweed organic fertilizer (*Ulva* sp.) and giving an-organic fertilizer (urea) on *Gracilaria* sp. seaweed production is compared. The experiments were performed for 50 days.

Materials and methods

Time and location

This research was conducted from May to June 2018. Seaweed sampling is conducted in Central Sulawesi seawaters.

Fertilizer is made in Laboratory of Fisheries, Faculty of Animal Husbandry and Fisheries, University of Tadulako, and the testing of growth hormone (auxin, gibberellin, cytokinin) in Laboratory of Agriculture, Agriculture Science Bogor. The implementation of giving organic fertilizer with seaweed material is conducted in Kampal Fish Seed Center (BBI), Installation of Mamboro Palu, Central Sulawesi, Indonesia.

Research stages

a. Stage of making fertilizer

In this stage, fertilizer is made in accordance with the procedure by considering the efficiency and effectivity of the produced fertilizer and then analyzing the chemical composition of the important elements contained in liquid seaweed fertilizer.

b. Stage of analyzing the testing of hormone growth content and seaweed agar quality.

In this stage, the analysis of growth hormone content of *Ulva* sp. seaweed (auxin, gibberellin, cytokinin) and *Gracilaria verrucosa* seaweed agar quality is conducted.

c. Stage of fertilizer effectivity testing conducted by compares the organic fertilizer with *Ulva* sp. seaweed and an-organic fertilizer applications on *Gracilaria verrucosa* seaweed production. Then, the testing of seaweed quality (agar) produced from cultivation with organic seaweed fertilizer *Ulva* sp. and an-organic fertilizer application (urea) is performed.

Observed variables

a. Absolute growth

The simplest method of reporting growth is the absolute increase in weight or absolute growth (Hopkins 1992):

$$W_t - W_i$$

where W_t is the weight at time, t is the final weight and W_i is the initial weight.

b. Analysis of growth controller substance from *Ulva* sp. fertilizer

Measurement of growth controller substance (growth hormone) from *Ulva* sp. seaweed fertilizer is analyzed in Laboratory of Faculty of Agriculture Technology, Bogor

Agricultural University, in accordance with Indonesian National Standard (SNI).

c. Analysis of agar content

Seaweed, which the agar content in the form of rendemen is examined, is analyzed with extract *agar* weight formula divided by dry seaweed weight and multiple by one hundred (SNI 1998). Seaweed sample which is analyzed is wet seaweed; then, it is dried and simultaneously taken on each treatment of its root and branch.

d. Measurement of water quality as cultivation media

Measurement of waters quality component in physics and chemistry is conducted every week simultaneously with weighing as the supporting data on *Gracilaria verrucosa* growth.

Data analysis

The data obtained during this research are analyzed using analysis of variance (Anova). If there is any influence of treatment on the tested organism, then it is continued by least significance difference (LSD). The data are analyzed by using minitab 16 and Microsoft Office Excel 2010. Meanwhile, the laboratory testing result of seaweed nutrient content, agar value, growth hormone, and water quality is descriptively analyzed.

Results and discussion

Result

Screening noneconomic seaweed

Noneconomic seaweed types which are found in several locations of Central Sulawesi waters are:

1. Seaweed found in central seawaters, Donggala Regency, consists of *Liagora* sp.; *Dictyopteris* sp.; *Halimeda* sp.; *Padina* sp.; and *Codium* sp.;
2. Seaweed found in Kabonga Besar waters, Donggala Regency, is *Padina* sp. and *Ulva* sp.;
3. Seaweed found in Morowali Regency waters is *Amphiroa* sp.
4. Seaweed found in Togeian waters, Tojo Una-Una Regency, is *Sargassum* sp. and *Caulerpa* sp.

Several of those types are found in considerable and sufficient numbers; thus, it satisfies to conduct nutrient content analysis (> 1 kg wet), as presented in Table 1.

Table 1 Nutrient contents of several noneconomic seaweed types found in several locations in Central Sulawesi waters

Seaweed	N (mg/L)	P (mg/L)	K (mg/L)	Fe (mg/L)	Mg (mg/L)
<i>Codium</i> sp.	0.49	2.53	7.902	0.788	2.753
<i>Ulva</i> sp.	0.45	7.67	11.063	1.192	3.195
<i>Padina</i> sp.	0.16	2.30	0.577	0.577	2.779
<i>Amphiroa</i> sp.	0.55	1.73	0.75	0.75	2.801

Fig. 1 The average of absolute growth of *Gracilaria verrucosa*

Absolute growth of *Gracilaria verrucosa* seaweed

Ulva sp. seaweed shows higher nutrient content than other seaweed types. Consequently, this seaweed is chosen as organic fertilizer material for *Gracilaria verrucosa* cultivation, and as a comparison, an-organic commercial fertilizer is used. The result of the research with different fertilizer applications (liquid fertilizer *Ulva* sp. and urea fertilizer) and without fertilizer given shows different absolute growth on *Gracilaria verrucosa* seaweed, as it can be seen in Fig. 1.

The result of analysis of variance (Anova) on absolute weight growth of *Gracilaria verrucosa* seaweed on various treatment in conservation media shows significant result ($p < 0.05$). Based on continuous test result, treatment A (seaweed liquid fertilizer) is not significantly different from treatment B (urea fertilizer), but it has significant difference with treatment without fertilizer given.

Content of hormone/growth regulator substance (ZPT)

The result of analysis on hormone or growth regulator substance content (auxin IAA, gibberellin, and cytokinin)

Table 2 Analysis result of growth regulation substance (ZPT) on liquid fertilizer *Ulva* sp. and urea fertilizer

Fertilizer type	Auxin IAA (ppm)	Gibberellin (ppm)	Cytokinin (kinetin) (ppm)
Liquid fertilizer <i>Ulva</i> sp.	0.3961	36.9595	3.3718
Urea fertilizer	0.4063	30.2047	0.0717

Fig. 2 Agar content of *Gracilaria verrucosa*

on liquid fertilizer of *Ulva* sp. seaweed and urea fertilizer is presented in Table 2.

Content of *Gracilaria verrucosa* agar

Agar content produced from *Gracilaria verrucosa* which is cultivated with fertilizer application and without fertilizer is shown in Fig. 2.

Discussion

Screening noneconomic seaweed

Central Sulawesi is the only province in Sulawesi Island which has three waters at once and not with other provinces in Sulawesi Island. The waters are Tomini Bay, Tolo Bay, and Makassar Strait/Sulawesi Sea (Directorate General of Maritime Affairs and Fisheries 2018). The result shows that Central Sulawesi coast has several kinds of seaweed. Moreover, according to the screening result and analysis of nutrient content in several seaweed types such as *Codium* sp., *Ulva* sp., *Padina* sp., and *Amphiroa* sp., it shows that the seaweeds may have potency to be used as organic fertilizer material. It is concluded by the result of mineral content analysis (Table 1) that all taken seaweeds have nutrient elements such as N, P, K, Fe, and Mg, in which those nutrients are very needed by plants including *Gracilaria verrucosa*. The content amount of macronutrient element of N, P, and K on sea plants is a potential resource to be organic fertilizer. It is supported by Zia (1990) cited in Zahid

(1999) that organic fertilizer from seaweed is very useful for growth and plant production increasing since it contains organic and an-organic materials which may boost nutrition absorption as well as help assimilation process between carbohydrate and protein of the plant. Furthermore, Nas-mia et al. (2017) also state that extract of *Sargassum* sp. seaweed may stimulate growth and extend time of micro-algae cells density increase since it contains nutrient element N (35,59 mg/L), P (3,81 mg/L), K (3,22 mg/L), Ca (2,02 mg/L), Fe (2,17 mg/L), S (6,67 mg/L), Cu (0,19 mg/L), and Zn (19,62 mg/L). Those nutrient elements function as energy and cell development sources as well as an important component to form protein.

Among several kinds of seaweed which are analyzed, *Ulva* sp. seaweed has better mineral content than other kinds of seaweed (*Codium* sp., *Padina* sp., *Amphiroa* sp.). The minerals within seaweed are diverse; it is supported by environment condition which has many minerals such as Ca, Mg, Na, K, and other minerals (Utomo and Asmawit 2012). In addition, Basmal (2009) expresses that seaweed not only can be used as functional food, but it may potentially become organic fertilizer because it contains trace metal which is quite diverse (Fe, B, Ca, Cu, Cl, K, Mg, and Mn). The organic material may be processed into alternative fertilizer so that may give advantage to repair land fertility, production increase, and natural resources conservation (Kaderi 2004).

Fertilizer is a material which contains a number of nutrient needed for seaweed to grow and survive. The utilization of seaweed liquid waste as organic fertilizer or addition material for fertilizer is expected to minimize waste in environment. It is supported by Dhargalkar and Periera (2005) that fertilizer made from seaweed extract may be degraded naturally, not contaminate, not poisonous, and safe for human and animal. Furthermore, Hamed et al. (2017) suggested the use of seaweed extracts for sustainable agriculture because seaweed contains micronutrients and macronutrients, which can help improve the nutrient status of the plants including several important nutrients such as N, P, K, Ca, S, Mg, Zn, Mn, and Fe.

Absolute growth of *Gracilaria verrucosa* seaweed

Based on the result of the research, fertilizer application (liquid fertilizer *Ulva* sp. seaweed, urea fertilizer, and without fertilizer) gives significant influence ($p < 0.005$) on absolute

growth of *Gracilaria verrucosa* (Fig. 1). The average of absolute growth *Gracilaria verrucosa* on various treatments with liquid fertilizer *Ulva* sp. is 71.0 g, urea fertilizer 52.4 g, and without fertilizer 37.9 g. This result shows that the highest *Gracilaria verrucosa* growth is on the treatment with seaweed liquid fertilizer and the lowest growth is in without fertilizer treatment. It shows that seaweed liquid fertilizer usage is more effective than urea fertilizer and without fertilizer. The analysis result shows seaweed liquid fertilizer from *Ulva* sp. contains higher nutrient element number (N:0.45 mg/L, P:7.67 mg/L, K:11.063 mg/L, Fe:1.192 mg/L, Mg:3.195 mg/L) than other seaweeds (*Codium* sp., *Padina* sp., *Amphiroa* sp.). Yustin et al. (2005) research result shows that potassium and chloride value of seaweed liquid fertilizer is greater than artificial fertilizer, respectively, 0.87–2.88% for potassium, and 1.37–2.41% for chloride.

Rosyida (2014) expresses in their research that *Gracilaria verrucosa* production may increase due to nutrient giving which contains macro- and micronutrients in which N, P, and Fe. Those nutrients will boost cells metabolism activity by entering into the cell bit by bit and then develop vacuole within the cells. Vacuole has an important role in live since plant live defense mechanism depends on vacuole capability to keep particular dissolved substance concentration within plants. Vacuole volume increases by the entering of nutrient into the cell and causes weight of tested plant increases as well (Lobban and Horrison 1994). In addition, Crouch and Van Staden (1993) also state that the magnitude response of plant growth increase by the usage of seaweed fertilizer is caused by growth regulator hormone which absorbs nutrients into plants.

Seaweed needs nutrients element either macro- or micro-nutrient elements. *Gracilaria verrucosa* seaweed needs nutrient in which widely used is nitrate and phosphate. Both of the elements are used as fertilizer, and both are obtained from *Ulva* sp. seaweed, while urea fertilizer only obtains nitrogen. Nitrogen availability in waters may cause plants to be fertile; however besides N element, seaweed also needs phosphate (P) to grow. In line with Lingga and Marsono (2007), phosphate is a very important element to stimulate thallus growth, accelerate, and strengthen juvenile plants to become full-grown plants, and causes high rate of growth. Angkasa et al. (2011) use fertilizer which contains nitrogen in amount of 10 kg/ha on the first 4 weeks of planting and use fertilizer which contains phosphate in amount of 5 kg/ha on 2 until 3 weeks later, which turns out to increase *Gracilaria* sp. growth. Moreover, Taha et al. (2011) express that the use of seaweed extract gives better result on cucumber plant growth rather than with control (without fertilizer).

Algae growth process may also occur because of active roles from phytohormone substance, in which it is an organic substance needed in small amount, but this small amount may determine the occurrence of physiological process.

Phytohormone is in the form of substances which help growth process, often called as growth stimulator substance or growth hormone. The result analysis of growth regulator substance (auxin, gibberellin, cytokinin) which is tested on liquid fertilizer *Ulva* sp. contains greater growth hormone than urea fertilizer. These growth hormones may increase growth and nutrient absorption and strengthen vegetative growth (Jensen 2004). The sufficiency of nutrient and growth hormone will make seaweed growth greater. It is in line with Yokoya and Handro (1996) statement that growth regulator substance can regulate growth, morphogenesis process, and red algae micropropagation.

Enhancing growth regulator substance IAA will stimulate callus formation which will form filament and act in plants physiological development process, such as cells extension and callus formation. Thus, it causes seaweed growth to increase. Briggs and Funge-Smith (1993) also state that rate of growth and photosynthesis, protein content, gel power, and melting point from *Gracilaria* raise along with nutrient enhancement into culture media either simultaneously and continuously or even gradually.

Other influence factor of seaweed growth is water quality. The result of water quality parameter measurement during research shows that normal estimation for *Gracilaria verrucosa* growth is: salinity 34–35 ppt, temperature 29.4–31.8 °C, and pH 7.3–8.2. It is in line with Trono and Fortes (1988) opinion that seaweed may grow better on particular salinity estimation depending on its tolerance and adaptation facing the environment. *Gracilaria* sp. may grow on high salinity estimation and withstanding up to 50 ppm. According to Aslan (1995), pH estimation which appropriate for seaweed cultivation tends to have base characteristic. Appropriate pH for seaweed cultivation is approximately between 7.0 and 8.5. Base water is productive base and encourages organic material change process to become minerals in water and can be assimilated by phytoplankton (Susilowati et al. 2012).

The content of hormone/growth regulator substance (ZPT)

The analysis result of content of hormone or growth regulator substance in liquid organic fertilizer *Ulva* sp. and urea fertilizer is shown in Table 2. Hormone content in *Ulva* sp. has higher value, generally, than urea fertilizer. Liquid fertilizer *Ulva* sp. has auxin IAA hormone to the value of IAA 0.3961 ppm, gibberellin 36.9595 ppm, and cytokinin (kinetin) 3.3718 ppm, and urea fertilizer has auxin IAA hormone to the value of 0.34063 ppm, gibberellin 30.2047 ppm, and cytokinin (kinetin) 0.0717 ppm.

The result of this research shows that seaweed has good potency to be organic fertilizer and environment-friendly because it has higher hormone content than urea fertilizer, and seaweed also contains macro- and microelements which

are much needed in plants growth. It is in line with what is expressed by Sedayu et al. (2014) that produced seaweed liquid fertilizer with semi-anaerobic composting that containing growth regulator hormone in which auxin, gibberellin, and cytokinin is higher than commercial liquid fertilizer. Giving organic fertilizer may repair land characteristics such as physical, chemical, and biological characteristics and also able to reduce excessive an-organic fertilizer use (Amilia 2011). Dhargalkar and Periera (2005) also express that fertilizer is made from seaweed and can be naturally degraded, not poisonous, not contaminate, and safe for human and animal.

Growth regulator substance (ZPT) content on fertilizer either liquid seaweed or urea fertilizer has different functions. Auxin compound has function in plants physiological process, such as growth, cells splitting, and protein synthesis (Gardner et al. 1991). Auxin stimulates protein in plasma membrane of plants cell to pump ion H^+ to cells wall which activate enzyme. After plants' cells extension process, cells will continue to grow by re-synthesizing material of cells wall and cytoplasm. Hormone contents in seaweed growth are diverse. It is affected by several factors such as season factor and seaweed growth phase (Mooney and Van Staden 1984).

Gibberellin which is produced naturally by plants has function as growth regulator substance (growth hormone). Besides in plants, gibberellin is also found in seaweed, moss, and ferns (Basmal 2009). From testing result of hormone content (ZPT), it shows that fertilizer *Ulva* sp. (Table 2) has gibberellin content (36.9595 ppm) higher than in urea fertilizer (30.2047 ppm). It shows that *Ulva* sp. seaweed has good potency to be organic fertilizer.

Gibberellin is a hormone compound which stimulates tube network development and stimulates cells splitting on cambium vessels so that it supports diameter expansion on branch/thallus. Gibberellin also has an important role in seeds sprouting in plants. Seeds which need particular environment condition to sprout, such as low temperature, will immediately sprout after sprayed by gibberellin (Basmal 2009).

Quality of agar *Gracilaria verrucosa* content

The obtained agar *Gracilaria verrucosa* content in this research with three treatments is without fertilizer (32.57%), urea fertilizer (38.62%), and with liquid fertilizer *Ulva* sp. (40.89%) (Fig. 2). The high value of *Gracilaria* agar content shows that giving liquid fertilizer *Ulva* sp. may give good and effective contribution in providing nutrient and growth hormone. As supported by Mackie and Preston (1974), nutrient absorption process which goes well is really needed to form polysaccharide such as agarose and agarpectin saved in cells wall as the

main material of agar. Agar is formed by two mixtures of polysaccharide in which agarose and agarpectin are the main component which determine gel and viscosity agar. Phycocolloid quality in the form of produced agar from *Gracilaria* sp. is affected by species, environment factor, season variation, and extraction method (Rao and Khaladaran 2003). Gioelea et al. (2017) also express that the main factor which influences agar result and quality is algae species, physiological factor, location, season, and environment condition. The number and quality of carageenan derived from seaweed cultivation are varied, not only based on strain, but also plants age, light, nutrient, temperature, and salinity (Hurtado 2001; Mendoza et al. 2006).

Meanwhile, the low agar content in without fertilizer treatment is suspected because of nutrient less availability in preservation media so that the absorption process in diffusion does not go very well. The assumption is in line with statement by Heddi (1986) that agar formation process in seaweed cells wall occurs with cells development by absorbing many nutrients and through photosynthesis process changed to various polysaccharides including agar. Meanwhile, physiological effect from growing seaweed includes biochemistry and biophysical processes that change simple molecules CO_2 , H_2O , to become sugar, amino acid, and polysaccharide. Those results of the process will increase agar content of seaweed (Wattimena 1988). Different agar contents on cultivated seaweed are hypothesized due to physiological process and ecological adaptation which varied on nutrient availability. Hence, it affects nutrient absorption process.

Conclusion

Based on the result of the research, it can be concluded there are significant differences between tested treatments ($p < 0.05$). The highest growth of *Gracilaria verrucosa* is on the use of liquid fertilizer with material *Ulva* sp. (71.0 g) with dose 400 mL/15 L water. The result of quality *Gracilaria verrucosa* agar by using liquid fertilizer *Ulva* sp. is 40.89%, urea fertilizer 38.62%, and without fertilizer 32.57%. Hormone content of *Ulva* sp. growth is IAA (0.3961 ppm), gibberellin (36.9595 ppm), and kinetin (3.3718 ppm), and urea fertilizer contains IAA (0.4063 ppm), gibberellin (30.2047 ppm), and kinetin (0.0717 ppm).

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