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Application of Seaweed *Gracilaria verrucosa* Tissue Culture using Different Doses of Vermicompost Fertilizer

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ABSTRACT

Market demand on the need of agar in Indonesia continues to increase from year to year, so it takes seaweed cultivation technology to improve the quantity and quality of seaweed especially for *Gracilaria verrucosa*. The purpose of this research is to analyze the doses of vermicompost fertilizer for the quality of agar (viscosity, gel strength) and nutrient content (carbon, nitrogen and phosphorus) of *G. verrucosa* seaweed from tissue culture. This research was conducted in seaweed pond of Pangkah Kulon Village, Ujung Pangkah District, Gresik Regency, East Java, from April to July 2017. The experimental design used in this study was completely randomized design (CRD) with 6 treatments and repeated 3 times. The experimental treatment was a different dose of vermicompost fertilizer, consisting of 0 ppm (Treatment A), 400 ppm (Treatment B), 425 ppm (Treatment C), 450 ppm (Treatment D), 475 ppm (Treatment E) and a dose of 500 ppm (Treatment F). The results showed that the quality of agar viscosity (cps) was best in the treatment of 450 ppm dosage for 67 cps. The quality of agar gel strength (g/cm^2) was best at treatment of 400 ppm dosage with a value of 77.2 g/cm^2. While the nutrient content of seaweed *G. verrucosa* form of carbon (%) was best in treatment of 450 ppm dosage with a value of 25.74%, the content of nitrogen and phosphorus (%) was best at treatment of 400 ppm dosage with values of 2.02% and 0.26% respectively.

INTRODUCTION

Gracilaria verrucosa is a species of seaweed of the class Rhodophyceae which is included in a producer group of gelatin. The main function of gelatin is as a stabilizing ingredient, stabilizers, emulsifiers, fillers, purification, gelmakers and others. Some industries which utilize the gel-forming property are food, pharmaceutical, cosmetics, skin, photography and microbial growth industries. *G. verrucosa* seaweed is one of the marine biological resources which has an important economic value. The development of *G. verrucosa* cultivation in Indonesia will provide great benefits as the demand on agar is increasing (Imaniar et al. 2013).

East Java itself is one of the top 10 largest seaweed producer regions in Indonesia. With the potential of marine water which is very suitable for the development of seaweed cultivation, it is not surprising that East Java transformed into one of the national seaweed production barn. Seaweed area in East Java reached more than 166 thousands ha from 158 thousands ha. High export market demand on seaweed also spur the business in seaweed production center, and the seaweed cultivation spread in a number of areas (BPS East Java Province 2013).

Market demand on agar in Indonesia continues to increase from year to year, so it takes the marine seaweed cultivation technology in terms of improving the quantity and quality. According to Arhan (2008), during 2004-2008, the price of dry *Gracilaria* seaweed per kg decreased from Rp. 5,000-15,000 with seaweed production ranging from 27,874-69,264 tons and agar production from 5,574-7,696 tons. Indonesia has not sufficiently high demand for *Gracilaria* seaweed during 2004 to 2008 as the raw material for producing agar (Febriko et al. 2008).

According to Mukhtar (2008), in 2007 Indonesia seaweed production reached 94 thousand tons. At the end of 2008, the supply of seaweed to market demand was reduced by 13.1%. This is because the productivity of seaweed cultivation farms decreased due to poor management of ponds, environmental pollution and environmental destruction. This causes a decrease in the quality and quantity of *G. verrucosa* seaweed. One of the success factors for increase in quality and quantity is the input of superior cultivation technology by way of tissue culture. Purification of seaweed seedlings has a goal to get the seeds free from disease or pure seeds. Then the seeds are given nutrients from fertilizers, which can repair and reproduce new cells in the thallus seaweed, so the quantity and quality of seaweed are growing (Mustafa et al. 2008).

Fertilizers are materials that contain a number of nutrients needed for seaweed. The use of fertilizers is usually done on plants that live on land while the use of fertilizer for aquatic plants is very rare. This is due to the water which is considered to be able to provide sufficient nutrients for plant growth. Provision of fertilizer on the culture medium is not enough without knowing the nutrient needed by the plant to produce the best quality and quantity. The lack of nutrition in the cultivation media will affect the production of seaweed. Formulation of the fertilizer can promote the growth and quality of seaweed agar *G. verrucosa*.

Fertilizer vermicompost is an organic fertilizer, which contains carbon, nitrogen and phosphorus needed by plants and is transformed through the activity of microorganisms into a form that is more easily absorbed by plants (Hernandez et al. 2014). Fertilizer with the right dose and ratio can produce the quality to make the best seaweed. So it needs to discover the formulations of carbon, nitrogen and phosphorus proper media for vermicompost fertilizer to generate the best growth and quality of seaweed *G. verrucosa*. Carbon is the main constituent element in the formation of plant carbohydrates, while nitrogen and phosphorus are the important elements of amino acid consisiting DNA, RNA, energy producers. Nitrogen and phosphorus are then converted into elements that synthesize carbohydrates. The purpose of this research is to analyze the appropriate dose of vermicompost fertilizer on the quality of agar (viscosity, gel strength) and nutrient contents (carbon, nitrogen and phosphorus) of *G. verrucosa* seaweed from tissue culture.

MATERIALS AND METHODS

Time and place of the study: Study as conducted in Tissue Culture Laboratory of Faculty of Agriculture University of Brawijaya Malang, Laboratory of Agricultural Technology University of Brawijaya Malang and Seaweed pond of Pangkah Kulon Village, Ujung Pangkah District, Gresik Regency, East Java, from April to July 2017.

Preparation of materials: Sterilized containers and tools were used in the research. Sterilization of seawater having a salinity of 25 ppt and explants (cell) of the seaweed was also carried out. Further, the explants were cut to a size of approximately 8-10 cm using a razor (Fadilah et al. 2010). They were each planted in glass bottles containing sterile marine water and the medium Conway, and placed at room temperature 25-27°C in a laminar flow chamber. The pH of the tissue culture medium was kept at 7.5. The explants were placed on a seaweed tissue culture stirrer shelf. Glass bottles that had been planted with explants, were placed on a stirrer rack in a sterile place, given the lighting from 40 watt fluorescent lamps. The lights were put on the top of the stirrer shelf and given a distance of 30 cm from the culture bottle with the lighting programmed for 24 hours continuously. After 14 days of tissue culture and emerging new shoots adaptation, the seeds of the tissue culture were applied to the pond cultivation with different doses of vermicompost fertilizer and cultivation carried out for 42 days.

Observation parameters: Quality of agar viscosity was determined by using a viscometer (cps), and gel strength using handcrank rheometer (g/cm^2). Seaweed contents of carbon, nitrogen and phosphorus (%) were estimated by using UV-VIS spectrophotometer.

Research design: Using the vermicompost dosage treatment with best composition, according to Afriansyah (2010), 300 g cow dung, 100 g soil (basic media), 300 g straw and 300 g grass, with dose of earthworm *Lumbricus* sp. @ 26 g/kg of material with the composition of C%:N%:P% 20:2:1 (Source: preliminary research data 2014). The experimental design used in this study was completely randomized design (CRD) with 6 treatments repeated 3 times so that there were 18 experimental units. The experimental treatment was to provide different doses of vermicompost fertilizer, consisting of 0 ppm (Treatment A), 400 ppm (Treatment B), 425 ppm (Treatment C), 450 ppm (Treatment D), 475 ppm (Treatment E) and 500 ppm (Treatment F).

Statistical analysis: Statistical analysis used was ANOVA (Analysis of Variance), by looking at the effect of vermicompost fertilizer on the quality of agar viscosity (cps), gel strength (g/cm^2), nutrient content of carbon, nitrogen and phosphorus (%) of *Gracilaria verrucosa* on the tissue culture. If the effect is significant ($p<0.05$), then it will be done with Tukey's advanced test, which aims to see the difference between the different doses of vermicompost fertilizer. Statistical analysis tool used was SPSS version 16.

RESULTS AND DISCUSSION

Quality of Agar Seaweed *Gracilaria verrucosa*

Viscosity (cps): The results show that the best quality of agar viscosity (cps) of *G. verrucosa* seaweed is present at 450 ppm with a viscosity of 67 cps (Fig. 1). The results of analysis of variance (ANOVA) show that, vermicompost fertilizer dose treatment has a significant effect on quality improvement for viscosity ($p <0.05$). Tukey's further tests show that the treatment doses of vermicompost 0 ppm fertilizer gives significant difference with 450 ppm treatment on quality improvement for viscosity ($p<0.05$). While the treatment of 400, 425, 450, 475 and 500 ppm do not give significant difference to the quality of agar viscosity in *G. verrucosa* ($p>0.05$).

The average viscosity range of this study is 40-67 cps (Fig. 1). According to Luthfy (1998), seaweed viscosity ranges from 5 to 800 cps. The high viscosity is due to the

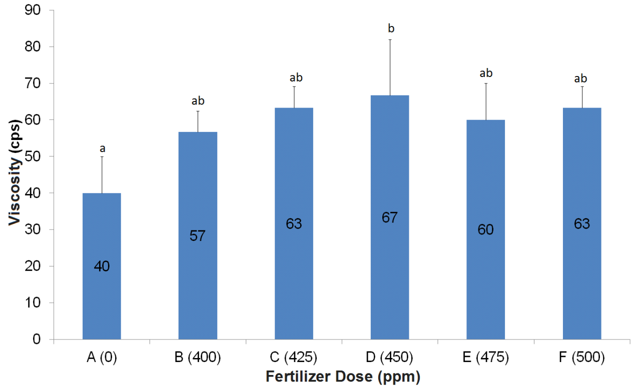

Fig. 1: The quality of agar viscosity (cps) of seaweed *G. verrucosa*.

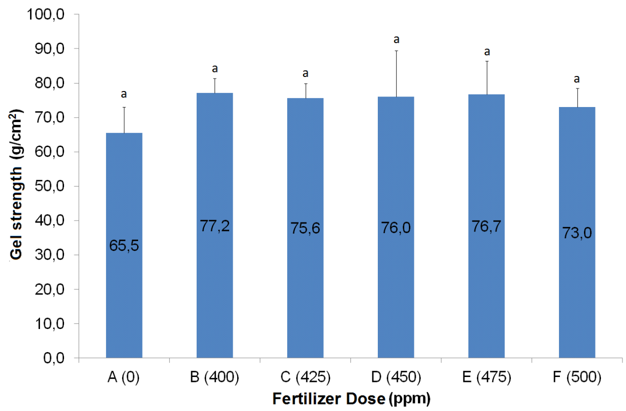

Fig. 2: Quality of agar gel strength of seaweed *G. verrucosa*.

rejecting forces between groups of ester which are loaded with water molecules bound in agar, thus causing higher viscosity (Pine et al. 2008). According to Aslan et al. (2009), the minimum seaweed viscosity level should not be less than 35 cps, and the higher the viscosity content, the higher the economic value of the seaweed.

Gel strength (g/cm^2): The results show that the best gel strength was obtained at a treatment of 400 ppm dose of 77.2 g/cm^2 (Fig. 2). From the analysis of variance (ANOVA), the dosage of vermicompost fertilizer do not give significant effect to the increase of *G. verrucosa* seaweed gel strength ($p > 0,05$) so that there is no further Tukey test conducted.

The average *G. verrucosa* gel strength range in this study is 65.5-77.2 g/cm^2 (Fig. 2), which is in the range proposed by Mudjarrab (2000) for *Gracilaria* species i.e. from 0-211.8 g/cm^2, with the highest gel strength obtained in the type of *G. verrucosa* ranged from 20-211.8 g/cm^2. According to Suryaningrum et al. (1994), the range of *G. verrucosa* gel strength in fishpond is 34.67-97.33 g/cm^2. While the results obtained by Subaryono & Mardinah (2011), the gel strength of *G. verrucosa* is 98.57-119.28 g/cm^2. Moirano (1997) stated that gel formation is a precipitation process involving ionic bonds to form gel thickness, the more viscous gel is marked by the decreasing strength, but otherwise the gel strength will be higher.

Nutrient Content of SeaWeed *G. verrucosa*

The content of carbon, nitrogen and phosphorus: The best carbon content of *G. verrucosa* is obtained at 450 ppm treatment which is 25.74%, and the lowest (15.76%) is in the treatment of 0 ppm (control). The best nitrogen content of 400 ppm treatment is 2.02%, the lowest at 0 ppm (control) treatment was 0.85%. Then the best phosphorus content in the treatment of 400 ppm is 0.26% and the lowest in the treatment of 0 ppm (control) is 0.20% (Fig. 3). Result of analysis of variance (ANOVA) shows that the dose of vermicompost fertilizer gives significant effect to the increase of carbon in the seaweed *G. verrucosa* ($p<0.05$). While the dosage of vermicompost fertilizer does not give a significant effect to the increase of nitrogen and phosphorus in the seaweed ($p>0.05$), so there is no further Tukey test done. Tukey's further test results in that the treatment 0 ppm (control) shows a significant difference with the treatment of 450 ppm, with the increase of carbon of the seaweed ($p<0.05$). While the treatment of 400, 425, 450, 475 and 500 ppm does not give significant difference ($p> 0.05$).

The carbon content of *G. verrucosa* during the study is 15.76-25.74% (Fig. 3). Erlania (2013) stated that the range of carbon in *G. verrucosa* cultivated on the coast is 21.38-24.57%. While the range of carbon content for *G. verrucosa* species cultivated in ponds is 15.34-39.84% (Hambali et al. 2004). Carbon is the nutrient element with the largest content in vermicompost fertilizer that is around 19.23-21.32% (Mashur 2001). Nutrient carbon is the main nutrient needed by seaweed in the process of photosynthesis to produce carbohydrates which are the main constituent components for seaweed (Wakhid et al. 2013).

The range of *G. verrucosa* nitrogen content during the study is 0.85-2.02% (Fig. 3). The nitrogen content cultivated offshore ranges from 0.44 to 4.73% (Yuniarsih et al. 2014). Meanwhile, according to Mulatsih et al. (2008) the nitrogen content of seaweed *G. verrucosa* cultivated on a laboratory scale ranges from 1.72 to 2.32%. The nitrogen content of vermicompost fertilizer ranges from 1.5 to 2.5% (Mashur 2001). The high nitrogen content in the dosage of 400-500 ppm of vermicompost fertilizer is due to the high nitrogen content in the maintenance container compared to the control media 0 ppm. According to Balmori et al. (2013) vermicompost fertilizer is an important source of nitrogen, but most of the nitrogen in the fertilizer is insoluble and not

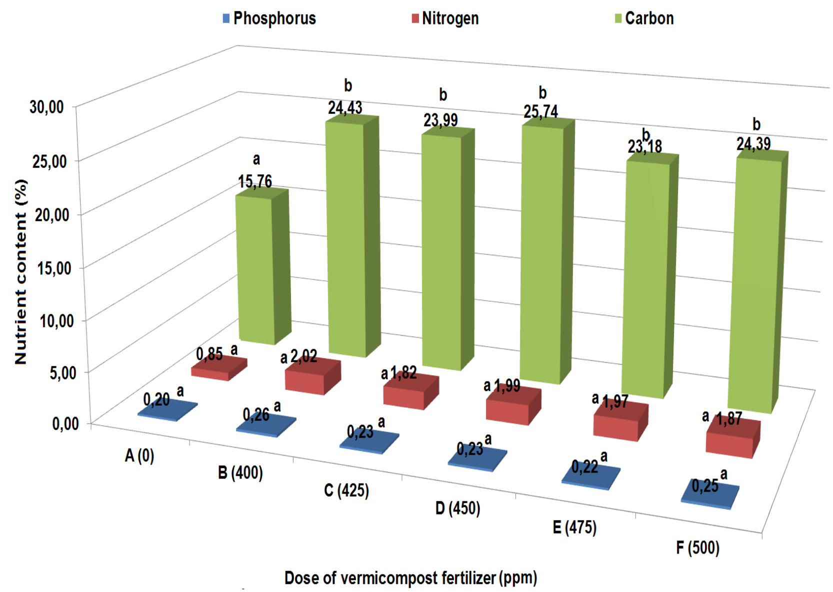

Fig. 3: Nutrient content of seaweed *G. verrucosa*.

immediately available for plant use.

The range of phosphorus content of *G. verrucosa* during the study is 0.20-0.26% (Fig. 3). The content of phosphorus cultivated offshore ranges from 0.06-1.07% (Yuniarsih et al. 2014). Mulatsih et al. (2008) stated that phosphorus content of seaweed *G. verrucosa* on a laboratory scale ranges from 0.03 to 0.10%. Optimal growth of plants was obtained with a phosphorus range of 0.3-0.5% (Engelstad 2007). Phosphorus content of vermicompost fertilizer ranges from 0.5 to 1.0% (Mashur 2001). The vermicompost fertilizer contains the phosphorus elements required by the plant in relatively large quantities and is also included in the macro element. However, the amount of phosphorus in plants is smaller than that of carbon and nitrogen, the role of phosphorus is crucial for plants as components of proteins, cell nuclei, cell walls, formation of high-energy compounds, and as components of RNA and DNA (Choirina et al. 2013).

CONCLUSION

The conclusion from this study is:

1. The best quality of agar viscosity (cps) was in the treatment of 450 ppm fertilizer dose with 67 cps.
2. The best quality of agar gel strength (g/cm^2) was in the treatment of 400 ppm fertilizer dose with 77.2 g/cm^2.
3. The best nutrient content of *G. verrucosa* in this study was carbon 25.74% with dose of 450 ppm, nitrogen 2.02% and phosphorus 0.26%, both with dose of 400 ppm.

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