

*Effect of Seaweed Extract from Water, Methanol, and Ethanol Extraction as Biostimulant on Growth and Yield of Upland Rice (*Oryza sativa* L.) in Ultisol*

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Abstract— Seaweed extract contains polysaccharides, protein, unsaturated fatty acids, polyphenols, micronutrients, macronutrients, minerals and growth regulators that have the potential as biostimulants. This study aims to determine the effect of 4 seaweed species as biostimulants to increase the growth and yield of upland rice on ultisol soils and to determine the effect of solvent type on seaweed extract. The solvents used in this study were water, methanol, and ethanol with a sample-solvent ratio 1:20 (w/v). The data obtained were analyzed using a completely randomized design (CRD) consisting of 2 factors with 3 replications. Factor A is the type of seaweed which consisted of 1. *Padina minor* 2. *Sargassum crassifolium* 3. *Sargassum cristaefolium* 4. *Turbinaria decurrens* and factor B is the type of solvent which consisted of 1. Water 2. Methanol 3. Ethanol. The results showed that *Padina minor* was the best seaweed to increase the productivity of upland rice, which showed from root wet weight, grain weight per clump, and 100-grain weight. Methanol is the best type of solvent for extracting seaweed. The best interaction between the two factors is found in *Padina minor* dissolved in methanol

Keywords— Seaweed extract, Upland rice, Extraction, Solvent

I. INTRODUCTION

Biostimulants are natural organic compounds which able to stimulate and improve the physiological functions in plants, such as respiration, photosynthesis, nucleic acid synthesis, nutrient absorption (Abbas, 2013), and increasing the plant response to stress (Du Jardin, 2015). The use of biostimulants can increase hormone and nutrient absorption (Kavipriya and Thangaraju, 2012), besides it gives good effect on germination, growth and increase yields that are environmentally friendly (Pise and Sabale, 2010). Biostimulants can be obtained from various sources, such as amino acids, humic substances, seaweed extracts, plant extracts, and mycorrhizae (Du Jardin, 2012).

In the research conducted by Hadi, Zakaria and Syam (2016), on Kasiak Gadang Island, Nirwana Beach, Padang City, West Sumatra, several types of seaweed were found including species from the Phaeophyta group (*Padina minor*, *Sargassum crassifolium*, *Sargassum cristaefolium*, and *Turbinaria decurrens*). These four species of seaweed have the potential to be studied as biostimulants. Seaweed has biologically active compounds that can increase agricultural productivity (Hernandez *et*

al., 2018).

Most seaweeds contain polyphenols, lipids (n-3 and n-6 fatty acids), micronutrients (B, Co, Cu, Fe, Mn, Mo, Ni, Si, and Zn) and macronutrients (Ca, K, Mg, Na, P and S). It also contains components of bioactive compounds in the form of growth regulators, including gibberellins and cytokinins (Tarakhovskaya, Yu, and Shishova, 2007).

The active substance from seaweed that will be used as a biostimulant can be obtained by extraction. According to Purnama (2004) extraction is a way to take bioactive compounds from plant parts by using a solvent that is in accordance with the polarity of the substance to be extracted. The principle of extraction is to dissolve polar compounds in polar solvents and non-polar compounds in non-polar solvent. In this study, the polar solvents used were water, methanol and ethanol by maceration method.

Methanol can dissolve hormones, such as auxin and cytokinin, as well as secondary metabolites including alkaloids, steroids, saponins, terpenoids, polyphenols and flavonoids (Sedayu *et al.*, 2013; Rachman *et al.*, 2017 and Podungge *et al.*, 2018). Ethanol can dissolve polyphenols (Cahyaningrum *et al.*, 2016), while water can dissolve secondary metabolites (flavonoids, saponins and terpenoids), various macronutrients (Ca, K, Mg, Na, P), and micronutrients (S, B, Co, Cu, Fe, Mn, Mo, Ni, Si, and Zn) (Septiana *et al.*, 2012; Godlewska, 2016).

II. METHODOLOGY

2.1 Experimental Design

Seaweed sampling was carried out at Nirwana Beach, Padang City. Seaweed extraction was carried out at the Plant Physiology Laboratory, and planting was carried out at the wire house of the Department of Biology, Faculty of Mathematics and Natural Science, Andalas University from January 2019 to August 2019. The experimental design used was Completely Randomized Design (CRD), the treatment consisted of 2 factors with 3 replications. Factor A - types of seaweed: *Sargassum crassifolium*, *Sargassum cristaeifolium*, *Turbinaria decurrens*, and *Padina minor*. Factor B - the type of solvent: water, methanol, and ethanol. The dried seaweed was grounded into powder, then weighed, the ratio of sample to solvent was 1:20 (w/v). Then it was shaken for 24 hours at room temperature and the sample was filtered using Whatman filter paper to obtain its extract (Soamole *et al.*, 2018).

2.2 Application of Seaweed Extract

The seaweed extract was sprayed evenly as much as ± 25 ml per plant for 15 days after planting. Spraying was carried out in the morning when the relative humidity of the air is still close to saturation (Kalaivanan, 2012).

2.3 Data Collection

The parameters of growth and yield measured were plant height, number of tillers, root wet weight, root dry weight, crown wet weight, crown dry weight, number of productive tillers, grain weight per clump, 100-grain weight, and leaf chlorophyll content.

2.4 Statistical Analysis

Data analysis used was analysis of variance (ANOVA) which then continued with Duncan's New Multiple Range Test (DNMRT) at 5% level. Data were analyzed following standard procedures using SPSS version 20.

III. RESULT AND DISCUSSION

The administration of all four species seaweed extract did not showed any significant effect on plant height, number of tillers, root dry weight, crown wet weight, crown dry weight, number of productive tillers, and 100-grain weight. In the other hand, significant effect was observed on roots wet weight and grain weight per clump.

Table 1. The Effect of Four Species Seaweed Extract on the Growth and Yield of Upland Rice

Seaweed species	Plant height (cm)	Number of tiller (stem)	Root wet weight (gr)	Root dry weight (gr)	Crown wet weight (gr)	Crown dry weight (gr)	Number of productive tillers (stem)	Grain weight/ clump (gr)	100-grain weight (gr)
<i>P.minor</i>	96,14 A	19,08 A	101,66 B	26,60 A	207,28 A	94,66 A	17,74 A	1,87 B	2,73 B
<i>S.crassifolium</i>	94,50 A	17,66 A	76,74 AB	22,66 A	182,77 A	74,65 A	14,91 A	1,85 B	2,71 A
<i>S.cristaefolium</i>	91,78 A	18,83 A	91,49 AB	22,09 A	188,08 A	84,51 A	16,24 A	1,60 AB	2,71 A
<i>T.decurrens</i>	91,75 A	18,25 A	72,22 A	18,69 A	187,52 A	77,91 A	15,41 A	1,44 A	2,71 A

Description : The numbers followed by the same letter in the same column shows results that are not significantly different at the 5% level of the DNMRT test.

From Table 1, it is known that the *Padina minor* extract showed a higher average value than the other 3 seaweed species. The high content of nitrogen and phosphorus nutrients in *P. minor* extract is known to be able to stimulate cell division and enlargement which able to support the increase in plant height. Nitrogen is an important constituent of chlorophyll, protoplasm, proteins, and nucleic acids, therefore it plays an important role in cell division and vegetative growth of plants. In addition, nitrogen is needed for all enzymatic reactions in plants and plays a direct role in the photosynthesis process ((Imran dan Gurmani, 2011 dan Rajasekar *et al.*, 2017). Phosphorus (P) plays a role in stimulating the formation of roots, flowers, fruits, seeds, and plant cell division as well as enlargement of cell tissue (Iskandar, 2003).

The content of secondary metabolites contained in the extract synergistically stimulates the process of cell division and elongation. In this process, zinc, iron, copper, manganese, and boron acts as cofactors to activate various enzymes in increasing cell metabolism and photosynthesis to stimulate the growth of upland rice. The research by Aisyah *et al.*, (2018), reported that the best interaction to increase the total height and wet weight of soybean was obtained by the addition of *P. minor* extract (0.4% concentration). Sriyuni (2020) reported that the provision of seaweed extract with the addition of amino acids as a biostimulant increased the growth of upland rice, namely the increase in height and root dry weight. According to Prasedya (2019), the administration of biostimulants from solid and liquid extracts of *Sargassum crassifolium* can increase plant height and number of rice tillers.

Table 2. The Effect of Solvent Type of Seaweed Extract on Growth of Upland Rice

Type of solvent	Plant height (cm)	Number of tiller (stem)	Root wet weight (gr)	Root dry weight (gr)	Crown wet weight (gr)	Crown dry weight (gr)	Number of productive tillers (stem)	Grain weight/ clump (gr)	100-grain weight (gr)
Control	79,79 A	17,16 A	123,47 B	29,99 B	202,84 B	94,02 B	15,33 A	1,57 A	2,70 A
Water	94,22 B	18,41 AB	78,51 A	16,82 A	195,04 B	85,16 B	15,75 A	1,49 A	2,72 B
Methanol	104,40 C	20,33 B	78,10 A	24,51 AB	214,36 B	89,41 B	18,74 B	2,29 B	2,72 B
Ethanol	95,98 B	17,91 AB	62,03 A	18,71 A	153,42 A	63,16 A	14,50 A	1,42 A	2,71 B

Description : The numbers followed by the same letter in the same column shows results that are not significantly different at the 5% level of the DNMRT test.

Methanol was the best type of solvent to achieve highest value of the average plant height, number of tillers, crown wet weight, number of productive tillers, grain weight per clump, and 100-grain weight. This results proved that the seaweed extract diluted with methanol is able to extract the phytochemical compounds better. Methanol has a polar group and a non-polar group, thus giving a tendency to attract polar and non-polar compounds (Astarina *et al.*, 2013). According to Supiyanti (2010), methanol can extract a large number of phytochemical compounds, in this case it is nitrogen (N). Methanol is able to extract higher N and P elements compared to other solvents. Methanol has the ability to attract flavonoid compounds, saponins, tannins and terpenoids in plants (Astarina *et al.*, 2013). Flavonoids are secondary metabolites that play a role in light absorption and hindering the auxin inhibitor, thus affecting the plant height (Kabera *et al.*, 2014).

Table 3. The Effect of Interaction Between Type of Seaweed Extracts and Type of Solvent on the Growth of Upland Rice

Interaction of seaweed type and solvent type	Plant height (cm)	Number of tiller (stem)	Root wet weight (gr)	Root dry weight (gr)	Crown wet weight (gr)	Crown dry weight (gr)	Number of productive tiller (stem)	Grain weight/clump (gr)	100-grain weight (gr)
<i>P.minor</i> control	81,16 ab	19,00 a	107,60 bcde	24,63 abc	227,51 abc	98,91 ab	17,00 abc	1,95 cde	2,72 abc
<i>P.minor</i> water	93,20 bcde	20,66 a	117,79 cde	20,58 abc	247,94 bc	97,38 ab	16,00 abc	2,02 de	2,75 d
<i>P.minor</i> methanol	108,86 f	21,66 a	100,64 abcde	35,28 c	202,48 abc	103,85 b	21,66 c	1,93 bcde	2,73 cd
<i>P.minor</i> ethanol	101,33 def	19,66 a	80,60 abcde	25,91 abc	151,21 a	78,51 ab	16,33 abc	1,57 abcde	2,72 abc
<i>S.crassifolium</i> control	82,03 abc	19,33 a	116,15 cde	35,01 c	161,28 abc	83,89 ab	13,00 ab	1,11 a	2,70 ab
<i>S.crassifolium</i> water	91,20 bcde	17,33 a	46,27 ab	15,31 abc	144,70 a	62,27 ab	15,00 ab	1,47 abcd	2,72 abc
<i>S.crassifolium</i> methanol	103,43 ef	20,00 a	81,16 abcde	23,35 abc	260,65 c	97,45 ab	17,66 abc	3,20 f	2,73 cd
<i>S.crassifolium</i> ethanol	101,33 def	18,00 a	63,38 abc	16,98 abc	164,46 ab	54,98 ab	14,00 ab	1,65 abcde	2,71 abc
<i>S.cristaeifolium</i> control	74,43 a	17,00 a	134,03 de	29,63 bc	216,85 abc	98,30 ab	17,33 abc	1,63 abcde	2,70 abc
<i>S.cristaeifolium</i> water	98,40 def	19,66 a	98,70 abcde	21,86 abc	188,84 abc	90,72 ab	13,33 ab	1,22 ab	2,73 bcd
<i>S.cristaeifolium</i> methanol	101,53 ef	22,33 a	72,21 abcd	19,66 abc	194,20 abc	83,17 ab	18,33 abc	2,25 c	2,72 abc
<i>S.cristaeifolium</i> ethanol	92,76 bcde	20,66 a	61,03 abc	17,21 abc	153,44 ab	65,85 ab	16,00 abc	1,30 abc	2,71 abc
<i>T.decurrens</i> control	81,53 abc	17,33 a	136,11 e	30,68 bc	205,74 abc	94,97 ab	14,00 ab	1,61 abcde	2,70 ab
<i>T.decurrens</i> water	94,10 cde	20,00 a	51,26 ab	9,54 a	198,68 abc	90,23 ab	18,66 bc	1,24 abc	2,70 a
<i>T.decurrens</i> methanol	103,76 ef	22,00 a	58,40 abc	19,77 abc	200,12 abc	73,15 ab	17,33 abc	1,78 abcde	2,72 abc
<i>T.decurrens</i> ethanol	88,50 bcd	17,66 a	43,11 a	14,76 ab	145,57 a	53,30 a	11,66 a	1,15 a	2,71 abc

Description : The numbers followed by the same letter in the same column shows results that are not significantly different at the 5% level of the DNMRT test.

In Table 3, the best interaction was showed by *Padina minor* dissolved in methanol. In the interaction factor, the relationship between the two treatments gave the best value for plant height, root dry weight, crown dry weight, and the number of productive tillers was obtained from the treatment using *P. minor* extract with methanol as a solvent. This could be due to the influence of the macro and micro elements contained in the extract which able to increase the metabolism of upland rice. The extract of *P. minor* contains N, P, and K elements. The functions N (Nitrogen) element includes stimulating vegetative growth, gives plants a dark green color, and synthesizing amino acids and proteins. While the P (Phosphorus) element plays a role in stimulating the formation of roots, flowers, fruit, seeds. and plant cell division as well as enlargement of cell tissue, thereby stimulating the formation of leaves and branches in plants (Sudarmi, 2013). K (Potassium) element is a macronutrient needed by plants and is absorbed in the form of K^+ ions. Potassium is abundant in the cytoplasm.

The result of this study is in accordance with the research conducted by Mardawati *et al.* (2008), where the extraction using methanol as solvent resulted in a higher yield of mangosteen peel extract compared to ethanol solvent in the same concentration. Likewise, in barley seed extract, the highest yield was obtained using methanol as a solvent compared to ethanol and acetone (Liu and Yao, 2007). Methanol also showed better increase of the plant growth and development compared to other alcohol compounds such as ethanol (Rowe *et al.*, 1994).

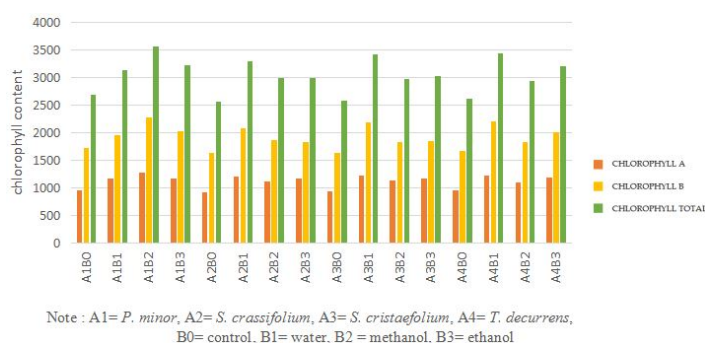


Figure: Graph of test results for total chlorophyll content at 7 days after administration of seaweed extract using water, methanol and ethanol as solvents.

The chlorophyll content of upland rice after the application of *S. crassifolium*, *S. cristaefolium*, *T. decurrens*, and *P. minor* extract using water, ethanol and methanol solvents showed different values. After the administration of *P. minor* extract using methanol solvent the highest chlorophyll a, b, and total chlorophyll content was obtained compared to the application of other seaweed extracts. The highest chlorophyll a content obtained was 1286.66 mg/g, highest chlorophyll b content was 2286.36 mg/g, and total chlorophyll obtained was 3571.76 mg/g.

The crop yields are also related to the vegetative growth of upland rice plants. From this study, it was known that the administration of *P. minor* biostimulant with methanol solvent resulted in better vegetative growth and increased the chlorophyll content of upland rice leaves. The chlorophyll content is related to plant physiological processes. With the increase of chlorophyll content it is assumed that the plant photosynthesis is also increases, thus resulted in higher photosynthate yields. These photosynthate will later be allocated to increase the grain weight per clump and the 100-grain weight.

Chlorophyll is a determining element of plant ability to carry out photosynthesis. The higher content of leaf chlorophyll means the higher photosynthesize ability of that plant (Aziez *et al.*, 2014). The availability of magnesium (Mg) and Nitrogen (N) elements in seaweed plays a role in the formation of chlorophyll, help the photosynthetic metabolic process, and stimulate the vegetative growth of plants. Chlorophyll is shaped like green granules and found in chloroplasts. Factors that affect the availability of chlorophyll are genetic factors, light, oxygen, temperature, water, carbohydrates, temperature, as well as macro (N and Mg) and micro (Fe, Mn, Cu, Zn) nutrients. Chlorophyll functions as an antenna, which collects light and transfers energy to the reaction center in the photosynthesis process. Chlorophyll a plays a direct role in the reaction of converting radiation energy into chemical energy, as well as absorbing and transporting energy to the molecular reaction center. Meanwhile, chlorophyll b functions as an absorber for radiation energy which is then forwarded to chlorophyll a (Atteya and Amer, 2018).

In the photosynthesis process, chlorophyll plays a role in absorbing and converting light energy into chemical energy. Chlorophyll can absorb and reflect light with different wavelengths, especially in blue (435 nm) and red light (660 nm). Chlorophyll b absorbs solar energy which then passed on to chlorophyll a to be used in the photosynthesis process stage 1 (light reaction). On the other hand, chlorophyll a directly converts sunlight energy into chemical energy and transports it directly to the molecular reaction center (Hopkins, 2006; NioSong and Banyo, 2011).

IV. CONCLUSION

This research showed that *Padina minor* was the best type of seaweed to increase the productivity of upland rice, particularly for root wet weight, grain weight per clump, and 100-grains weight. Methanol is the best type of solvent for seaweed extraction. The best interaction between the two factors is *Padina minor* dissolved in methanol.

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