Effect of liquid seaweed extracts as biostimulant on vegetative growth of soybean

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Abstract. Seaweeds are an important sustainable marine source and extracts from them have been applied as plant biostimulants. Seaweed-based extracts have been recently employed as sustainable tools to improve abiotic stress tolerance and increase growth and quality of plant. The research aimed to evaluate the effect of liquid seaweed extracts from some species of seaweeds with different concentrations on vegetative growth of soybean. Samples of seaweed were collected on Kasiak Gadang Island, Nirwana Beach, Padang, West Sumatra. Species of seaweed we tested were Padina minor, Sargassum crassifolium, Sargassum cristaefolium and Turbinaria decurrens and concentration of liquid extracts were control, 0.1%, 0.2%, 0.3%, and 0.4%. The result showed that P. minor liquid extract increasing some parameters including height, number of leaves, number of branches and fresh weight. While the dry weight of soybean was similar among the treatments in soybean. 0.4% concentration of seaweed extract significantly improves all parameters of vegetative growth of soybean.

1. Introduction
Soybean is an important crop commodity in Indonesia [1]. Soybean is one of the food sources of protein that favored by Indonesian society and mostly consumed in many products like tempeh, tofu, milk, etc [2]. The demand consumption of soybean is getting higher every year with an increasing population. However, domestic soybean production has been unable to qualify the demand of the community and causing dependence on imported soybean [3]. The import trend in 2016-2020 has a tendency to increase with an average percentage of 4.13% per year [4]. Therefore, better cultivation technology is needed to increase the productivity of soybean [3], one of the promising efforts to enhance production of soybean is the application of biostimulant [5].

Biostimulant is defined as organic materials and/or microorganisms that can enhance plant physiology processes such as respiration, photosynthesis, nucleic acid synthesis and ion absorption [6]. Biostimulant consists of several categories including microorganisms, protein hydrolyzates, humic acid, plant extract, and seaweed extracts [7,8]. Among the various alternatives, seaweed is one of the biostimulant group that has attracted the attention of many researchers because of its abundant availability. Nowadays, they are becoming more important in agriculture because of their positive effect on plant performance [9–11]. Seaweed extracts have been widely used as biostimulants due to presence of some growth regulators and macro micronutrients such as cytokinin, auxins, gibberellins, betaines, Ca, K, P, Fe, Cu, Zn, B, Mn, Co and Mo, which are necessary for improving plant growth and development [12]. They also contain chemical constituent that includes the complex polysaccharide, fatty acids and vitamins [13].
Many studies have revealed beneficial effects of seaweed extract on plants, such as improve seed germination, seedling development, vegetative growth, increasing crop productivity and quality of product [14,15]. Seaweed extract also enhances tolerate biotic and abiotic stress, pest and diseases on plants [13], increases the efficiency of water use and nutrient uptake [13,16]. In addition, seaweed extract as bio-stimulant is an effective and eco-friendly alternative to inorganic fertilizers [17]. Applications of seaweed extract have been reported to stimulate vegetative growth, productivity and quality of some crops including onion [17], cucumber [18], tomato [19], corn [11], common bean [20] and coffee [16].

Hadi et al. [21] reported that there are 4 species of seaweed in Kasiak Gadang Island, Nirwana Beach, West Sumatra including Padina minor, Sargassum crassifolium, Sargassum cristaefolium, and Turbinaria decurrens. However, screening of macro and micro nutrient of these seaweed and its potential to increase plant growth of soybean have not been studied. Thus, the present investigation was conducted to evaluate the effect of these liquid seaweed extracts with different concentrations on vegetative growth of soybean.

2. Methods

2.1 Experimental Design

The research was conducted from March until May 2018 in Limau Manis farmland, Padang and Plant Physiology Laboratory of Biology Department, Faculty of Mathematics and Natural Science, Andalas University, Padang. The study was arranged in Factorial Completely Randomized Design with 2 factors and 3 replications. The first factor was species of seaweed as bio-stimulant (Padina minor, Sargassum crassifolium, Sargassum cristaefolium, and Turbinaria decurrens). While the second factor was the concentration of seaweed extract (control, 0.1%, 0.2%, 0.3%, and 0.4%).

2.2 Preparation of Seaweed Extract

Seaweed was collected in Nirwana Beach, Padang, West Sumatera, Indonesia. Seaweed cleaned with seawater to remove sand and impurities and stored in polythene bag during transport. Upon arrival at the laboratory, samples were thoroughly washed with tap water, drained, cut into small pieces and then dried for four days and crushed into powder. 30 grams of seaweed powder were added to 300 ml of distilled water and left it for 2 days. The filtrate was centrifuged at 4250 rpm for 15 minutes and filtered through Whatman No. 1 filter paper. The supernatant was taken as 100% seaweed extract. Seaweed extracts were prepared in concentrations of 0.1%, 0.2%, 0.3% and 0.4% in each species of seaweed [22–24].

2.3 Application of Seaweed Extract

The application of seaweed extract was carried out through foliar spraying 15 days after planting (25 ml per plant). Spraying was carried out in the morning when relative humidity was close to saturation [25].

2.4 Data Collection and Statistical Analysis

Chemical content of seaweed extracts which includes macroelements (N, P, K), microelements (Na, Ca, Mg, S, Mn, Cl) and pH were analyzed. The analysis of variance (ANOVA) for various growth characters was performed following F test. When F was significant at the p < 0.05 level, treatments means were separated using Duncan’s New Multiple Range Test (DNMRT). Data were analyzed following standard procedure using SPSS software.

3. Results and Discussion

3.1 Analysis macro and microelements of seaweed extracts

The analysis macro and microelement of liquid seaweed extract is presented in Table 1. The nutrient analysis showed the presence of the macro and microelements N, P, K, Na, Ca, Mg, S, Mn and Cl in all seaweeds. Based on Table 1, P. minor especially rich in N, P, K, Ca, Mg, Mn and Cl than others. S. cristaefolium has a great amount of Na and S. On the other hand, the concentration macro and
micronutrient of *T. decurrens*, and *S. crassifolium* was similar. The pH values of all seaweeds were neutral.

3.2 Vegetative growth parameters

The analysis of seaweed liquid extract obtained from *P. minor*, *S. cristaefolium*, *T. decurrens*, and *S. crassifolium* on vegetative growth of soybean is presented in Table 2. Based on Table 2, the application of *P. minor* extract was the best treatment to increase plant height, number of leaves, number of branches and fresh weight of Soybean significantly among the other treatments. But, there was no significant effect of application of some species seaweed extract to dry weight of Soybean.

The effect of concentration seaweed extract treatments on the vegetative growth of Soybean is shown in Table 3. The application of 0.4% seaweed extract of all species significantly improved plant height, number of leaves, number of branches, fresh weight and dry weight of soybean compared with control and other concentration treatment.

Interaction of some species seaweed extract and concentration of extract is shown in Table 4. 0.4% *P. minor* extract had a beneficial effect on plant growth with higher values recorded in these plants compared to the other treatment. Interaction of some species seaweed extract and concentration of extract showed no significant influence on dry weight of Soybean.

**Table 1.** Chemical characterization of seaweed extract.

| Parameters | *S. crassifolium* | *P. minor* | *T. decurrens* | *S. cristaefolium* |
|-----------|------------------|------------|----------------|-------------------|
| N         | 0.868            | 1.459      | 0.856          | 0.937             |
| P         | 0.310            | 0.946      | 0.292          | 0.413             |
| K         | 0.548            | 0.588      | 0.539          | 0.537             |
| Na        | 0.627            | 0.661      | 0.649          | 0.667             |
| Ca        | 0.587            | 0.644      | 0.592          | 0.592             |
| Mg        | 0.432            | 0.489      | 0.472          | 0.482             |
| S         | 0.087            | 0.097      | 0.159          | 0.883             |
| Mn        | 0.159            | 0.176      | 0.159          | 0.156             |
| Cl        | 0.059            | 0.102      | 0.073          | 0.059             |
| pH        | 7.40             | 7.54       | 7.48           | 7.10              |

**Table 2.** Effects of foliar applications of some species of seaweed extract on Soybean growth parameters.

| Species of Seaweed | Plant Height (cm) | Number of Leaves | Number of branches | Fresh Weight (g) | Dry Weight (g) |
|--------------------|-------------------|------------------|--------------------|------------------|----------------|
| *S. crassifolium*   | 34.23<sup>D</sup> | 26.87<sup>C</sup>| 2.20<sup>C</sup>  | 28.30<sup>C</sup>| 8.44<sup>A</sup>|
| *P. minor*          | 37.53<sup>A</sup>| 31.80<sup>A</sup>| 3.40<sup>A</sup>  | 31.96<sup>A</sup>| 9.06<sup>A</sup>|
| *T. decurrens*      | 35.17<sup>C</sup>| 27.33<sup>BC</sup>| 2.47<sup>BC</sup> | 30.06<sup>B</sup>| 8.68<sup>A</sup>|
| *S. cristaefolium*  | 36.77<sup>B</sup>| 28.73<sup>B</sup>| 2.87<sup>AB</sup>| 30.25<sup>B</sup>| 9.15<sup>A</sup>|

Noted: Values with different letter are significantly different from each other according to DNMRT at *p* ≤ 0.05.
Table 3. Effect of some concentration of seaweed extract on Soybean growth parameters.

| Concentration (%) | Plant Height (cm) | Number of Leaves | Number of branches | Fresh Weight (g) | Dry Weight (g) |
|-------------------|-------------------|------------------|-------------------|------------------|---------------|
| Control           | 31.79D            | 21.42E           | 1.42D             | 22.44E           | 7.01C         |
| 0.1               | 32.88D            | 26.83D           | 1.92D             | 27.07D           | 7.75C         |
| 0.2               | 35.29C            | 29.17C           | 2.58C             | 30.08C           | 8.98B         |
| 0.3               | 38.42B            | 31.33B           | 3.42B             | 34.02B           | 9.58B         |
| 0.4               | 41.25A            | 34.67A           | 4.33A             | 37.09A           | 10.86A        |

Noted : Values with different letter are significantly different from each other according to DNMRT at $p \leq 0.05$

Table 4. Effects of concentration and species of seaweed extract on Soybean growth parameters.

| Treatments          | Plant Height (cm) | Number of Leaves | Number of branches | Fresh Weight (g) | Dry Weight (g) |
|---------------------|-------------------|------------------|-------------------|------------------|---------------|
| $S. crassifolium$ 0%| 30.67kl           | 21.33a           | 1.33a             | 21.69l           | 6.77a         |
| $S. crassifolium$ 0.1 % | 30.17l          | 25.33a           | 1.33a             | 29.24h           | 7.95a         |
| $S. crassifolium$ 0.2 % | 32.83h          | 25.67a           | 1.33a             | 29.68h           | 7.62a         |
| $S. crassifolium$ 0.3 % | 37.50f           | 30.33a           | 3.33a             | 29.03i           | 8.58a         |
| $S. crassifolium$ 0.4 % | 40.00ed          | 31.67a           | 3.67a             | 31.87e           | 11.29a        |
| $P. minor$ 0 %     | 33.00h            | 22.67a           | 1.33a             | 22.70jk          | 7.24a         |
| $P. minor$ 0.1 %   | 37.17fg           | 30.00a           | 2.67a             | 30.30fg          | 7.7a          |
| $P. minor$ 0.2 %   | 36.67fg           | 32.67a           | 3.33a             | 31.74ef          | 10.22a        |
| $P. minor$ 0.3 %   | 37.67f            | 33.00a           | 3.67a             | 35.34f           | 9.31a         |
| $P. minor$ 0.4 %   | 43.17a            | 40.67a           | 6.00a             | 39.69a           | 10.56a        |
| $T. decurrens$ 0 % | 31.17jk           | 21.33a           | 1.33a             | 23.29jk          | 7.07a         |
| $T. decurrens$ 0.1 % | 32.33hi          | 25.33a           | 1.33a             | 26.40j           | 7.00a         |
| $T. decurrens$ 0.2 % | 3.83ji           | 28.33a           | 2.67a             | 28.92i           | 8.07a         |
| $T. decurrens$ 0.3 % | 39.17e           | 30.00a           | 3.33a             | 32.43d           | 10.32a        |
| $T. decurrens$ 0.4 % | 41.33b           | 31.67a           | 3.67a             | 39.25b           | 10.94a        |
| $S. cristaefolium$ 0% | 32.33hi          | 20.33a           | 1.67a             | 22.07kl          | 6.59a         |
| $S. cristaefolium$ 0.1 % | 31.83ji          | 26.67a           | 2.33a             | 22.33jk          | 8.06a         |
| $S. cristaefolium$ 0.2 % | 39.83de          | 30.00a           | 3.00a             | 29.98g           | 9.99a         |
| $S. cristaefolium$ 0.3 % | 39.33de          | 32.00a           | 3.33a             | 39.29b           | 10.09a        |
| $S. cristaefolium$ 0.4 % | 40.50c           | 34.67a           | 4.00a             | 37.56b           | 10.65a        |
In the present study, we investigated the effect of some species of seaweed with different concentration of seaweed extract as biostimulant on vegetative growth of Soybean. Species of seaweed alone and a combination of species of seaweed and concentration of extract showed no significant influence on dry weight of Soybean, while notable significant effects was observed due to concentration of extract.

The results showed that the application of *P. minor* extract was the best treatment to increase significantly vegetative growth of Soybean such as plant height, number of leaves, number of branches and fresh weight (Table 2). The positive and significant response of Soybean to the application of *P. minor* extract could be due to the presence of macro and micro nutrient and various phytohormones that are present in *P. minor* extract. The extract of *P. minor* carries considerable amounts of nitrogen (Table 1), which would be utilized for the protein synthesis and eventually resulted in stimulated growth. Moreover nitrogen is an important component of amino acids and coenzymes, which have considerable biological importance [26,27].

Treatment with 0.4% seaweed extract resulted in the most increase in plant height, number of leaves, number of branches and fresh weight of all four species of seaweed (Table 3). According to Herrera et al. [14], the utilization of appropriate concentration of biostimulant can improved plant growth. The effect of biostimulant on plant growth is strongly dependent on the concentration of biostimulant [28]. In the present study, 0.4% extract of *P. minor* was the best combination to significantly enhanced plant height, number of leaves, and number of branches and fresh weight of Soybean (Table 4). Similar findings have been reported by Ramu and Nallamuthu [22] application of 0.4% *Padina tetrastromatica* extract enhanced vegetative growth of rice and mung bean [29]. Similar result reported by Kocira et al. [20] was 0.4% *Ecklonia maxima* extract significantly increased vegetative growth of *Phaseolus vulgaris* (common bean).

This paper provides information on screening and utilization of seaweed resources in Kasiak Gadang Island for technological development in agriculture. The high macro-micro nutrient in *P. minor* makes them the best choice in increasing vegetative growth of soybean. The utilization of *P.minor* extract as a biostimulant can reduce the use of chemical fertilizers and overcome the negative impact of chemical fertilizers on the environment such as environmental pollution and decreased soil organic matter. Therefore, the practice of applying eco-friendly seaweed extract can be recommended to help a sustainable agricultural system.

4. Conclusion

The present study demonstrated the potential benefit of applying different species of seaweed as bio stimulant to improve vegetative growth of Soybean This study shows that 0.4% extract of *P. minor* was most effective stimulating the vegetative growth of soybean, and therefore, they can be considered as a potential source of bio stimulant to enhancing the growth of plants in agriculture and horticulture.

5. References

[1] Aimon H and Satrianto A 2014 Prospek Konsumsi Dan Impor Kedelai Di Indonesia Tahun 2015 - 2020 J. Kaji. Ekon. 3 103411

[2] Riniarsi D 2018 *Outlook Kedelai Komoditas Pertanian Subsektor Tanaman Pangan*

[3] Roessali W, Ekowati T, Prasetyo E and Mukson 2017 *Supply Response of The Soybean in Indonesia* Proc. Int. Conf. Indonesian Regional Science Association

[4] Ningrum I H, Irianto H and Riptanti E W 2018 *Analysis of soybean production and import trends and its import factors in Indonesia* IOP Conf. Ser. Earth Environ. Sci. 142

[5] Szczepanek M, Wszelaczynska E and Poberezny J 2018 Effect of Seaweed Biostimulant Application in Sprng Wheat *AgroLife Sci. J.* 7 131–6

[6] Abbas S M 2013 The influence of biostimulants on the growth and on the biochemical
composition of vicia faba CV. Giza 3 beans *Rom. Biotechnol. Lett.* **18** 8061–8

[7] Pylak M, Oszust K and Frąc M 2019 Review report on the role of bioproducts, biopreparations, biostimulants and microbial inoculants in organic production of fruit *Rev. Environ. Sci. Biotechnol.* **18** 597–616

[8] Yakhin O I, Lubyanov A A, Yakhin I A and Brown P H 2017 Biostimulants in plant science: A global perspective *Front. Plant Sci.* **7**

[9] Mishra A, Sahni S, Kumar S and Prasad B D 2020 Seaweed - An Eco-friendly Alternative of Agrochemicals in Sustainable Agriculture *Curr. J. Appl. Sci. Technol.* **39** 71–8

[10] El Boukhari M E M, Barakate M, Bouhia Y and Lyamlouli K 2020 Trends in seaweed extract based biostimulants: Manufacturing process and beneficial effect on soil-plant systems *Plants* **9**

[11] Ertani A, Francioso O, Tinti A, Pizzeghello D and Nardi S 2018 Evaluation of seaweed extracts from laminaria and ascophyllum nodosum spp. As biostimulants in zea mays L. using a combination of chemical, biochemical and morphological approaches *Front. Plant Sci.* **9** 428

[12] Begum M, Bordoloi B C, Singha D D and Ojha N J 2018 Role of seaweed extract on growth, yield and quality of some agricultural crops: A review *Agric. Rev.* **39**

[13] Raj T S, Nishanthi P, Graff K H and Suji H A 2018 Seaweed Extract as A Biostimulant and a Pathogen Controlling Agent in *J. Trop. Agric.* **36**

[14] Hernández-Herrera R M, Santacruz-Ruvalcaba F, Briceño-Domínguez D R, Di Filippo-Herrera D A and Hernández-Carmona G 2018 Seaweed as potential plant growth stimulants for agriculture in Mexico *Hidrobiologica* **28** 129–40

[15] Mafakheri S and Asghari B 2018 Effect of seaweed extract, humic acid and chemical fertilizers on morphological, physiological and biochemical characteristics of Trigonella foenum-graecum L. *J. Agric. Sci. Technol.* **20** 1505–16

[16] Teixeira Fernandes A L, Oliveira Silva R, de Oliveira Bettini M and Broetto F 2019 Effect of Seaweed Extract Formulation on Coffee Plants at different irrigation levels *Asian Acad. Res. J. Multidiscip.* **6** 60–74

[17] Abbas M, Anwar J, Zafar-Ul-Hye M, Khan R I, Saleem M, Rahi A A, Danish S and Datta R 2020 Effect of seaweed extract on productivity and quality attributes of four onion cultivars *Horticulturae* **6**

[18] Valencia R T, Acosta L S, Hernández M F, Rangel P P, Robles M Á G, Cruz R del C A and Vázquez C V 2018 Effect of seaweed aqueous extracts and compost on vegetative growth, yield, and nutraceutical quality of cucumber (Cucumis sativus L.) fruit *Agronomy* **8** 1–25

[19] Yao Y, Wang X, Chen B, Zhang M and Ma J 2020 Seaweed Extract Improved Yields, Leaf Photosynthesis, Ripening Time, and Net Returns of Tomato (Solanum lycopersicum Mill.) *ACS Omega* **5** 4242–9

[20] Kocirc S, Kocirc A, Kornas R, Koszel M, Szmigielski M, Krajewksa M, Szparaga A and Krzysiak Z 2018 Effects of seaweed extract on yield and protein content of two common bean (Phaseolus vulgaris L.) cultivars *Legum. Res.* **41** 589–93

[21] Hadi F 2016 Diversity of Macroalgae in Kasiak Gadang Island Nirwana Beach , Padang - West Sumatra , Indonesia *J. Trop. Life Sci* **6**

[22] Ramu K and Nallamuthu T 2012 Effect of seaweed liquid fertilizers on the biostimulant on early seed germination and growth parameters of Oryza sativa L. *Int. J. Curr. Sci.* **3** 15–20

[23] Hernández-Herrera R M, Santacruz-Ruvalcaba F, Ruiz-López M A, Norrie J and Hernández-Carmona G 2014 Effect of liquid seaweed extracts on growth of tomato seedlings (Solanum lycopersicum L.) *J. Appl. Phycol.* **26** 619–28

[24] Godlewksa K, Michalak I, Tuh L and Chojnacka K 2016 Plant Growth Biostimulants Based on Different Methods of Seaweed Extraction with Water *Biomed Res. Int.* **2016**

[25] Kalaivanan C ; M C and V. and Venkatesalu 2012 Effect of seaweed liquid extract of Caulerpa scalpelliformis on growth and biochemical constituents of black gram ( Vigna mungo ( L. )
Rajasekar M, Associate R, Nandhini U and Balakrishnan K 2017 A review on role of macro nutrients on production and quality of vegetables Int. J. Chem. Stud. 5 304–9

Tripathi D K, Singh S, Singh S, Mishra S, Chauhan D K and Dubey N K 2015 Micronutrients and their diverse role in agricultural crops: advances and future prospective Acta Physiol. Plant. 37 1–14

Kocira S, Szparaga A, Kocira A, Czerwińska E, Wójtowicz A, Bronowicka-Mielniczuk U, Koszel M and Findura P 2018 Modeling biometric traits, yield and nutritional and antioxidant properties of seeds of three soybean cultivars through the application of biostimulant containing seaweed and amino acids Front. Plant Sci. 9

Kavipriya R, Dhanalakshmi P K, Jayashree S and Thangaraju N 2011 Seaweed extract as a biostimulant for legume crop, green gram J. Ecobiotechnology 3 16–9

Acknowledgment
This article is part of the master research grant from Ministry of Research, Technology and Higher Education of Indonesia. We thank to government for the funding support of the research project.