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Growth Hormones and Essential Elements in Extracts of Selected Brown Macroalgae from Lombok Stimulate Growth and Yield of Cucumber Plants

Sunarpi[1], Brigitta Amara Fanny Debra Geraldine[1], Sonia Ardilla Pebriani[2], Aluh Nikmatullah[3], Sri Widyastuti[4], Rina Kurnianingsih[2], Anggit Listyacahyani Sunarwidhi[5] and Eka Sunarwidhi Prasedya[1*]

[1]*Bioscience and Biotechnology Research Center, Faculty of Mathematics and Natural Sciences, University of Mataram, Jalan Majapahit 62, Mataram, West Nusa Tenggara 83125, Indonesia*

[2]*Faculty of Mathematics and Natural Sciences, University of Mataram, Jalan Majapahit 62, Mataram, West Nusa Tenggara 83125, Indonesia*

[3]*Faculty of Agriculture, University of Mataram, Jalan Pendidikan 37, Mataram, West Nusa Tenggara 83125, Indonesia*

[4]*Faculty of Food Technology and Agroindustry, University of Mataram, Jalan Majapahit 62, Mataram, West Nusa Tenggara 83125, Indonesia*

[5]*Department of Pharmacy, Faculty of Medicine, University of Mataram, Jalan Majapahit 62, Mataram, West Nusa Tenggara 83125, Indonesia*

The application of inorganic fertilisers causes many disadvantages, such as an increased production cost, decreased soil fertility and pollution to the environment. Therefore, exploring natural resources for the development of biostimulants and biofertilisers that are less expensive, easily available and eco-friendly is important. Brown macroalgae are well known as an important source of growth hormones and essential elements that could stimulate plant growth and increase the yield of food and horticultural crops. This article reports the presence of phytohormones and essential elements in the liquid and solid extracts of selected brown macroalgae (*Sargassum crassifolium*, *Sargassum cristaefolium* and *Turbinaria murrayana*) from Lombok, as well as the application of these macroalgal extracts in stimulating the growth and yield of cucumber plants. The application of 10% macroalgal liquid extracts as foliar spray once a week improved the growth and yield of the plants. Similar increase in plant growth and yield was also found with the application of 5% macroalgal solid extracts to the soil medium. The results suggested that solid and liquid extracts of *S. crassifolium*, *S. cristaefolium* and *T. murrayana* could be an important source for the development of biostimulants and biofertilisers, which are effective, easy to produce and safer for the environment.

Keywords: solid extract; liquid extract; growth; yield; cucumber

I. INTRODUCTION

The application of inorganic fertilisers in agricultural system causes many disadvantages, such as increased production cost, reduced soil fertility, and also pollution to the environment. Therefore, it is important to explore bioresources that are cheap, available in abundance, and eco-friendly as an alternative to the inorganic fertilisers. Many researchers reported that macroalgae are a potential source of plant growth hormones and essential elements that could be developed into biostimulants and organic fertilisers (Godlewska *et. al.*, 2016; Kiseleva *et. al.*, 2012; Tarakhovskaya *et. al.*, 2007; Zodape, 2001). The presence of these bioactive compounds and elements in macroalgae

*Corresponding author's e-mail: ekasprasedya@unram.ac.id

is part of their adaptive mechanism for survival in the face of stress in their habitat.

Previous studies have shown that the extracts of macroalgae can be used to improve the growth and yield of several crops. For example, Sivasankari *et al.* (2006) reported that liquid extracts of *Sargassum wightii* and *Caulerpa chemnitzia* increased the growth and biochemical constituents of *Vigna sinensis*. Liquid extract of *Sargassum myriocystum* was reported to stimulate the seedling growth of *Vigna mungo* (Kalaivanan & Venkatesalu, 2012). Hernández-Herrera *et al.* (2014) reported that liquid extracts of macroalgal species such as *Ulva lactuca*, *Caulerpa sertularioides*, *Padina gymnospora* and *Sargassum liebmanni*, were capable of stimulating seed germination and vegetative growth of seedlings in tomato. Liquid extracts of red marine algae like *Laurencia obtusa, Corallina elongata* and *Jania rubens* were also reported to enhance the growth of maize (Safinaz & Ragaa, 2013). Liquid extracts of *Gracilaria textorii* and *Hypnea musciformis* were shown to improve the germination and productivity of brinjal, tomato and chilli (Rao & Chatterjee, 2014). Liquid extract from green macroalga *Ulva lactuca* was also reported to improve the growth of sunflower (Chbani *et al.*, 2015). The enhanced growth of crops such as soybean (Kocira *et al.*, 2018) and *Ocimum sanctum* (Uthirapandi *et al.*, 2018) with the application of liquid organic fertiliser containing seaweed extracts have been documented.

A total of 88 macroalgal species was reported from the coastal area of West Nusa Tenggara (Sunarpi *et al.*, 2005). Seventeen of them were brown seaweeds which were known to have applications in stimulating germination, growth and production of crops (Sunarpi *et al.*, 2007; 2010). The potential for developing organic fertilisers from these seaweed species in the region was explored (Sunarpi, 2007). Some of the brown macroalgal species from Lombok had been reported to contain cytokinin, a plant growth hormone that induces cell division and elongation (Nikmatullah *et al.*, 2014). Therefore, it follows that the application of liquid extracts of brown macroalgae as foliar spray could enhance the growth and yield of rice plants (Sunarpi *et al.*, 2019).

This study analysed the plant growth hormones and essential elements contained in the solid and liquid extracts of selected brown macroalgae (*Sargassum crassifolium*, *Sargassum cristaefolium* and *Turbinaria murrayana*) collected from Lombok. The application of these macroalgal extracts in stimulating the growth and production of cucumber plants (*Cucumis sativus* L.) was also evaluated to provide baseline data for the prospective development of macroalgae-based biostimulants and organic fertilisers from the local resources in Lombok.

II. MATERIALS AND METHODS

A. Sample Collection and Extraction

Macroalgal samples of *S. crassifolium* and *S. cristaefolium* were collected from the coastal area of Batu Layar, West Lombok, while *T. murrayana* was sampled from Lendang Luar, North Lombok. The liquid and solid extracts of seaweeds were prepared according to the modified procedures in Godlewska *et al.* (2016) at the Biosciences and Biotechnology Research Center, Faculty of Mathematics and Natural Sciences, University of Mataram.

The macroalgal samples were first rinsed with seawater and air-dried in the shade for 3 d. The air-dried samples were then cut into small pieces with a pair of scissors, ground into fine powder using a blender, and 250 mg of the seaweed powder of each species was separately placed in a 3 L flask. The seaweed powder was suspended in 1.25 L of distilled water, and the suspension was homogenised using a magnetic stirrer for 30 min prior to heating in a 95°C water bath for 30 min. The suspension was then centrifuged at 4,500 rpm for 5 min and filtered using a Whatman no. 1 filter paper. The supernatant was taken as the 100% stock macroalgal liquid extract, while the pellet or seaweed residue represented the solid extract of macroalga. These extracts were applied as foliar spray (10% liquid extract) and fertiliser (5% or 100 g of the solid extract for every 2 kg of organic fertiliser) to evaluate their effectiveness in improving the growth and yield of cucumber plants.

B. Experimental Design and Cultivation of Cucumber Plants

The experiment was carried out with a completely randomised design in a plastic growth house in Jatisela,

West Lombok, from July to October 2018. The experiment consisted of a control and six treatments with separate application of the liquid and solid extracts of three brown macroalgal species (*S. crassifolium*, *S. cristaefolium* and *T. murrayana*). For the control and treatment groups with 10% liquid extracts of each brown seaweed species applied as foliar spray, the potted cucumber plants were grown in a medium consisted of 6 kg of soil and 2 kg of organic fertiliser (manure). For treatments with solid extracts of each seaweed species applied as 5% organic fertiliser, the planting medium consisted of 6 kg of soil, 1.9 kg of manure, and 0.1 kg of seaweed residue.

The cucumber seeds were soaked in water for 1 h, and placed on a paper towel for germination overnight. Germinated seeds were sowed in a medium containing a mixture of soil and sand at a 3:1 ratio. After 14 d, cucumber seedlings were transplanted to 14 L plastic pots containing the planting medium composed of soil and organic fertiliser as mentioned above for each treatment. Each replicate consisted of two seedlings grown in a pot, and each treatment was performed in three replicates. Each replicate for treatments with seaweed liquid extracts was given 15 mL of 10% seaweed extract as foliar spray once a week during the vegetative growth of the cucumber seedlings for 3 times starting from day 21 after planting. At the same time, the control plants were sprayed with the same amount of water.

The growth parameters, including plant height, leaf number, shoot dry weight and root dry weight, fruit number and fruit weight per plant, as well as the nutrient status (nitrogen, phosphorus and potassium content) in leaf tissue, were determined after harvest at 40 d after planting.

C. Analysis of Phytohormone Content in Liquid Macroalgal Extracts Using HPLC

The identity and concentration of plant growth hormones in the liquid extracts of *S. crassifolium*, *S. cristaefolium* and *T. murrayana* was determined using the high performance liquid chromatography (HPLC). Standards for several plant growth hormones, such as indole acetic acid (IAA), naphthalene acetic acid (NAA), gibberellic acid (GA_3), kinetin, abscisic acid and 2,4 dichlorophenoxyacetic acid (2,4-D) were purchased from SIGMA-Aldrich. A Shim-pack CLC-ODS column (Shimadzu, Japan) was used to separate the components in the samples of seaweed liquid extracts. Each sample was appropriately diluted before automatically injected into the HPLC column and separated at a column temperature of 30°C, pressure of 50 kg/cm^2, continuous flow rate of 0.5 mL/min, using methanol/distilled water (7:1 v/v) as the mobile phase. Analysis was conducted by comparing the chromatogram of each sample with those of the plant growth hormone standards.

D. Elemental Analysis for Solid Macroalgal Extracts and Leaf Tissue Using ICP-OES

The essential elements were measured using the Agilent 725 ICP-OES (inductively coupled plasma optical emission spectrometry) system, according to the modified procedure in Godlewska *et al.* (2016). The samples (0.5 g of solid extract or leaf tissue) were first digested with 5 mL of nitric acid at 320°C using Maspion electric stove. Digestion was considered completed once the solution reached its maximum opacity. The samples were then diluted ten times with ultra-filtered water (Milli-Q Integral system, Millipore Sigma, US) and the concentration of essential elements in the diluted samples was determined using ICP-OES. All samples were analysed in duplicate.

E. Data Analysis

The analysis of variance was used to determine whether the nutrient content of leaf tissue, growth and yield of cucumber plants were significantly different after treatment with seaweed extracts. The honestly significant difference (HSD) test was conducted to make pairwise comparisons between the treatment groups and control, and to determine the error rate between the experimental groups at 5% significance level. All statistical analyses were performed using the SPSS software. The values presented in the graphs and tables were means of three replicates ± standard error.

III. RESULTS AND DISCUSSION

A. Phytohormone Content in Liquid Macroalgal Extracts

The content of plant growth hormones found in the liquid extracts of brown macroalgae, *S. crassifolium*, *S.*

cristaefolium and *T. murrayana*, varied among species (Table 1). The liquid extracts of *S. crassifolium* contained GA_3, kinetin and NAA at a concentration of 0.04, 0.01 and 0.01 mg/mL, while that of *T. murrayana* contained GA_3, kinetin and abscisic acid at a concentration of 0.08, 0.01 and 0.01 mg/mL, respectively. On the other hand, the liquid extract of *S. cristaefolium* contained a significant amount of IAA at 0.19 mg/mL.

B. Essential Element Content in Solid Macroalgal Extracts

The concentrations of six essential elements (N, P, K, Ca, Fe and Mn) in solid extracts of *S. crassifolium*, *S. cristaefolium* and *T. murrayana* were shown in Table 2. Except for calcium, the concentrations of all macroelements analysed in the solid extracts of the *Sargassum* species were higher than those of *T. murrayana*. The N content in solid extracts of *S. crassifolium* and *S. cristaefolium* were higher than that of *T. murrayana* by 50% and 68%. Similarly, the P content in solid extracts of *S. crassifolium* and *S. cristaefolium* were 40% and 64% higher than that of *T. murrayana*. The K content in solid extracts of *S. crassifolium* and *S. cristaefolium* were also higher than that of *T. murrayana*, by 17% and 23%. On the other hand, the concentration of calcium in solid extract of *T. murrayana* at 0.60% was higher than that of *S. crassifolium* at 0.49%, but the concentration was still lower compared to that of *S. cristaefolium* at 0.70%. The Fe content in the solid extracts of *S. crassifolium* and *S. cristaefolium* were 36.72% and 41.18%, respectively, compared with 21.58% in that of *T. murrayana*. The solid extracts of *S. crassifolium* and *S. cristaefolium* contained 3.36% and 6.38% Mn, compared with 2.44% Mn in the solid residue of *T. murrayana*.

C. Effect of Macroalgal Extracts on the Nutrient Content in Leaf Tissue

The effect of the liquid and solid extracts of *S. crassifolium*, *S. cristaefolium* and *T. murrayana* on the N, P and K content in leaf tissue of cucumber plants was shown in Table 3. In general, the application of liquid extracts of all brown algal species as foliar spray significantly increased the content of N, P and K in leaf tissue, but the effect was not significantly different among liquid extracts of different species (Table 3). Similarly, the application of solid extracts of all brown algal species to the soil medium also significantly increased the N and K content of leaf tissue (Table 3). In contrast, the application of *T. murrayana* solid extract to the soil medium resulted in a significant increase in the P content of leaf tissue, but not the treatment with seaweed residues of *S. crassifolium* and *S. cristaefolium*.

Leaf nutrient content informs the status of soil nutrients used to support plant growth. We consider the improved nutrient content in the leaf tissue of cucumber plants following the application of seaweed extracts an indication of enhanced plant growth associated with increased

Table 1. Composition of plant growth hormones in liquid extracts of *Sargassum crassifolium*, *Sargassum cristaefolium* and *Turbinaria murrayana*.

Seaweed species	**Concentration of plant growth hormone in seaweed liquid extract (mg/mL)**				
	GA_3	**Kinetin**	**Abscisic acid**	**NAA**	**IAA**
Sargassum crassifolium	0.04	0.01	-	0.01	-
Sargassum cristaefolium	-	-	-	-	0.19
Turbinaria murrayana	0.08	0.01	0.01	-	-

Values are expressed as average of two replicates.

Table 2. Concentration of essential elements in solid extracts of *Sargassum crassifolium*, *Sargassum cristaefolium* and *Turbinaria murrayana*.

Seaweed species	Essential element content in seaweed solid extract (% dry weight)					
	N	P	K	Ca	Fe	Mn
Sargassum crassifolium	0.33 ± 0.13	0.04 ± 0.01	4.51 ± 1.70	0.49 ± 0.19	36.72 ± 13.87	5.36 ± 2.02
Sargassum cristaefolium	0.37 ± 0.14	0.04 ± 0.02	4.72 ± 1.75	0.70 ± 0.26	41.18 ± 15.30	6.38 ± 2.37
Turbinaria murrayana	0.22 ± 0.09	0.03 ± 0.01	3.84 ± 1.44	0.60 ±0.23	21.58 ± 4.52	2.44 ± 0.91

Values are expressed as average of three replicates ± standard error.

Table 3. NPK content in leaf tissue of cucumber plants treated with liquid and solid extracts of *Sargassum crassifolium, Sargassum cristaefolium* and *Turbinaria murrayana*.

Treatment	Nutrient content (% dry weight)		
	N	P	K
Control	0.72 ± 0.10 a	0.12± 0.00 a	2.02 ± 1.00 a
Seaweed liquid extract			
Sargassum crassifolium	1.43 ± 0.20 b	0.51 ± 0.10 b	6.01 ± 2.00 bc
Sargassum cristaefolium	1.61 ± 0.20 b	0.43 ± 0.10 b	6.02 ± 2.00 bc
Turbinaria murrayana	1.64 ± 0.10 b	0.43 ± 0.10 b	7.04 ± 2.00 c
Seaweed solid extract			
Sargassum crassifolium	1.44 ± 0.20 b	0.23 ± 0.10 a	4.01 ± 1.00 ab
Sargassum cristaefolium	1.42 ± 0.20 b	0.32 ± 0.20 ab	4.04 ± 1.00 ab
Turbinaria murrayana	1.23 ± 0.20 b	2.02 ± 0.10 c	4.03 ± 2.00 ab

Mean values in each column followed by different letters were significantly different according to the HSD test ($p < 0.05$).

nutrient uptake and assimilation. Plant growth hormones present in the seaweed liquid extracts (Table 1) might have stimulated an increased uptake of soil nutrients which were assimilated for growth, with the growth reflected as increased nutrient content in the leaves of cucumber plants. The application of solid extracts of seaweeds containing essential elements (Table 2) probably has improved the availability of essential elements in the soil, and thus nutrient uptake by the roots for metabolism and growth of cucumber plants which was indicated by the increased leaf nutrient content.

C. Effect of Macroalgal Extracts on Growth and Yield of Cucumber Plants

Growth parameters measured in this experiment were plant height, leaf number, shoot dry weight and root dry weight, while the yield was expressed as generative growth parameters including the number and weight of fruits per plant.

The effect of liquid and solid extracts of brown macroalgae on the height of cucumber plants was shown in Figure 1. The results showed that the application of liquid extracts of all brown macroalgal species significantly increased the cucumber plant height, probably due to the plant growth hormones like GA_3, kinetin, NAA and IAA present in the extracts (Table 1). It seemed that the effect of plant growth hormones on plant height was dependent on the concentration of the hormones in seaweed liquid extracts. The highest plant height was recorded in treatment with liquid extract of *S. cristaefolium* which contained IAA at a rather high concentration. In contrast to the liquid extracts, the application of solid extracts of brown macroalgae did not significantly increase the height of cucumber plants (Figure 1). This indicates that the essential elements contained in the seaweed solid extracts were not enough to support the division of meristematic cells to result in significant growth in plant height.

Both liquid and solid extracts of the tested brown macroalgal species significantly increased the leaf number of cucumber plants compared to the control (Figure 2). In line with that, the shoot dry weight also increased significantly with the application of liquid and solid extracts of seaweed (Figure 3). In contrast, plants treated

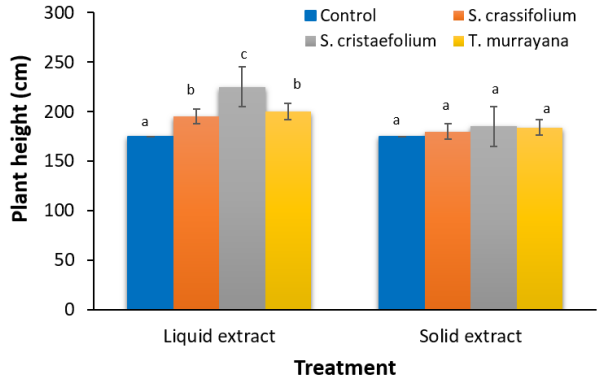

Figure 1. Effect of liquid and solid extracts of *Sargassum crassifolium*, *Sargassum cristaefolium* and *Turbinaria murrayana* on the cucumber plant height. Mean values followed by different letters were significantly different according to the HSD test ($p < 0.05$).

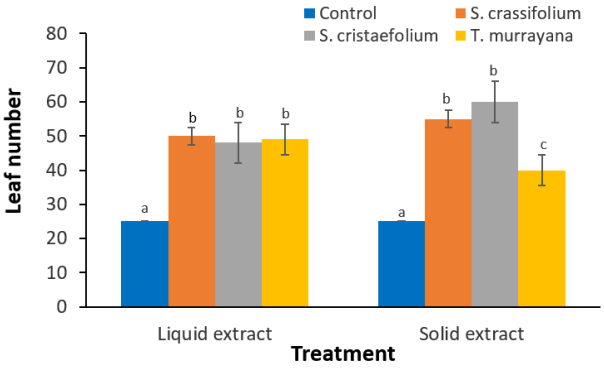

Figure 2. Effect of liquid and solid extracts of *Sargassum crassifolium*, *Sargassum cristaefolium* and *Turbinaria murrayana* on the leaf number of cucumber plants. Mean values followed by different letters were significantly different according to the HSD test ($p < 0.05$).

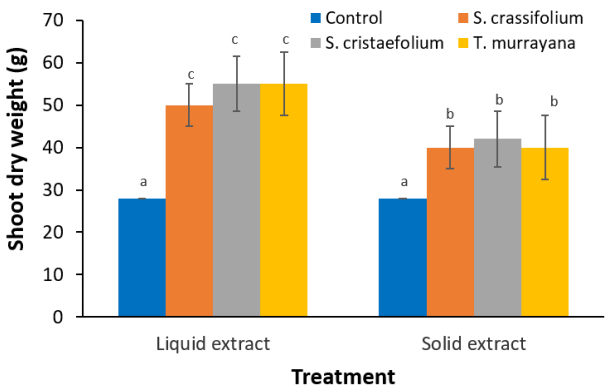

Figure 3. Effect of liquid and solid extracts of *Sargassum crassifolium*, *Sargassum cristaefolium* and *Turbinaria murrayana* on the shoot dry weight of cucumber plants. Mean values followed by different letters were significantly different according to the HSD test ($p < 0.05$).

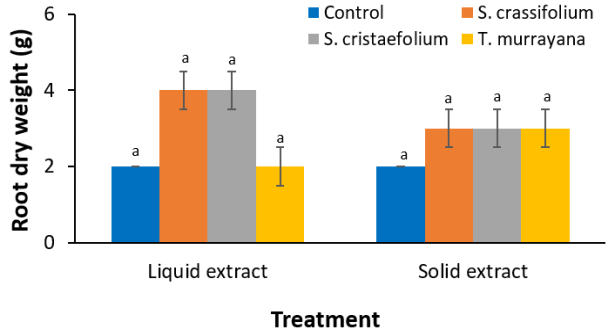

Figure 4. Effect of liquid and solid extracts of *Sargassum crassifolium*, *Sargassum cristaefolium* and *Turbinaria murrayana* on the root dry weight of cucumber plants. Mean values followed by different letters were significantly different according to the HSD test ($p < 0.05$).

with seaweed extracts showed a higher root dry weight compared with the control, but the effect of both liquid and solid extracts of brown macroalgae on the root dry weight of cucumber plants was not significant (Figure 4). Although liquid extracts of seaweeds were reported to contain zeatin that induced rooting in plants (Finnie & van Staden, 1985), our study found that root dry weight of the cucumber plants was not significantly improved following the application of seaweed extracts.

The shoot to root ratio of cucumber plants treated with seaweed extracts was more than 1 for all treatments, indicating that the plants used in this experiment were healthy, in which the elements taken up were used with priority in supporting the growth of the shoot system. It

explains why the application of liquid and solid extracts of seaweeds only significantly improved the growth of shoot system, but not the root system. This argument had been documented in many studies (Salisbury & Ross, 1991; Taiz & Zeiger, 1998; Buchanan *et al.*, 2000).

The effects of liquid and solid extracts on the number and weight of cucumbers per plant were shown in Figures 5 and 6. The application of liquid extracts of all brown macroalgal species as foliar spray significantly increased the number of cucumbers produced (Figure 5). Similarly, the application of solid extracts of the tested brown macroalgal species also significantly increased the fruit number in cucumber plants compared to the control (Figure 5). In line with the significant increase in the number of fruits produced with the application of seaweed extracts, the weight of fruits per plant also increased significantly when the plants were sprayed with seaweed liquid extracts or grown in the soil medium added with seaweed solid extracts (Figure 6).

Application of seaweed liquid extracts containing plant growth hormones (Table 1) as foliar spray during the vegetative growth of cucumber plants might have stimulated nutrient uptake required for cellular metabolism, which in turn significantly improved plant growth in terms of plant height, leaf number and shoot growth (Figures 1–3), and the yield of cucumber (Figures 5 & 6). Solid extracts of seaweeds contained several essential elements (Table 2) and their application as organic ffertiliser might have increased the availability of essential elements in the soil and improved the uptake of essential elements from the soil, thereby stimulating the development of leaves (Figures 2 & 3) and thus increase in the yield of cucumber (Figures 5 & 6). A higher leaf number which may translate to higher leaf area would allow greater light interception to support a higher photosynthesis rate and produce more metabolites required for better plant growth and development.

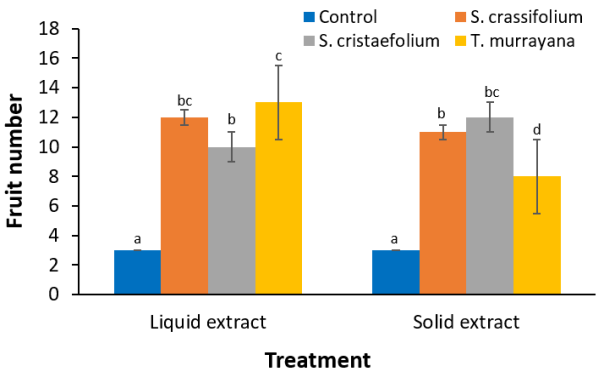

Figure 5. Effect of liquid and solid extracts of *Sargassum crassifolium*, *Sargassum cristaefolium* and *Turbinaria murrayana* on the number of fruit per cucumber plant. Mean values followed by different letters were significantly different according to the HSD test ($p < 0.05$).

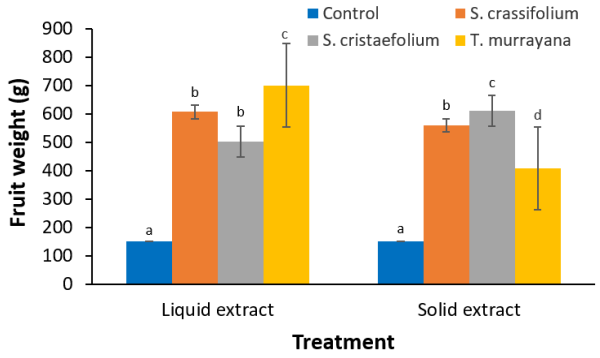

Figure 6. Effect of liquid and solid extracts of *Sargassum crassifolium*, *Sargassum cristaefolium* and *Turbinaria murrayana* on the weight of fruit per cucumber plant. Mean values followed by different letters were significantly different according to the HSD test ($p < 0.05$).

Various studies indicated that seaweed extracts exhibit plant growth-stimulating capacity because they contained essential elements and compounds like amino acids, vitamins, cytokinins, auxins and abscisic acid that could affect cellular metabolism in a manner which leads to enhanced growth and crop yields. Our findings also confirmed that the growth-stimulating feature of brown macroalgal extracts was partially attributed to the plant growth hormones as well as essential elements present in the seaweed extracts. Stephenson (1974) suggested that seaweed biostimulants contain the precursors of elicitor compounds that could increase plant germination rates. The plant growth and yield promoting effect of seaweed extracts could also be attributed to the presence of

polysaccharides as sugars that are known to improve plant growth in a way similar to hormones (Rolland *et al.*, 2002). Furthermore, brown macroalgal extracts also contain several kinds of betaines and betaine-like compounds (Blunden *et. al.*, 1986; Ghoul *et al.*, 1995). Betaines might be used as a nitrogen source when provided in low concentration and serve as osmolyte, a compatible solute that alleviates osmotic stress induced by salinity and drought stress, at higher concentration in plants (Naidu *et al.*, 1987). Besides that, betaines are also known to play a part in successful formation of somatic embryos from cotyledonary tissues and mature seeds of tea plant (Akula *et al.*, 2000).

Therefore, liquid and solid extracts of brown seaweeds have the potential to be developed into biostimulants and organic fertilisers as they contain bioactive compounds and nutrients that can improve plant growth and thus crop production. Similar improvement in the plant growth and yield following the application of seaweed extracts or seaweed-based fertilisers had been reported in various crops, such as *Vigna sinensis* (Sivasankari *et al.*, 2006), tomato (Hernández-Herrera *et al.*, 2014), maize (Safinaz & Ragaa, 2013), brinjal, tomato, chilli (Rao & Chatterjee, 2014), *Lepidium sativum* (Godlewska *et al.*, 2016) and soybean (Kocira *et al.*, 2018).

IV. CONCLUSION

Liquid extracts of *S. crassifolium*, *S. cristaefolium* and *T. murrayana* from Lombok contained several plant growth hormones including GA_3, kinetin, NAA and IAA, all of which might have a role in stimulating the mineral uptake, and thus improved the growth and yield of cucumber plants. The application of the solid extracts of the selected brown macroalgae which contained essential elements to the planting medium was also shown to improve the growth and yield of cucumber plants. Based on our observation, the usage of brown macroalgal extracts on cucumber plants as foliar spray and biofertiliser at a low concentration resulted in neither harmful effect to the plants nor to the medium (environment) in this study. These findings suggested that the liquid and solid extracts of those selected brown macroalgae from Lombok could be developed into biostimulant and biofertiliser in an effort to promote sustainable agricultural practice on the island.

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