

Evidence for the presence of growth-promoting factors in Lombok Turbinaria murayana extract stimulating growth and yield of tomato plants (*Lycopersicum esculentum* Mill.)

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Evidence for the presence of growth-promoting factors in Lombok *Turbinaria murayana* extract stimulating growth and yield of tomato plants (*Lycopersicum esculentum* Mill.)

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ABSTRACT

Many studies have evidenced the negative effects of synthetic fertilizers toward the environment and also the crop products itself. Hence, the improvement of organic fertilizers is necessary to resolve this problem. Seaweeds or marine macroalgae are considered to be an excellent natural resource in different aspects of agricultural fields. Brown macroalgae *Turbinaria murayana* (TM) is often found in Indonesian coastal areas with high bioavailability throughout the year. This study evaluated the potentials of TM as biofertilizer in tomato plants (*Lycopersicum esculentum* Mill.). Evaluation of phytohormones present in TM crude extract was conducted with high performance liquid chromatography (HPLC). Macro and micronutrients available in TM crude extract were determined by atomic absorbance spectroscopy (AAS). A concentration of 4% TM crude extract was applied to tomato plants. The vegetative properties of the treated tomatoes were observed such as growth and yield. Current results evidenced the presence of growth-promoting factors (kinetin, gibberellin and ABA) and essential elements (N, P, K, Ca, Fe and Mn) in TM crude extract. Application of 4% TM extract to tomato plants significantly increased vegetative growth compared to untreated plants; plant height, leaf number and branch number, approximately 58.4%, 87.5% and 200%, respectively. Similar results were also found in the response of generative growth. Application of 4% liquid extract significantly improved flower and fruit number compared to control; 53.6% and 125%, respectively. These results suggest that TM crude extract is a potential source for developing organic fertilizers.

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Introduction

The marine environment is well known to produce various natural resources which remain largely unexplored (Blunt et al. 2016). Seaweeds or marine macroalgae are among the important marine living resources with broad aspects of biological properties (Stengel and Connan 2015). Indonesia is well known for its biodiversity of marine resources such as macroalgae. However, most remain largely unexplored (Unsworth et al. 2018; Williams et al. 2017).

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Chemical fertilizers have been proven to induce negative effects toward the environment. Increased nitrate leaching in the environment is among the results of excessive usage of inorganic fertilizers in agriculture and horticulture systems (Corte et al. 2014; Mardani-Talaei et al. 2017; Negahban-Azar and Sharville 2018; Yao et al. 2016). Nitrate leaching influences the quality of groundwater and crop (Wang et al. 2017). Furthermore, consuming crops with excessive nitrate could harm human health, due to the transformation of nitrate (NO_3) into nitrite (NO_2) (Hakeem et al. 2017). This chemical reaction could occur when the edible crops are heated in boiled water (Yao et al. 2016). Hence, the usage of organic fertilizers with high macro and micro-nutrient contents would be more preferable compared to inorganic fertilizers (Zhu et al. 2015).

Macroalgae is well known to contain a broad range of bioactive compounds which could promote plant growth. Seaweed extracts enhanced germination of seeds, increased uptake of plant nutrients, affected ripening of fruits and increasing shelf-life of horticultural products (Blunden, Challen, and Woods 1968; Ghahriardakani et al. 2019; Kurepin, Zaman, and Pharis 2014). Previous research also reported *Sargassum wightii* and *Caulerpa chemnitzia* extracts contained growth hormones, bioactive metabolites and trace elements stimulating the growth of *Vigna sinensis* (Sivasankari et al. 2006). Another species of algae, such as *Ulva lactuca*, *Caulerpa sertularioides*, *Padina gymnospora* and *Sargassum liebmannii* extracts, also contains bio-stimulants which induced germination and growth of tomato plants. Red marine algae extracts sprayed to maize plants, increased phosphorus uptake and growth of the plant (Safinaz and Ragaa 2013). It was also reported that liquid extract from *Gracilaria textorii* and *Hypnea musciformis* stimulates germination and productivity of vegetable crops (Rao and Chatterjee 2014). Green macroalgae *U. lactuca* extracts stimulated chlorophyll and proline biosynthesis, which ultimately increased growth and stress tolerance of sunflower (Ghani et al. 2015).

However, information concerning the effect of Indonesian seaweed extracts on growth and yield of agricultural and horticultural plants remains limited. This article reported the evidence for the presence of growth-promoting factors (kinetin, gibberellin and ABA) and essential elements (N, P, K, Ca, Fe and Mn) in Lombok *Turbinaria murayana* (TM) extract that stimulates growth and yield of tomato plants. During vegetative growth, the application of 4% extract to tomato plants significantly increased plant height, leaf number and branch number compared with those of control plants. Similar results were also found in the response of generative growth. Application of 4% liquid extract significantly improved flower and fruit numbers compared to plants untreated with seaweed fertilizer. Further study regarding the bioactivity of brown seaweed TM in promoting plant growth would be reasonable for its development as a potential organic fertilizer.

Materials and methods

Experimental design

The experiment was designed as completely randomized design in a plastic greenhouse, Jatisela West Lombok Indonesia. The experiment was repeated two times, January–April 2018 (Experiment I), and in June–September 2018 (Experiment II). Each experiment consisted of two treatments: control, non-treated plants; and TM, treated plants with 4% TM extract. Each treatment was replicated three times, therefore, there were six experimental pots for each experiment (Experiment I and Experiment II).

Seaweed extraction

Seaweed species, Lombok TM, was collected in Batu Layar coastal beach area, West Lombok district, West Nusa Tenggara Province, Indonesia. The samples were then cleaned from sand and

other debris⁴. Then, samples were rinsed using distilled water. Samples were then subjected to extraction according to the modified procedure by Godlewska et al. (2016). Seaweed samples (500 g dry weight) were cut into small pieces⁴ and ground with mortar. Powdered seaweed samples were soaked in 500 mL of distilled water for 30 min. The mixture was centrifuged in 4200 rpm for 15 min. Obtained supernatant was filtered using Whatman no. 1 filter paper. Filtrate was considered as 100% liquid seaweed extract.

Analysis of growth hormones using high performance liquid chromatography (HPLC)

Growth hormones present in the liquid extract⁴ of TM were determined using HPLC (Breeze QS-Waters, MA USA). Standard solution of growth hormones, such as indole acetic acid (IAA), 6-naphthalene acetic acid (NAA), gibberellic acid (GA3), zeatin (ZA), kinetin, abscisic acid (ABA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were obtained from Sigma-Aldrich. HPLC (Breeze QS-Waters, MA, USA) analyses were carried out using a Shimpack CLC-ODS column (Shimadzu, Japan). Each sample was injected automatically after appropriate dilution onto the HPLC column and separated at a column temperature⁴ of 30 °C, pressure of 50 kg/cm², a continuous retention speed of 0.5 mL per minute and using methanol/distilled water (7:1, v/v) as the mobile phase. Analysis was conducted by comparing the chromatogram of each sample with the standards.

Analysis of essential elements using inductively coupled plasma optical emission spectroscopy (ICP-OES)

Measurement of essential elements in seaweed biomass³ was also conducted according to the modified procedure⁴ of Godlewska et al. (2016). Seaweed samples (0.5 g dry weight) were treated from 5 mL of nitric acid in hot plate at 400 °C. The samples were then diluted 10 times with ultra-filtered water (Milli-Q Integral, Millipore Sigma). All samples were analyzed in two replicates. Finally, the content of essential elements was measured using ICP-OES (Agilent Technologies, USA).

Preparation of plants and application of extract

Seeds of tomato plants (100 seeds⁴) were imbibed with 100 mL of water in 150 mL beaker glass. Then, after water was dried, the seeds were sowed in a medium consisting of soil and sand with composition 3:1. The type of soil used was regosol with sandy texture. It was collected from top soil in the area of vegetable cultivation in Central Lombok, Indonesia. Nutrient content of the soil, such as N, P and K, were 0.14, 0.16, and 0.02%. After 14 days, the seedlings were planted in medium consisted of soil and organic fertilizer with composition 3:1. Organic fertilizer was produced by a local company, "Tegeng Karya". The fertilizer was composed of chicken manure, rice husk and sawdust, with nutrient composition of 16.7% water, 0.78% N, 15.9% C organic, 20.15% C/N ratio, 0.60% P₂O₅ and 1.55% K₂O. Finally, 4% liquid extract was sprayed once a week during vegetative growth, which occurs 4 weeks after planting, four times. During vegetative growth, the parameters like plant height, leaf number and branch number, were measured every week. The number of flowers was also measured during generative growth. The number of fruits was measured during harvesting time. The total number and weight³ of fruit was the yield per tomato plant. The data were analyzed using "ANOVA" followed honestly significant different (HSD) test at 5% significant level. Then, the mean of three replicates ± SE and significantly separation symbols were presented in tables and graphics.

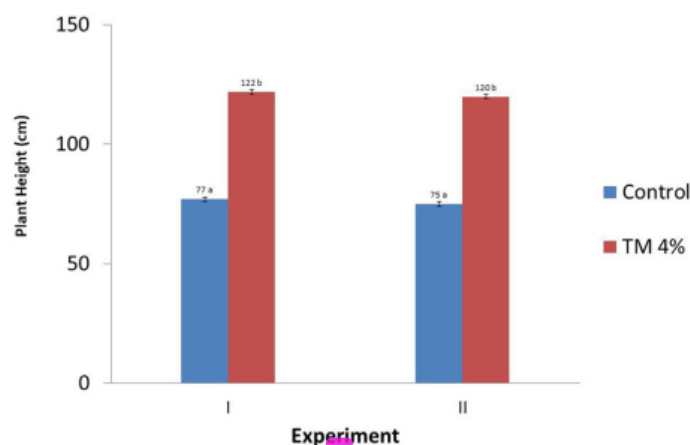


Figure 1. Effect of application of 4% Turbinaria extract on plant height of tomato plants, both in Experiment I (left) and Experiment II (right). The value in bar chart followed by different alphabets indicating significantly different based on HSD test at 5% significant level.

Results and discussion

Concentration of growth-promoting factors in Lombok Turbinaria murayana extract

The presence of growth-promoting factors in Lombok TM was detected using HPLC. Spectrum of growth-promoting factors in the extract is shown in Figure 1. Based on the peak characteristics of the standard compounds, three dominant compounds known as gibberellin, kinetin and ABA were present in TM crude extract. These growth hormones, kinetin and gibberellin, are known to play an important role in stimulating growth. On the other hand, ABA induces senescence abscission of leaves and fruits of several crops (Salisbury and Ross 1991; Anderson and Beardall 1991; Raven, Evert, and Eichhorn 1992). The detection of G, K and ABA suggests that Lombok TM extract could stimulate cell division, growth and production of plants.

The concentration of growth hormones in TM crude extract varied (Table 1). Gibberellin shows the highest concentration of growth hormone in TM extract (0.079 mg/mL). The other hormones, such as kinetin and ABA, show lower concentration than gibberellin, approximately 0.014 and 0.006 mg/mL extract. Furthermore, the concentration of hormones in TM differed with those reported in different species of seaweed (Chojnacka et al. 2012). Other seaweeds contained lower concentrations of cytokinin, auxin, gibberellin and ABA compared to TM crude extract. Hence, current results indicate TM extract may simulate both cell division and elongation during vegetative growth and also senescence and abscission promoting remobilization of macromolecules and deposition of small molecule to fruit during generative growth. This probably is due to the present of kinetin, gibberellin (GA3) and ABA in TM extract (Figure 1 and Table 1), hormones which play an important role stimulating cell division and elongation during vegetative growth (kinetin and GA3); and promoting senescence, abscission, remobilization and translocation during generative growth (ABA) as stated in many literatures (Salisbury and Ross 1991; Teiz and Zeiger 1998). Since liquid extract of TM contained gibberellin, kinetin and ABA, therefore, this extract could be developed as biostimulant which has stimulatory effects on nutrient uptake, growth and yield of plants.

Concentration of macro and micronutrients in Lombok Turbinaria murayana extract

Concentration of essential elements, such as N, P, K, Ca (macronutrients), Fe and Mn (micronutrients) were detected using atomic absorbance spectroscopy (AAS). Concentration of macro and

Table 1. The concentration of growth hormone in Lombok *Turbinaria murayana* extract.

Growth hormone	Concentration (mg/mL)
Gibberellin	0.079
Kinetin	0.014
Absciscic acid	0.006

Table 2. The concentration of N, P and K in leaf tissue of tomato plants sprayed with or without 4% TM extract, both in Experiment I and Experiment II.

Treatments	Concentration of NPK (% dry weight)					
	Experiment I			Experiment II		
	N	P	K	N	P	K
Control	0.40 ± 0.01 a*	0.09 ± 0.01 a	0.45 ± 0.05 a	0.45 ± 0.05 a	0.07 ± 0.02 a	0.40 ± 0.03 a
4% TM extract	0.70 ± 0.05 b	0.15 ± 0.03 b	0.65 ± 0.05 b	0.77 ± 0.03 b	0.16 ± 0.02 b	0.70 ± 0.05 b

* signifies the treatment has significant effects to control.

a and b indicates significant difference among treatments.

Table 3. The concentration of macro and micronutrients of Lombok *Turbinaria murayana* extract.

Macronutrients	Concentration (% dry weight)
N	0.28 ± 0.01
P	0.03
K	4.85
Ca	0.76
Micronutrients	
Fe	24.77
Mn	3.09

micronutrients in TM crude extract is shown in Table 2. Concentration of macronutrient was varied in the extract, in which, potassium was the highest macronutrient content, approximately 4.85%. The concentration of other macronutrients, such as calcium, nitrogen and phosphorus were subsequently lower; 0.76%, 0.28% and 0.03% respectively. In addition, the highest concentration of micronutrient was Fe followed by Mn, 24.77 mg/kg DW and 3.09 mg/kg DW, respectively. The concentration of those elements found this experiment was slightly higher than those reported in *Lepidium sativum* extract (Godlewska et al. 2016). This evidence suggests that TM extract has the potential to support macromolecule synthesis, cell division, growth and production of plants (Teiz and Zeiger 1998; Buchanan et al. 2000). Since the content of essential elements was measured in seaweed powder, then it could be that solid extract contained essential elements. Based on the fact, the solid extract could be developed as organic fertilizer, increasing the availability of mineral nutrition to support growth and yield of plant.

Effect of extract on growth of tomato plants

TM crude extract containing growth-promoting factors (Figure 1 and Table 1), and essential elements (Table 3), were tested for its effect on growth (leaf height, leaf number and branch number) and yield (flower and fruit number) of tomato plants. The extract consistently influenced plant height (Figure 2), leaf number and branch number (Figure 3) of tomato plants both in Experiment I and in Experiment II. Tomato plants sprayed with 4% extract was significantly (58.4%) higher compared to those which were not sprayed by extract (control plants). As reported by previous researchers that seaweed extract could induce the growth of plants because it contains growth hormones and trace elements (Zodape 2001). Since gibberellin and kinetin were dominant hormones found in TM crude extract, the application of TM extract would induce

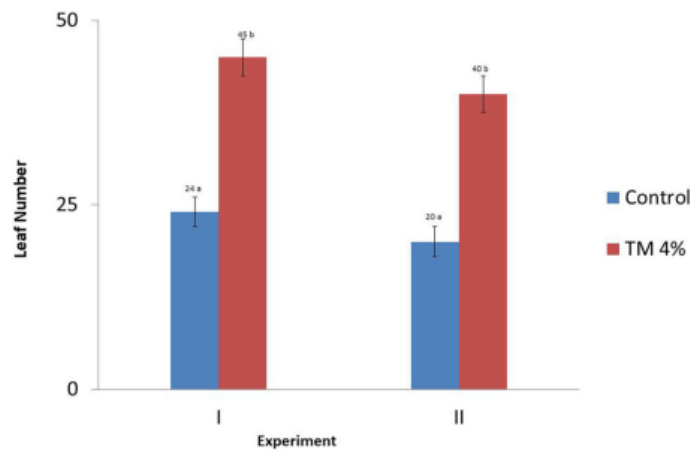


Figure 2. Effect of the application of 4% Turbinaria extract on leaf number of tomato plants, both in Experiment I (left) and Experiment II (right). The value in bar chart followed by different alphabets indicating significantly different based on HSD test at 5% significant level.

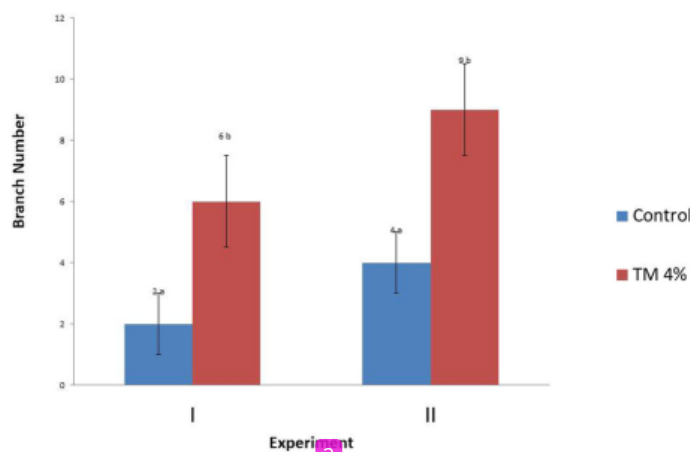


Figure 3. Effect of the application of 4% Turbinaria extract on branch number of tomato plants, both in Experiment I (left) and Experiment II (right). The value in bar chart followed by different alphabets indicating significantly different based on HSD test at 5% significant level.

elongation of tomato plants, because these two hormones play an important role in cell division and elongation (Raven, Evert, and Eichhorn 1992).

Similar phenomena were also found on leaves and branch number of tomato plants applied with TM crude extract (Figure 3). The plants sprayed with TM crude extract produced larger number of leaves and branches (45 leaves and 6 branches) compared to untreated plants (24 leaves and 2 branches). Those values were significantly different based on HSD test at 5% significant level. Furthermore, increased growth in treated tomato plants is probably caused by the stimulation of plant growth hormones presence in TM crude extract (Figures 2 and 3) and to absorb essential elements (Table 2). It seems that this effect is rather biostimulatory effect than providing essential elements, as 4% liquid extract sprayed containing growth hormones, such as gibberellin, kinetin and ABA. This phenomenon probably induces macromolecular biosynthesis, cell division and growth, as documented in the literature (Salisbury and Ross 1991; Anderson and Beardall 1991; Raven, Evert, and Eichhorn 1992; Teiz and Zeiger 1998; Buchanan et al. 2000). Seaweed Turbinaria extract also seem to effect shoot dry weight of tomato plants compared to

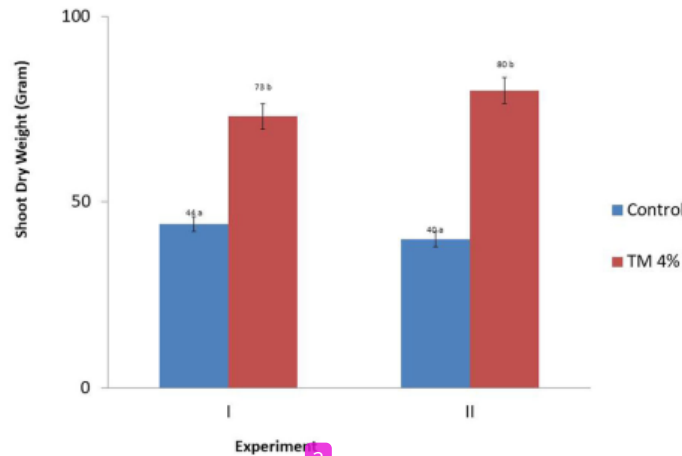


Figure 4. Effect of the application of 4% Turbinaria extract on shoot dry weight of tomato plants, both in Experiment I (left) and Experiment II (right). The value in bar chart followed by different alphabets indicating significantly different based on HSD test at 5% significant level.

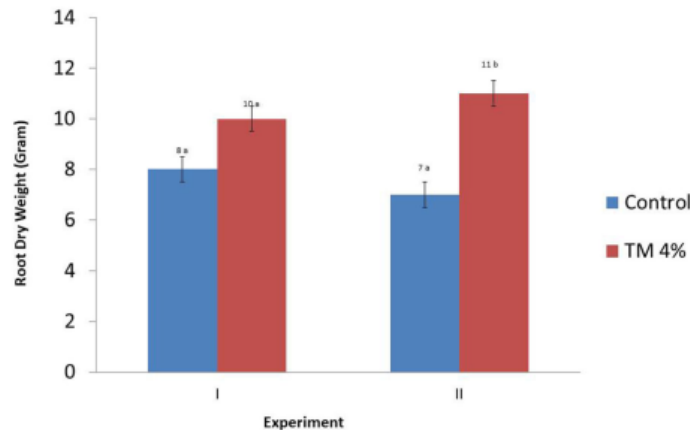


Figure 5. Effect of the application of 4% Turbinaria extract on root dry weight of tomato plants, both in Experiment I (left) and Experiment II (right). The value in bar chart followed by different alphabets indicating significantly different based on HSD test at 5% significant level.

control (Figure 4). The additional number of leaves and branches of tomato plants maybe are the result of cell division and promotion of shoot, which are stimulated by kinetin, one member of cytokinin, present in the seaweed extract (Raven, Evert, and Eichhorn 1992). Similar arguments were applied seaweed extracts, such as *S. wightii* and *C. chemnitzia* could induce the growth of *V. sinensis* plants, because it contained kinetin (Sivasankari et al. 2005). Similar reason also for induction of red algae extract on growth of maize (Safinaz and Ragaa 2013), *U. lactuca* on the growth of tomato plants (Hernández-Herrera et al. 2014; Chbani et al. 2015), *G. textorii* and *H. musciformis* on the growth of vegetable crops (Rao and Chatterjee 2014).

Effect of extract on yield of tomato plants

As it effects on vegetative growth, Lombok TM extract also influenced generative growth, such as flower number and fruit number (Figures 5 and 6). Tomato plants sprayed with the extract

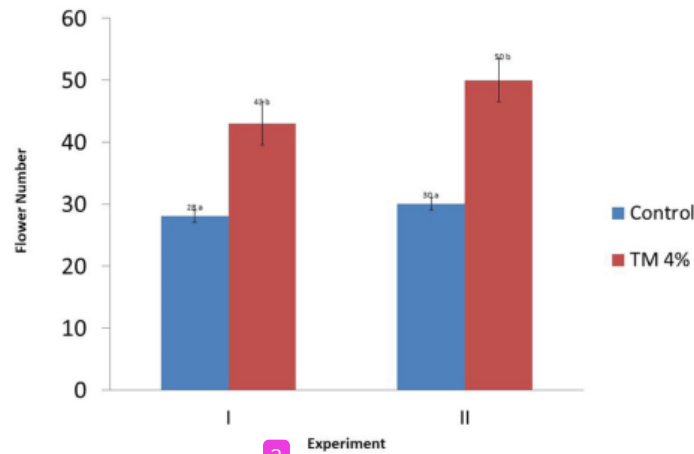


Figure 6. Effect of the application of 4% Turbinaria extract on fruit number of tomato plants, both in Experiment I (left) and Experiment II (right). The value in bar chart followed by different alphabets indicating significantly different based on HSD test at 5% significant level.

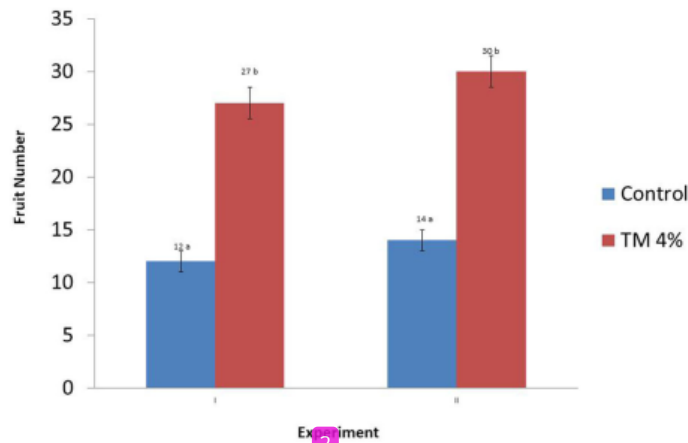


Figure 7. Effect of the application of 4% Turbinaria extract on root dry weight of tomato plants, both in Experiment I (left) and Experiment II (right). The value in bar chart followed by different alphabets indicating significantly different based on HSD test at 5% significant level.

Table 4. The percentage of flower to become fruit of tomato plants sprayed with or without 4% of TM extract, both in Experiment I and Experiment II.

Treatments	Percentage of flower to become fruits (%)	
	Experiment I	Experiment II
Control	42.80 ± 6.51 a	42.33 ± 6.81 a
4% TM extract	62.80 ± 1.53 b	61.00 ± 2.65 b

a and b indicates significant difference among treatments.

produced higher number of flowers (43) and fruits (27) than those of control plants which produced only 28 flowers and 27 fruits per plant, respectively. Based on statistical analysis, "ANOVA which was continued by honestly significant different" at 5% significant level, the value was significantly different between treated and control plants, both in Experiment I and Experiment II as pointed in figure ban 70. This means that treated plants produced flowers and fruits approximately 53.6% and 125% significantly higher than those of control plants. The data probably indicate that gibberellin and kinetin (Table 1) which were present in Lombok TM extract, stimulate

the absorption of mineral from soil media (Table 2), macromolecule biosynthesis, and translocation of those molecules into storage organ like pod, fruit and seed (Anderson and Beardall 1991; Sunarpi and Anderson 1998; Buchanan et al. 2000).

Existence of macro and microelements in Lombok TM extract (Table 3) also improved the absorption of mineral nutrition (Table 2). This condition also supports the absorption of macromolecule due to increase length of roots (Figure 7). Also, its biosynthesis in leaves, which provides more reserve for remobilization of macromolecules into small molecules which are ready to be translocated into young leaves or storage organs. It seems that this mechanism is induced by the presence of ABA in the extract, which is well known as this hormone involved in the senescence mechanism in plant leaf (Raven, Evert, and Eichhorn 1992; Buchanan et al. 2000). Senescence mechanism in plant is closely related to the action of proteolytic enzyme activities to remobilize macromolecules into small molecules, which finally these molecules are translocated into tomato fruits. This is the reason why treated plants have more fruits than those of control plants (Figure 6).

The number of flowers to become fruits was also influenced by the application of Lombok TM extract (Table 4). The number of flowers to become fruits was significantly higher in treated plants (62.8%) than those of control plants (42.8%). In another word, in treated tomato plants, approximately 46.7% higher flower to become fruits compared with those of control plants (Figure 5). Based on statistical analysis, "ANOVA followed by HSD test" at 5% significant level, the value was significantly different between treated and control plants as indicated in Table 4. This suggests that the presence of ABA in Lombok TM extract protects flowers to fall off, as it is well known that ABA plays an important role not only in inhibiting growth, but also it inhibits abscission process in plants (Salisbury and Ross 1991; Raven, Evert, and Eichhorn 1992; Teiz and Zeiger 1998). This phenomenon was also reported in soybean (Kocira et al. 2018) and gold cherry (Polo and Mata 2018).

Conclusions

Current results demonstrated the application of TM significantly increased vegetative and also generative growth of tomato plants. Results from this study evidenced the presence of essential plant growth hormones in brown seaweed TM, such as gibberellin, kinetin, and ABA. Furthermore, macro and microessential elements were also detected in TM crude extracts. High contents of K, Mn, and Fe could be suggested to contribute to the growth of tomato plants applied with TM crude extract. Further studies regarding the mechanism of TM biological activity in promoting plant growth would be reasonable for its development as biostimulant and organic fertilizer.

Conflict of interest

The authors declare no conflict of interest.

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