

## Effect of Solid and Liquid Extracts of Lombok *Sargassum cristafolium* on Growth and Yield of Rice Plants (*Oryza sativa* L.)

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**Abstract:** An excessive use of inorganic fertilizers in agriculture cultivation system causes many disadvantages, such as increase cost production, reduce farmer's income and soil fertility, and harm our environment. Therefore, it is needed to find out raw materials for development of organic biostimulant and biofertilizer inducing essential element absorption, growth and yield of plants, which are adaptive to our environment as well. Previous researches reported that seaweed contained plant growth hormones and essential elements in liquid and solid extracts respectively. This article reports effect of solid and liquid extracts of *Sargassum cristafolium* on growth and yield of rice plants. The extracts were extracted according to modified procedure.. Solid extract (350 gr) of *Sargassum cristafolium* was mixed with soil (6650 gr) to become 7 kg soil media. Moreover, liquid extract (10%) of *Sargassum cristafolium* was sprayed to rice plants once a week during vegetative growth. The results shown that addition of 350 gr of solid extract to soil media increased growth (plant high, tiller number, shoot dry weight and root dry weight) and yield (penicle number and grain weight per plants) significantly compared with those of plants which were not supplied with solid extract. However, spraying liquid extract of *Sargassum cristafolium* did not affect significantly growth and yield of rice plants, even small change happen in growth and yield parameters of the rice plants supplied with solid extract. This indicates that solid extract is a potential source for development of biofertilizer which is adaptive to our environment to support sustainable agriculture system.

**Keywords:** *liquid extract, solid extract, Sargassum cristafolium, growth, yield, rice plants*

### Introduction

The excessive use of inorganic fertilizers in agricultural production system, creates many problems, such as increases cost production, reduces soil fertility and farmers income, and harms our environment. Therefore, it is needed an understanding a natural resources which can be used as sources for developing organic biostimulants and biofertilizers which are adaptive

to our environment and could support sustainable agriculture. Previous researchers reported that macroalgae contained plant hormones and essential elements in liquid and solid extracts respectively, supporting growth and yield of plants (Zodape et., 2001; Godlewska et al., 2016).

Based on macroalgae content, extracts of some species of macroalgae have been reported to affect several growth phases, such germination, seedling, vegetatif and generative growth of plants.

This is due to the argument documented in some literatures that growth plant hormones, like gibberellin, IAA and NAA plays an important role as signal transductions for catalytic enzymes activities, like protease, amylase, nuclease and lipase in grain seeds which are in the process of germination (Raven *et al.*, 1992; Salisbury dan Ross, 1991; Teiz dan Zeiger, 1998; Buchanan *et al.*, 2000).

Since liquid extracts contained plant growth hormones (Zodapr, 2001; Goldlewska *et al.*, 2016), therefore, imbibition of several vegetable seeds with liquid extracts of seaweeds, induced germination (Rao dan Chatterjee, 2014). Except germinatin, liquid extracts also stimulated growth of tomato seedlings (Hernandez-Herrera, 2013). These indicates that plant growth hormones in liquid extracts, stimulates enzymes involve in germination process, like amylase, protease and lipase to remobilize macromolecules to become small molecules to support growth as occurred in soybean cotyledones (Sunarpi dan Anderson, 2005). Based on this argument, seaweed liquid extracts induced the growth of *Vigna mungo* L. (Kalaivanan dan Venkateslu, 2012), and tomato plants (hernandez-Herrera, 2013).

Several researchers also reported that liquid extracts of macroalgae influenced vegetative growth of several plant species. When vegetative growth begin, plants start to absorb essential elements from environment and it is transported to leaf. On the other hand, photosynthetic compounds are translocated into young leaf and root system. Therefore, liquid extracts of seaweed containing plant growth hormones, which could induce essential element absorption, photosynthesis, growth and production of plants.

Beside liquid extract, researchers also reported that solid extracts affected the growth plants, since this extracts contained macro and micro essential elements as reported by Godlewska *et al.* (2016). For example, biofertilizer developed based on solid extract of red algae, could increase the growth of green bean (Sivasankari *et al.*, 2005), maize plants (Safinaz and Raga, 2013). In addition, the other researchers also reported that biofertilizers developed from solid extract of green algae, influenced chlorophyll and proline content, and growth of sunflower plants (Chbani *et al.*, 2015; Majed *et al.*, 2015). Similar effects of biofertilizers developed from solid extracts, were also reported in vegetable (Li *et al.*, 2017), soybean (Kocira *et al.*, 2018), rice (Sunarpi *et al.*, 2018), cucumber (Sunarpi *et al.*, 2019) and tomato plants (Sunarpi *et al.*, 2020). These facts are correlated with arguments that the application of biofertilizers containing solid extracts which are rich with essential elements will

provide the availability of essential elements in soil media. This condition will induce mineral absorption, photosynthesis and growth of plants as documented in many literatures (Buchanan *et al.*, 2000).

This article reported the effect of liquid and solid extracts of *Sargassum cristafolium* on rice plants (*Oryza sativa* L.). The results shown that liquid extracts did not affect growth and yield of rice plants significantly. However, solid extracts influenced significantly all growth and yield parameters of rice plants. This indicates that solid extract of *Sargassum cristafolium* is a potential source for development of organic biofertilizers which provide sufficient essential elements in soil to induce the growth and yield of plants in order to support sustainable agriculture.

## Materials and Methods

### Experimental design

The experiment was designed with completely randomized design. There were four treatments: L0, no spraying with liquid extract of *Sargassum cristafolium*; LS, spraying with liquid extract of *Sargassum cristafolium*; P0, no addition with solid extract of *Sargassum cristafolium* in soil media; PS, addition with solid extract of *Sargassum cristafolium* in soil media. Each treatment was repeated three times. Therefore, there were 12 experimental pots.

### Preparation of soil media and rice seedlings

Soil media was prepared as follows. P0, plastic pots were added with 7 kg of soil, no addition with solid extract of. P1, plastic pots were added with 6650 gr of soil and 350 gr of solid extract. In addition, rice seedlings were prepared by sowing rice seeds in a plastic pot containing 7 kg of soil. After that, the germination process begins with the entry of water into the dry seeds. The dry seeds will absorb water from the environment and grow until 21 days after being sown, at the time the seedlings were ready to be transplanted.

### Sample collection and extraction

Sample of *Sargassum cristafolium* was collected from Batulayar coastal beach West Lombok. The sample was then rinsed and dried in shadow place without direct contact with sun light. After that, dried samples were extracted using distilled water according to the procedure developed by Godlewska *et al.* (2016). The samples were cutted in small pieces using scissor. The cut samples were mixed with distilled water with the comparison 1 kg of sample plus 3 L of water. The mixed samples were boiled at 95° C boiled water for 30 minute. The mixed samples were then filtered using whatman paper no. 1. Finally,

solution and pelled obtained were called as liquid and solid extracts of *Sargassum cristafolium* respectively.

### Plant culture and extract treatments

21 days old rice seedlings are transferred to plastic pots containing 7 kg of mixed soil media. Then it is allowed to grow until harvest, 90 days after planting. The application of liquid extracts were conducted by spraying 10% liquid extract of *Sargassum cristafolium* once a week during vegetative growth. The maintenance of water, insect and disease was conducted according to recommended cultivation of rice plants (Sunarpi, *et al.* 2019). Growth parameters, such as plant height (cm), tiller number, shoot (gr) and root dry weight (gr), were measured both during vegetative growth and after harvesting time. Similarly, yield parameters, like panicle number and grain weight per pot (gr), were also measured after harvesting time. The data were analyzed by using analysis of variance (ANOVA) which were followed by t-test at 5% significant level. The data were expressed as mean  $\pm$  SD followed by letters (a/b/c) showing significance of the data.

### Phytohormones analysis in liquid extract using HPLC

Plant growth hormones in liquid extract of *Sargassum cristafolium* were detected using HPLC as modified procedure developed by Godlewska *et al.* (2016). Firstly, 0.1% IAA standard solution was prepared. Secondly, liquid extract of *Sargassum cristafolium* was filtered using X filter before it was injected into HPLC column. The column used in this experiment was a Shimadzu CLC-ODS column (Shimadzu, Japan). Then, the sample was injected into HPLC column using automatic sampler injection, which was automatically diluted in the column. After that, the sample was separated at temperature 30° C, pressure 50 kg/cm<sup>2</sup>, retention speed 0.5 mL per minute using methanol/water (7:1, v/v) as moving phase. Finally, growth plant hormone content was analyzed by comparing between sample and standard chromatogram. The data were expressed as mean value  $\pm$  SD.

### Essential elements analysis in solid extracts using AAS

Essential elements, such as nitrogen, phosphorus, potassium, calcium, manganese and iron in solid extract of *Sargassum cristafolium*, were detected using Atomic Absorbance Spectroscopy (AAS) according to modified procedure developed by Godlewska *et al.* (2016). Firstly, 0.1% standard solution for nitrogen, phosphorus, potassium, calcium, manganese and iron were prepared. After that, the solid extract (10 gr) was destructed using 100 mL HCl 90% at 400° C temperature. Then, standard and sample solutions were

injected into AAS column as recommended procedure. Essential elements content in sample were determined by comparing between standard and sample chromatograms. The data were expressed as mean value of three replicates  $\pm$  SD.

## Results and Discussion

### Morphology and ecosystem of *Sargassum cristafolium*

*Sargassum cristafolium* is included in the Phaeophyceae class and can be found in tropical and subtropical waters. The morphology and ecology of *Sargassum cristafolium* is presented in Figure 1.



Figure 1. Morphology of *Sargassum cristafolium*

The algae was grouped into algae which have dichotomous branches with oval leaves and thick. Some dominant morphological characters in *Sargassum cristafolium* are shape of receptacles, leaves, vesicles and stems. This species has flattened triangular receptacles. Moreover, this species has duplicated leaves with fine teeth. The thalli of this algae is up to 9 cm high with discoid holdfast. Main and primary branches of this algae have terete and smooth characteristics, where cryptostomata are present. Cryptostomata are randomly distributed in the main and primary branch of algae. In addition, *Sargassum cristafolium* also has been reported that the habitat of this algae is coral flats and subtidal zone. They usually grow in the lower portions of the intertidal zone on rock substrates or shallow subtidal zones (Shams *et al.*, 2015).

### Plant growth hormone and essential element content of *Sargassum cristafolium*

Hormones and essential elements are important to support plant growth. Essential minerals need in physiological processes and help enzymes work. Plant growth hormone in liquid extract and essential

elements in solid extract of *Sargassum cristafolium* was shown in Table 1.

**Table 1.** The plant growth hormone and essential element contents in liquid and solid extract of *Sargassum cristafolium*

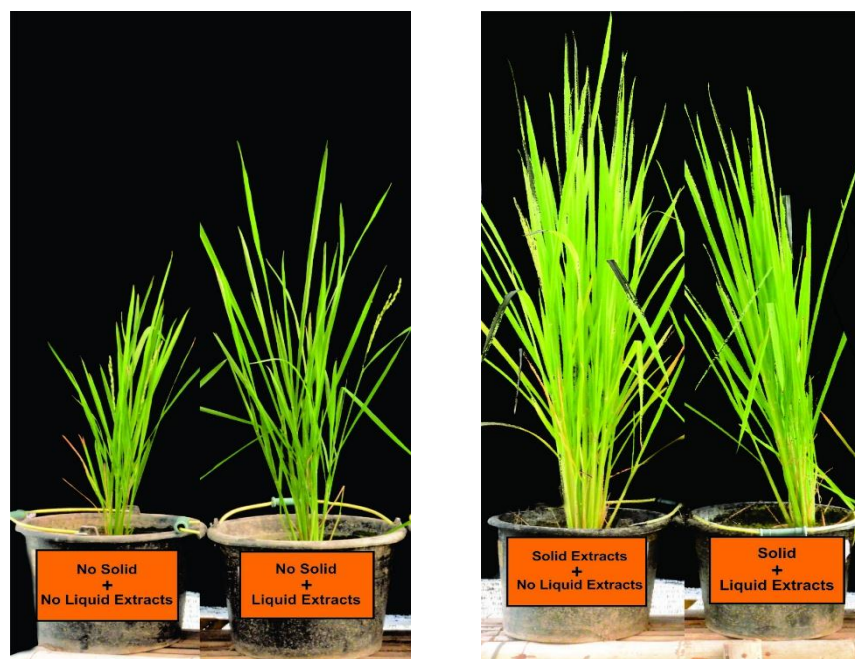
Liquid extract	Solid extract	Plant hormone contents(mgml <sup>-1</sup> )	Essential element content (% d.w.)
IAA		0.191±0.01	
	N		0.52±0.02
	P		0.08±0.002
	K		6.11±0.28
	Ca		0.85±0.07
	Fe		55±2.10
	Mn		8.5±0.26

The data in table 1 shows that liquid extract of *Sargassum cristafolium* contained IAA. On the other hand, solid extract of *Sargassum cristafolium* contained several macro and micro essential elements, such as N, P, K, Ca, Fe and Mn. Macro and micro essential content of solid extracts were relatively similar with those found in plant tissue (Salisbury dan Ross, 1995 dan Teiz dan Zeiger, 1998). Eventhough K, Ca, Mn and Fe relatively high in solid extract of *Sargassum cristafolium*, 0.52, 0.08, 6.11, 0.85, 55, and 8.5% dry weight. The data indicate that liquid extract could stimulate the growth, and solid extract provide

essential elements in soil media, which ultimately both extracts could support growth and production of plants.

#### Effect of liquid and solid extract of *Sargassum cristafolium* on growth

The use of liquid and solid extract of *Sargassum cristafolium* affects the growth of rice plants. Effect of liquid and solid extract of *Sargassum cristafolium* is presented in Figure 2.

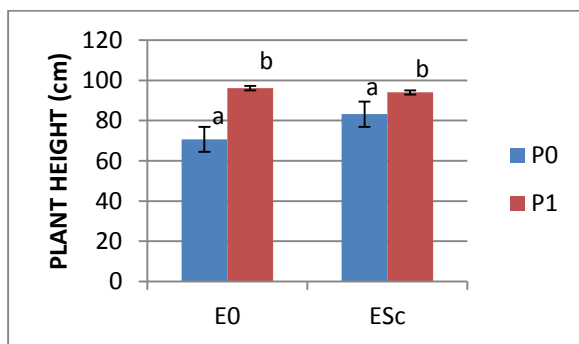


**Figure 2.** Effect of liquid and solid extract of *Sargassum cristafolium*; Control (No Solid+No Liquid Extract); P0+Esc (No Liquid+Liquid Extract); P+E0 (Solid Extract+No Liquid Extract); P+Esc ( Solid+Liquid Extract).

The performance of the plants shown in figure 2 shown that growth of rice plants were affected by application of solid and liquid extracts of *Sargassum cristafolium*. The rice plants grown in soil media containing solid extract, were grown better than the rice plants which were not supplied with solid extracts. This data indicates that solid extract in soil media provide essential elements to support growth and production of rice plants. This phenomena is consistent with the results reported by previous researchers (Godlewska et al., 2016; Sunarpi et al., 2018; Sunarpi et al., 2019; Sunarpi et al., 2020).

Similar to the effect of solid extract, liquid extract also slightly influenced the growth of rice plants (Figure 2). This effect seemly dependent on the availability of solid extract in soil media. For instance, in the plants which were not supplied with solid extract in soil media, the liquid extract slightly increased the growth of rice plants. In contrast, in the rice plants which were supplied with solid extract in soil media, the spraying 10% of *Sargassum cristafolium* extract slightly decreased the growth of rice plants. This suggests that abundant essential elements in soil media inhibits stimulation of growth by plant growth hormone in liquid extract of *Sargassum cristafolium*. Theoretically, it could be explained that external plant growth hormone, like IAA, induces activity of inhibitor involve in absorption mechanism in root system when essential elements were abundant in soil media as documented in many literatures (Salisbury and Ross, 1991; Taiz and Zeiger, 1998; Buchanan et al., 2000).

Rice plant growth parameters consist of plant height, tiller number, shoot dry weight, root dry weight, pinnacle number, and grain dry weight. One of growth parameters measured was the height of the rice plant. The provision of solid and liquid extracts has an effect on plant height of rice plants. The Effect of solid and liquid extracts on plant height of rice plants is presented in Figure 3.

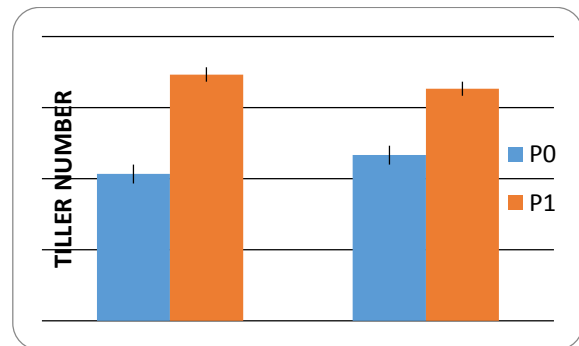


**Figure 3.** Effect of solid and liquid extracts on plant height of rice plants. The data presented were mean of

three replicates. The values were followed by different letter shown significancy at 0.05 significant level

The growth performance presented in Figure 2, plant height of rice plants was response differently on solid and liquid extracts of *Sargassum cristafolium* (Figure 3). Solid extract increased plant high significantly in the plants spraied without or with 10% of liquid extract. In contrast, liquid extract did not induce plant high significantly either in the plants were added with or without solid extract in soil media. The data indicate that solid extract are able to provide enough essential elements in soil media to support plant high of rice plants. However, IAA contained in liquid extract is not able to induce plant high.

The tiller numbers is influenced by environmental factors related to the environmental conditions for growing rice plants. Effect of solid and liquid extracts on tiller number of rice plants is presented in Figure 4.

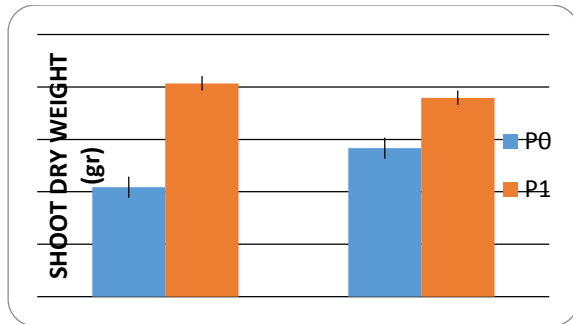


**Figure 4.** Effect of solid and liquid extracts on tiller number of rice plants. The data presented were mean of three replicates. The values were followed by different letter shown significancy at 0.05 significant level

Tiller number of rice plants were also response similarly to application of solid and liquid extracts of *Sargassum cristafolium* (Figure 4). Solid extract induced tiller number significantly of rice plants both in the plants spraied without and with liquid extract of *Sargassum cristafolium*. In contrast, liquid extract did not increase tiller number significantly both in the plants added without and with solid extracts in soil media. Therefore, it could be conclude that solid extract could provide enough essential elements in soil media to support growth and to form tiller. However, liquid extract containg IAA could not induce tiller number formation.

Since shoot dry weight is manifestation of pant high and tiller number of rice plants, therefore, shoot dry weight was response similarly to solid and liquid extracts with plant high and tiller number of

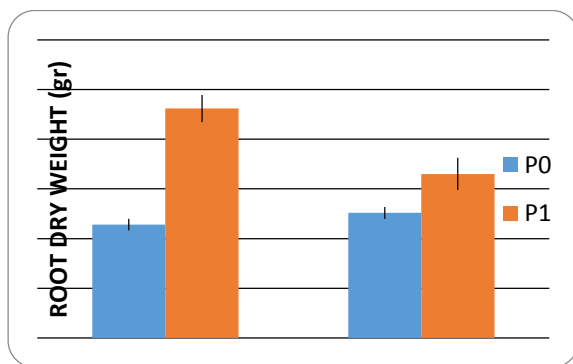
rice plants. Effect of solid and liquid extracts on shoot dry weight of rice plants is presented in Figure 5.



**Figure 5.** Effect of solid and liquid extracts on shoot dry weight of rice plants. The data presented were mean of three replicates. The values were followed by different letter shown significance at 0.05 significant level.

The addition of solid extract in soil media, induced shoot dry weight both in the plant sprayed both without and with 10% liquid extracts of *Sargassum cristafolium*. On the other hand, spraying 10% of liquid extract during vegetative growth of rice plants did not induce shoot dry weight of the rice plants which were added both without and with solid extracts. This phenomena indicates that solid extract is able to provide essential element in soil media to support growth of plant height and tiller number which ultimately increase shoot dry weight. In contrast with that, amount of IAA in liquid extract is unable to stimulate growth of rice plants, as also reported by previous researchers (Prasedya *et al.*, 2019).

Root system of rice plants was response slightly different with shoot system to application of solid and liquid extracts. Effect of solid and liquid extracts on root dry weight of rice plants is presented in Figure 6.



**Figure 6.** Effect of solid and liquid extracts on root dry weight of rice plants. The data presented were mean of three replicates. The values were followed by

different letter shown significance at 0.05 significant level.

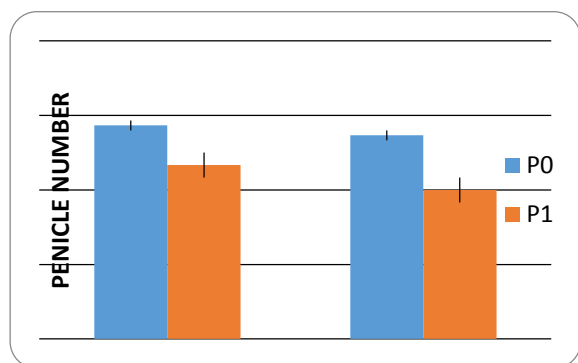
In the plants which were not sprayed with 10% liquid extract of *Sargassum cristafolium*, addition of solid extract in soil media induced root dry weight. However, in the plants which were sprayed with 10% liquid extract of *Sargassum cristafolium*, addition of solid extract did not induce root. This mean that spraying 10% liquid extract inhibited root growth of the rice plants supplied with solid extract in soil media. But this phenomena did not occur in the plants which were not sprayed with 10% liquid extract of *Sargassum cristafolium*. The phenomena suggests that IAA contained in liquid extract give priority to support shoot system rather than root system in the condition essential elements are available in optimum quantity in soil media. This theoretical hypothesis is documented in many plant physiology literatures (Salisbury and Ross, 1991; Taiz and Zeiger, 1998; Buchanan *et al.*, 2000).

Similar argument can be seen in another way (Figure 6). The rice plants which were not added with solid extract in soil media, spraying 10% liquid extract of *Sargassum cristafolium* did not induce root growth, due to not significantly different in term of root dry weight between two type of plants. In contrast, in the plants which were supplied with solid extract in soil media, the spraying 10% liquid extract of *Sargassum cristafolium* inhibited root growth, due to significantly different in term of root dry weight between two type of rice plants (Figure 6).

Generally, shoot and root growth phenomena of rice plants were response consistently to application of solid and liquid extracts of *Sargassum cristafolium* to rice plants. Solid extract provide essential elements in soil, which are ready to be absorbed by root system to support plant height, tiller number, which ultimately shoot system. This phenomena has been reported by many researchers in other plants, maize (Safinaz and Raga 2013), *Lepidium sativum* (Godlewska *et al.*, 2016), rice (Sunarpi *et al.*, 2018), soybean (Kocira *et al.*, 2019), cucumber (Sunarpi *et al.*, 2019) and tomato plants (Sunarpi *et al.*, 2020). However, IAA contained in liquid extract is not enough quantity to stimulate the growth of shoot system of rice plants. This argument is documented in previous literature (Raven *et al.*, 1995).

#### Effect of liquid and solid extract of *Sargassum cristafolium* on yield

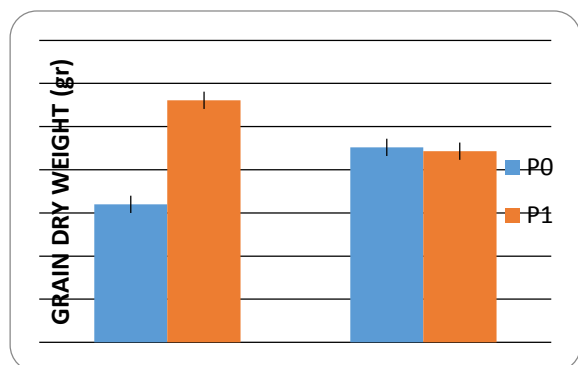
As phenomena shown in shoot system, solid extract induced panicle number of rice plant significantly, compared with those of rice plants which were not supplied with solid extract in soil media. The effect of solid and liquid extracts on panicle number of rice plants was shown in Figure 7.



**Figure 7.** Effect of solid and liquid extracts on panicle number of rice plants. The data presented were mean of three replicates. The values were followed by different letter shown significance at 0.05 significant level

This phenomena could be seen in both type of plants which were spried without and with 10% liquid extract of *Sargassum cristafolium*. In contrast with the data, liquid extract did not affect significantly panicle number of rice plants. This suggest that solid extract provide essential elements in soil which are ready to be absorbed by root system to support growth and panicle number of rice plants. On the other hand, IAA contained in liquid extract of *Sargassum cristafolium* is not in optimum concentration to support growth of shoot system and panicle of rice plants.

As effect of solid extract on shoot and panicle growth of rice plants, solid extract finally increased grain dry weight of rice plants significantly compared with the plants which were not supplied with solid extract. However, spraying 10% liquid extract of *Sargassum cristafolium* to rice plants during vegetative growth did not induce significantly grain dry weight of rice plants. Grain weight response of rice plants to solid and liquid extracts of *Sargassum cristafolium* was presented in Figure 8.



**Figure 8.** Effect of solid and liquid extracts on grain weight per pot of rice plants. The data presented were mean of three replicates. The values were followed by different letter shown significance at 0.05 significant level.

These data implies that solid extract provide essential elements in soil which are ready to be absorbed by root system to support growth and production of rice plants. On the other hand, IAA contained in liquid extract is not in optimum concentration to stimulate growth and production of rice plants. Such kind of argument has been documented in many literatures previoudly (Salisbury and Ross, 1991; Raven et al., 1995; Taiz and Zeiger, 1998; Buchanan et al., 2000). That is the reason why macroalgae could induce growth and production of several plants, such green bean (Siyasankari et al., 2005), maize (Safinaz and Raga, 2013), vegetable (Rao and Chatterjee, 2014), soybean (Kocira et al., 2018), cucumber (Sunarpi et al., 2019) and tomato (Sunarpi et al., 2020).

## Conclusion

Based on the results and discussion, it could be concluded that solid extract of *Sargassum cristafolium* induced growth and yield of rice plants. However, liquid extract of that algae, did not stimulate growth and production of rice plants. This suggest that solid extract of *Sargassum cristafolium* provide essential elements in soil media to support growth and yield of rice plants. In contrast, liquid extract is unable to plant growth hormone which was in optimum concentration to stimulate growth and yield of rice plants.

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