Growth and yield of rice plants (*Oryza sativa*) grown in soil media containing several doses of inorganic fertilizers and sprayed with lombok brown algae extracts

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**Abstract.** The use of inorganic fertilizers in rice production system in Indonesia, increases to an excessive level of application, which was 300 kg urea, 100 kg TSP, and 100 kg KCl per hectare, respectively. This application increases cost of rice production, reduces soil fertility, and farmers income, and harm environment. This article reports the effect of Lombok brown algae extracts on growth and yield of rice plants grown in soil media containing several doses of inorganic fertilizers. Lombok brown algae, such as *Sargassum crassifolium*, *Sargassum cristaefolium*, *Sargassum aquifolium*, and *Turbinaria murayana*, were collected in Lombok Indonesia coastal beach. Each liquid extract 10% of brown algae, was sprayed to rice plants grown in soil media containing 0, 50%, or 100% dose of inorganic fertilizers recomended by Indonesian ministry of agriculture. The result showed that effect Lombok brown algae liquid extracts on chlorophyll content in leaf, N, P, K content in tissue, growth, and yield of rice plants, depends on dose of inorganic fertilizer applied in soil media. Similar phenomena were also found in growth and yield parameters. An interesting result found in this experiment that there were no significant effect of brown algae liquid extract on chlorophyll content in leaf, N, P, K content in tissue, growth and yield of rice plants grown in media containing 50% and 100% inorganic fertilizers. This indicates that the application of inorganic fertilizers could be reduced to 50% when the rice plants were also sprayed with brown algae liquid extract to gain the same yield of these supplied with 100% inorganic fertilizers.

1. **Introduction**

Rice plants are main crop in Indonesia, as a result of nearly 90% of Indonesian population eat rice recently. In fact, Indonesian farmers including in West Nusa Tenggara (NTB) Province, use on inorganic...
fertilizers, such as urea, TSP and KCl increases to excessive dose level, 300 kg urea, 100 kg TSP and 100 kg KCl per hectar recently. A way use of inorganic fertilizers in rice production system, creates several disadvantage impacts as decrease soil fertility [1, 2, 3], pollute environment [4, 5], and increase crop production cost [6]. Nitrate leaching from excess application of inorganic fertilizers, like urea, to food crop production system, resulted high nitrate accumulation in edible parts of some vegetables, which can harm human health [7]. Moreover, since production cost increased and on the other hand rice price decreased during harvesting time, therefore farmers income was very low. The market demand for agriculture organic products with good price is increased recently [8]. Therefore, understanding natural resources for development of organic biostimulants and fertilizers inducing absorption of minereal nutrition, growth, and production of plants in low level of inorganic fertilizers in soil media, is an important study now a days.

Previous researcher reported that seaweeds, were a potential source for developing organic biostimulants and fertilizers. Because it contains plant growth hormones in liquid extract, and macro- micro essential elements in solid extracts [9, 10, 11]. Effect of red algae (Laurencia obtuse, Corallina elongate and Jania rubens) extracts on growth of corn has also been reported [12]. In addition, application of single liquid red algae extracts increased the growth of maize variably around 48-62%. The increase of corn production was higher when red algae extracts were applied in mixture form of red algae extracts, approximate by 72.4% compared with control plants.

Effect of liquid extract of Kappaphycus alvarezii, Gracilaria edulis, Caulerpa racemosa, and Sargassum crassifolium on stimulating growth, germination, productivity, and quality of rice seedlings were also reported [13, 14, 15]. The extract influenced growth of shoot and root system. Similar effect was also found in Gracilaria textorii and Hypnea musciformis liquid extracts when it applied to brinjal, tomato and chili plants [16]. These extracts increased growth and yield of those plant significantly.

The effect of liquid of algae except growth, liquid extract of algae also influenced biochemical constituent of several species’ plants [17, 18, 19]. Previous researchers reported that application of 20% of Sargassum wightii and Caulerpa chemnitzia stimulated growth of shoot and root, chlorophyll, and carotenoid biosynthesis of Vigna sinesis plants [20]. Effect of Ulva lactusa liquid extract was also reported to increase growth, chlorophyll, and proline biosynthesis in sunflower plants [21], gold cherry [22] and soybean [23]. Even though, all the research reported only the effect of liquid extract on growth and yield of several species of plants, but there is no report about the effect of liquid extract on essential elements absorption, growth, and yield of plants growing in several levels of inorganic fertilizers in soil media.

There were 88 species of macro algae grown in coastal area of West Nusa Tenggara (NTB), 17 species of them were brown algae, which were found to be a potential for developing growth stimulant and organic fertilizers [24]. It was found that Lombok brown algae extracts influenced growth and yield of rice plants, cucumber [15], tomato plants [16]. The effect of liquid extract of Lombok brown algae on growth and yield of rice plants grown in soil media containing several doses of inorganic fertilizers. The results shown that application of 10% liquid extract of Lombok brown algae induced absorption of NPK, chlorophyll content, growth and yield of rice plants grown in soil media containing 50% and 100% of inorganic fertilizers. Statistically, there were no different effect of liquid extract of algae on rice plants grown in soil media containing 50% and 100% inorganic fertilizers. The results indicate that application of inorganic fertilizers could be reduced into 50% dose to produce maximum yield when the rice plants seprayed with 10% liquid extract of Lombok brown algae during vegetative growth.

2. Materials and methods

2.1. Design, time and place of experiment

The experiment was consisted of two factors, Lombok brown algae liquid extracts and dose of inorganic fertilizers. Lombok brown algae extract consisted of five treatments: E0, non treated with Lombok brown algae liquid extracts, Scras, Sargassum crassifolium extract; Scris, Sargassum cristafolium extract, Saq, Sargassum aquifolium extract, Tm, Turbinaria murayana extract. Dose of inorganic
fertilizers consisted three treatments: N0, no application of inorganic fertilizers in soil media; N50, application of 50% dose inorganic fertilizers; N100, application of 100% doses of inorganic fertilizers. Therefore, there were 15 combination treatments. Since each treatment was replicated three times, therefore, there were 45 experimental pot.

2.2. Sample collection and extraction
Seaweed samples, such as *Sargassum crassifolium*, *Sargassum cristafolium*, *Sargassum aquifolium* and *Turbinaria murayana*, were collected in coastal beach area of Lombok, West Nusa Tenggara Indonesia. The samples were extracted according to modified procedure developed by [11]. Firstly, the samples were cleaned by rinsed with sea water and they were win dried in shadow place for three days. Then, each species of samples was cut using scissor into small pieces, and they were blundered to make fine powders. Powder Lombok brown algae (1 kg powder of each species) was placed in 15 L chemical flash separately. After that, 10 L distillated water was added to each flash, then they were homogenized by using magnetic stirrer for 30 minutes. Moreover, the mixtures were boiled in 95°C water bath for 30 minutes. Finally, the mixtures were filtered using whatman filter paper no.1. Supernatant obtained was known as 100% of liquid extract of brown algae.

2.3. Preparation of soil media and rice seedlings
Soil media was prepared by addition 7 kg soil obtained from rice field in West Lombok. Inorganic fertilizers, such as urea, TSP and KCl, were added to soil media at day 10 and 30 after transplanting as procedure as follows: N0, no addition of inorganic fertilizers; N50, addition of 50% dose of NPK at day 10 and 30 after planting; N100, addition of 100% dose of NPK at day 10 and 30 after planting. In addition, rice seedlings were prepared by sowing rice seeds in plastic pot containing 7 kg soil and they were left to grow. The seedlings were ready to be transplanted when 21 days age.

2.4. Cultivation and treatments
After 21 days age, the rice seedlings were transplanted into plastic pot containing 7 kg soil. Application of inorganic fertilizers according to treatments, was applicated two times at day 10 and 30 respectively. Moreover, the application of liquid extract of Lombok brown algae liquid extract, was conducted by sepraying 10% of extract one a week during vegetative growth. Then, the plants were left to grow until harvesting time.

2.5. Analysis of chlorophyl, N, P and K content
Chlorophyll content was measured using spectrophotometer according to procedure developed by [25]. Moreover, analysis of N, P, and K content in tissue was conducted according to modified procedure of [9, 26]. Seaweed samples 0.5 g dry weight were added with 5 mL of nitric acid in hot block in 400°C. Then, the samples were diluted ten times with ultra-filtered water (Milli-QIntegral, Millipore Sigma). All samples were analyzed in two replicates. Finally, the content of essential elements was measured using ICP-OES (Agilent technologies, US).

2.6. Data analysis and presentation
Data were analyzed using analysis of variance (ANOVA) which was continued by honestly significant different (HSD) analysis at 5% significant level. The value presented both in graph and table, is mean of three replicates, followed by ±SE bar.

3. Results and discussion

3.1. Effect of liquid extracts and dose of inorganic fertilizers on chlorophyll content
In control plants which were not sprayed with algae extracts, chlorophyll content to a level of significantly different when the plants were grown in soil media containing 100% dose of inorganic fertilizers. This indicates that application of 100% dose of inorganic fertilizers induces biosynthesis of
chlophyll, an essential compound for photosynthesis [18]. However, when the plants were sprayed with 10% of \textit{Sargassum crassifolium} and \textit{Sargassum cristafolium} extracts to the plants grown in soil media containing 50% of inorganic fertilizers during vegetative growth, the chlorophyll content of leaf increased significantly compared with those of control plants to a level of chlorophyll content which were not significantly different with those of plants supplied with 100% dose of inorganic fertilizers (Figure 1). Even though, these phenomena could not be seen on the plants sprayed with \textit{Sargassum aquifolium} and \textit{Turbinaria murayana} extracts. This indicate that the effect of liquid extracts of brown algae depend on type species. Two type of species, \textit{Sargassum crassifolium} and \textit{Sargassum cristafolium} have enough amount of plant hormone to stimulate chlorophyll biosynthesis. In contrast, the other two species, \textit{Sargassum aquifolium} and \textit{Turbinaria murayana} do not have enough amount of phytohormones to induce biosynthesis of chlorophyll in their leaf [26, 27, 28].

![Figure 1. Effect of Lombok brown algae Extract on Chlorophyll Content of rice plants grown in soil media containing 0, 50% or 100% doses of inorganic fertilizers. The value in bar chart followed by different alphabets indicating significantly different based on HSD test at 5% significant level.](image1)

3.2. Effect of liquid extracts and dose of inorganic fertilizers on N, P and K content in tissue

Nearly all species of brown algae extracts tested, were influenced N content in leaf of rice plants (Figure 2). These effects were dependent on dose of inorganic fertilizers supplied in soil media. Except the application of \textit{Sargassum aquifolium} extract, other extracts, like \textit{Sargassum crassifolium}, \textit{Sargassum cristafolium} and \textit{Turbinaria murayana} extracts, increased nitrogen of leaf of those plants supplied with 50% dose of inorganic fertilizers to the level which were not significantly different with the plants supplied with 100% dose of inorganic fertilizers in soil media (Figure 2).

![Figure 2. Effect of Lombok brown algae extracts on N content of rice plants grown in soil media containing 0, 50 or 100% dose of inorganic fertilizers. The value in bar chart followed by different alphabets indicating significantly different based on HSD test at 5% significant level.](image2)

All brown algae extract induced P leaf content of those rice plants supplied with 50% dose of inorganic fertilizers to a level which were not significantly different with those of plants supplied with 100% dose of inorganic fertilizers in soil media (Figure 3).
These phenomena demonstrate clearly that application 10% of brown algae extracts to the leaf of rice plants, could induces absorption of N, P and K from soil media containing 50% dose of inorganic fertilizers. However, the absorption of N, P, K by root system could not be increased eventhough the plants were supplied with 100% dose of inorganic fertilizers supplied in soil media. The theoritical argument for these facts that enzymes involve in absorption mechanism of N, P and K became saturated when substrate abundant in soil media [27, 29].

3.3. Effect of liquid extracts and dose of inorganic fertilizers on growth
As other parameters, growth parameters such as tiller number (Figure 5), were influenced by spraying 10% of brown algae extracts. These effects were also dependent on dose of inorganic fertilizers in soil media. Tiller numbers of rice plants grown in soil media containing 50% dose of inorganic fertilizers and sprayed with 10% of brown algae extracts, were increased significantly compared with control plants which were not sprayed with 10% brown algae extracts. Eventhough, the tiller number increased, did not significantly different compared with those of rice plants supplied with 100% dose of inorganic fertilizers.

Similar effects were also found in other growth parameter, like shoot (Figure 6). Since tiller number is an important contributor to shoot biomass, therefore, increasing tiller number will increase shoot dry weight.
The effect of liquid extract on dose of inorganic fertilizers on root dry weight (Figure 7) was also affected by application 10% of brown alga extract. Therefore, generally it can be concluded that spraying 10% of brown algae extracts increased growth of rice plants grown in soil media containing 50% and 100% dose of inorganic fertilizers. Eventhough, there were not significantly different between rice plants grown in soil media containing 50% and 100% dose of inorganic fertilizers which were sprayed with 10% of brown algae extracts. These indicates that rice plants only need 50% dose of inorganic fertilizers in soil media to support maximum growth when the plants sprayed with 10% brown algae extracts. This due to the fact that maximum capacity of enzymes involves in ion absorption by root system and transport mechanism in xilem to support growth depend on the concentration of substrat in soil media [27, 29, 30]. Therefore, application of 100 % dose of inorganic fertilizers in soil media, is uptaken maximally only 50%, and another 50% leaching to environment as toxic ion polluting environment.

3.4. Effect of liquid extracts and dose of inorganic fertilizers on yield
Generative parameters, such as panicle number (Figure 8), grain weight (Figure 9) and weight of 100 grain (Figure 10) of rice plants were response similarly with growth parameters to application 10% of brown extracts to the rice plants grown in soil media containing 0, 50% or 100% doses of inorganic fertilizers.
The panicle number of rice plants were increased significantly by addition of inorganic fertilizers in soil media (Figure 1). However, there were no significant different between the plants supplied with 50% and 100% inorganic fertilizers. In contrast, in the plants which were not supplied with inorganic fertilizers, there were no significant effect on panicle number (Figure 8). However, in the plants supplied with 50% and 100% inorganic fertilizers, there were significant effect of spraying 10% of brown extracts on panicle number of rice plants.

Similarly, since the addition of 50% and 100% inorganic fertilizers increased panicle number, therefore these treatments increased grain weight per of plants (Figure 9). In addition, in the plants which were not added with inorganic fertilizers, the spraying 10% of brown algae liquid extracts effected significantly the grain weight per plants of rice plants (Figure 9). In contrast, in the plants supplied with 50% and 100% inorganic fertilizers, the spraying 10% of brown algae liquid extracts did not affect significantly the grain weight per plant of rice plants.

Panicle numbers, grain weight and weight of 100 grain of rice plants, were increased significantly when the plants grown in soil media containing 50% dose of inorganic fertilizers. This increase was not significantly different compared with those plants grown in soil media containing 100% dose of inorganic fertilizers and sprayed with 10% of brown algae extracts. This indicates that growing rice plants in soil media containing 50% dose of inorganic fertilizers could achieve maximum absorption of essential elements, growth, and yield when the plants sprayed with 10% of brown algae extracts. Theoretically, addition 50% inorganic fertilizers in soil media, provide maximum concentration of substrate to support absorption and growth [28, 30].
Like other yield parameters, the weight of 100 grain was also increased significantly by addition of 50% and 100% of inorganic fertilizers in soil media. However, there were no significant different on that parameter between the plants supplied with 50% and 100% inorganic fertilizers in soil media (Figure 10). There was significant effect of the application of brown algae liquid extract on the weight of 100 grain of rice plants grown in soil media containing 50% inorganic fertilizers. However, these were no significant effect of the liquid extract on that parameter of the rice plants grown in soil media containing 100% inorganic fertilizers.

4. Conclusion
Application of 10% brown algae extracts to rice plants during vegetative growth, induced mineral nutrition absorption, growth and yield of rice plants significantly compared with control plants. These effects depend on dose of inorganic fertilizers supplied in soil media. Maximum mineral absorption, growth, and yield of rice plants, could be achieved when the plants supplied with 50% dose of inorganic fertilizers and seprayed with 10% of brown algae extracts during vegetative growth of rice plants. It is recommended to grow rice supplied with 50% dose of inorganic fertilizers and with 10% of brown algae during vegetative growth. The future study is very important to understand the effect of dose of organic fertilizer and liquid extract brown algae on photosynthetic rate, carbohydrate, and protein disposition from leave to grain of rice plant.

References
[1] Shen J, Li R, Zhang F, Fan J, Tang C and Rengel Z 2004 Crop yield, soils fertility and phosphorus fractions in response to long-term fertilization under the rice monoculture system on a calcareous soil. Field Crop Res. 86(2-3): 225-38
[2] Rasool R, Kukal G S and Hira G S 2007 Soil physical fertility and crop performance are affected by long-term application of FYM and inorganic fertilizers in rice-wheat system. Soil Till. Res. 96(1-2): 64-72
[3] Gosal S K, Gill G K, Sharma S, Walia S S 2018 Soil nutrient status and yield of rice affected by long-term integrated use of inorganic and organic fertilizers. J. Plant Nutri. 41(4): 539-44
[4] Chien H, Prochnow L I and Cantarella H 2011 Recent development of fertilizer production and use to improve nutrient efficiency and minimize environmental impacts. Adv. Agr. 102: 267-322
[5] Panjaitan E, Sidauruk L, Indrodewa D, Martono E and Sartohadi J 2020 Impact of agriculture on water pollution in deli serdang regency, North Sumatra Province, Indonesia. Organic Agr.: 1-9
[6] Rahman K M and Zhang D 2018 Effect of fertilizer broadcasting on excessive use of inorganic fertilizers and environmental sustainability. Sustainability 10(3): 759
[7] Chen H, Yang X, Wang P, Wang Z, Li M and Zhao F 2018 Dietary cadmium intake from rice and vegetables and potential health risk: a case study in Xiangtan, Southern China. Sci. Total Environ. 639: 271-7
[8] Zodape S T 2001. Seaweed as a biofertilizer J. Sci. Industr. Res., 60: 378-82
[9] Khan W, Rayirath U P, Subramanian S, Jithesh M N, Rayorath P, Hodges D M, Critchley A T, Cragie J S, Norrie J and Prithiviraj B 2009 Seaweed extracts as biostimulants of plant growth and development. Plant Growth Regul. 28: 386-99
[10] Chojnacka K, Saeid A, Witkowska Z and Tuhy L 2012 Biologically active compounds in seaweed extracts - the prospects for the application. Open Conf. Proc. J., 3(1): 20-8
[11] Godlewksa K, Michalak I, Tuhy L and Chojnacka K 2016 Plant growth biostimulants based on different methods of seaweed extraction with water. BioMed Res. Inter. 2016: 1-11
[12] Safinaz A F and Ragaa A H 2013 Effect of some red marine algae as biofertilizers on growth of maize (Zea mays L.) plants. Inter. Food Res. J. 20(4): 1629-32
[13] Dumale J V, Ramosa G R and Divina C C 2016 Plant growth promoting effect, gibberlic acid and auxin like activity of liquid extract of caulerpa racemosa on rice seed germination. Inter. J.
[14] Layek J, Das A, Idapuganti R G, Sarkar S and Ghosh A 2018 Seaweed extract as organic bio-stimulant improves productivity and quality of rice in Eastern Himalayas. *J. Appl. Phycol.* 30(1): 547-58

[15] Sunarpi H, Pebriani S A, Ambana Y, Putri F E, Nikmatullah A, Ghazali M, Kumianingsih R, and Prasedya E S 2019 Effect of inorganic fertilizer and brown alga solid extract on growth and yield of rice plants. AIP Conference Proceeding 2199(1): 1-5

[16] Rao G M N and Chatterjee R 2014 Effect of Seaweed Liquid Fertilizer From Gracilaria textorii and Hypnea musciformis on Seed Germination and Productivity of Some Vegetables Crops *Univ. J. Plant Sci.* 2(7): 115-20

[17] Sivaskari S, Venkatesalu V, Anantharaj M and Chandrasekaran M 2006 Effect of seaweed extracts on the growth is influenced biochemical constituent of vigna sinensis. *Bioresour. Tech.* 97(14): 1745-51

[18] Ramya S S, Nagaraj S and Vijayanand N 2011 Influence of seaweed liquid extracts on growth, biochemical and yield characteristics of cyamopsis tetragonolaba (L.) Taub. *J. Phytol.* 3(9): 37-41

[19] Vijayanand N, Ramya S S and Rathinavel S 2014 Potential of liquid extracts of sargassum wightii on growth, biochemical and yield parameters of cluster bean plant. *Asian Pacific J. Reprod.* 3(2): 150-5

[20] Venkatesalu V and Kalaivanan C 2012 Utilization of seaweed sargassum myriocystum extracts as a stimulant of seedlings of vigna mungo (L.) hepper. *Spanish J. Agri. Res.* 10(2): 466-70

[21] Chbani M A, Mawlawi S and Kammoun M 2015 The use of seaweed as a bio-fertilizer: Does it influence proline and chlorophyll concentration in plants treated? *Arabian J. Medi. Aromatic Plants* 1(1): 67-77

[22] Polo J and Mata P 2018 Evaluation of a biostimulant (pepton) based in enzymatic hydrolyzed animal protein in comparison to seaweed extracts on root development, vegetative growth, flowering, and yield of gold cherry tomatoes grown under low stress ambient field conditions Front. *Plant Sci.* 8: 2261

[23] Kocira S, Szparaga A, Kocira A, Czerwiriska E, Wojtowicz 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: 1-18

[24] Sunarpi, Jupri A, Kumianingsih R, Julisaniah N I and Nikmatullah A 2010 Effect of seaweed extracts on growth and yield of rice plants. *Nusantara Biosci.* 2(2): 73-7

[25] Granger S and Izumi H 2001 Water quality measurement methods for seagrass habitat In: Short FT Coles RG editor. *Global Seagrass Research Methods* (Amsterdam: Elsevier Science B V)

[26] Sunarpi H, Kumianingsih R, Ghazali M, Fanani R A, Sunarwidhi A L, Widyastuti S, Nikmatullah A and Prasedya E S. 2020. Evidence for the presence of growth-promoting factors in Lombok Turbinaria murayana extract stimulating growth and yield of tomato plants (Lycopersicum esculentum Mill.) *J. Plant Nutri.* 43(12): 1813-23

[27] Salisbury F B and Ross C W 1991 *Plant Physiology Fourth Edition* (USA: Wadsworth Publishing Company, Belmont California) 682p

[28] Anderson J W and Beardall J 1991 *Molecular activities of plant cells: an introduction to plant biochemistry* (UK: Blackwell Scientific Publications) p 384

[29] Taiz, L. and Zeiger E. 1998. *Plant Physiology 2nd* (Sunderland, Massachusetts: Sinauer Associates Publisher)

[30] Buchanan B B 2015 *Biochemistry and Molecular Biology of Plants 2nd Edition.* (USA: American Society of Plant Biologists, Wiley Blackwel)