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Analysis of leaf chlorophyll content of paddy plants during vegetative stage grown in soil media containing macroalgae organic fertilizer

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Abstract. Chlorophyll is a green pigment in leaves that functions to absorb light energy for photosynthesis. The value of chlorophyll concentration in leaves indicates the health status of a plant and healthy plants will produce more fruit. The purpose of this study was to determine the effect of chemical fertilizer (CF) and solid algae fertilizer (SAF) with different doses on the concentration of chlorophyll in paddy leaves. This research is important to do to determine the potential of macroalgae in reducing the dose of chemical fertilizer use in agricultural activities. In this study, paddy plants were given four different treatments, namely control (without CF and SAF); 100% chemical fertilizer (CF); 100% solid algae fertilizer (SAF); 50% chemical fertilizers and 50% solid algae fertilizers (CF+SAF). Each treatment consisted of ten pots. The results showed that the administration of SAF in the form of a combination of SAF 50% and CF 50% had a significant effect on the increase of chlorophyll b content of the leaves of paddy plants. However, there were no significant difference in chlorophyll a content between CF and CF+SAF group. Current results show that addition of SAF could potentially increase growth quality in paddy plants due to the increase of chlorophyll b content which absorbs higher frequency of blue light for photosynthesis.

1. Introduction

Paddy plants is the main agricultural product in Indonesia. Nearly 90% of the Indonesian population eats paddy and as a staple food [1]. The paddy produced provides 35-85% of the total calories needed by the body. Every year the need for paddy for food continues to increase. In current practice, farmers mostly use inorganic fertilizers such as Urea, TSP, and KCL to increase their production yields [2]. The use of chemical fertilizers continues to increase every year so that it can cause various negative impacts such as increasing crop production costs, reducing soil fertility, and explaining the environment [3-5]. Therefore, it is necessary to make an alternative effort to reduce the use of inorganic fertilizers and substitute them with organic fertilizers that are more environmentally friendly.

West Nusa Tenggara (NTB) has warm waters so that various types of macroalgae can grow well. A total of 88 types of macroalgae that grow on the coast of NTB and 17 of them are types of algae that have the potential to be used as biostimulant and organic fertilizers [6]. According to several studies, it is reported that liquid extracts of macroalgae can affect the vegetative growth of several plant species. The root system of the plant will absorb important elements from the environment and be carried to the young leaves when vegetative growth begins. On the other side, the products of photosynthesis are transported to the young leaves and root system. This requires hormones to activate the activity of the enzymes involved in the mechanism. Macroalgae solid extract is also reported to affect plant growth and production because of the essential element content of macroalgae solid extract. Biofertilizers developed from solid extracts of red algae can increase the growth of mung bean and corn plants. Meanwhile, biofertilizers developed from solid extracts of green algae affected the chlorophyll and proline content as well as the growth of sunflower, vegetable, soybean, cucumber, and tomato plants [7].

Chlorophyll is the green pigment in plants, algae and photosynthetic bacteria [8-9]. In plants, chlorophyll is used in the process of photosynthesis to absorb and convert light energy into chemical energy. Chlorophyll is called the central pigment of the photosynthetic reaction because it can accommodate light that is absorbed by other pigments through photosynthesis [10]. Chlorophyll content is one indicator of photosynthetic activity [11]. Many researchers state that the ratio between chlorophyll a and b is 3:1. These values differ as a function of plant growth and development, the cultivar of the plant concerned and several environmental factors. Chlorophyll also plays a role in the process of plant organogenesis [12].

According to some studies, the synthesis of chlorophyll depends on mineral nutrition. Mineral nutrition significantly affects the dynamics of leaf surface formation and leaf surface area, which is reflected in the total leaf surface amount, photosynthetic potential, and pure photosynthetic productivity. Of all the macrometabolic elements, the greatest influence on plant development in general and on the leaf surface is nitrogen, whose effects are enhanced by phosphorus and potassium [13]. However, information on the content of beneficial micronutrients and phytohormones in brown macroalgae found in Lombok coast is still limited. This study aimed to determine the effect of liquid macroalgae extract of Lombok on leaf chlorophyll content and the effect of solid extract of Lombok macroalgae on vegetative growth of paddy plants.

2. Materials and Methods

2.1 Manufacture of macroalgae-based organic fertilizer

Fresh macroalgae were taken from the waters of Lombok, Batu Layar, West Lombok (8°31'03.1"S 116°03'40.8"E) and then dried for approximately 5 days. The macroalgae used were *Sargassum cristaefolium*, *Sargassum crassifolium*, and *Sargassum polycystum*. The three ingredients are mixed with dilute EM4. EM4 serves to speed up the fermentation process in the manufacture of fertilizers. The final stage is incubation for 30 days to be ready for use in testing.

2.2 Soil media preparation

The mixture of soil media used weights 7 kg. In this study used 4 treatments, namely control, consisting of 7 kg of soil in the media; CF, consisting of 7 kg of soil plus 100% inorganic fertilizers; SAF, consisting of 6 kg of soil and 1 kg of organic fertilizer based on macroalgae; CF + SAF, consisting of 50% inorganic fertilizer and 6.5 kg of soil + 0.5 kg of macroalgae-based organic fertilizer.

2.3 Planting paddy in pots

Paddy seeds that were 3 weeks old were planted on media that had been prepared according to treatment. Plant maintenance up to the time is carried out in accordance with the procedures recommended for paddy plants. The application of inorganic fertilizers is carried out according to a predetermined dose. The application of inorganic NPK fertilizer was carried out when the plants were 14 and 30 days old.

2.4 Collection and preparation of oaddy plant leaf samples

Paddy leaves were taken from a plastic house in Jatisela Village, West Lombok (8°33'08.7"S 116°05'27.3"E). Paddy leaves were taken randomly from 10 pots in each treatment and then collected in 1.5 ml eppendorf tubes. The leaves were then taken to the laboratory of Pusat Unggulan Biosains dan Bioteknologi, University of Mataram. The leaves were cut into small pieces and weighed as much as 0.05 mg. Furthermore, the leaf pieces were soaked in 1.5 ml of Eppendorf using 1 mL of 80% acetone and incubated at room temperature for 24 hours [14].

2.5 Analysis of chlorophyll

Chlorophyll content was calculated using a spectrophotometer. After 24 hours soaked in leaf acetone and then centrifuged for 5 minutes, 15,000 g. The supernatant was taken 100 l and put into 96-well plates. The absorption was measured using Thermo Scientific Multiskan GO 3.2 Spectrophotometry at two wavelengths 646 and 663 nm. Chlorophyll a, b, and total chlorophyll were calculated according to the Lichtenthaler's equation (A) [15] and Arnon's equation (b) [16].

$$A. \text{ Chlorophyll a } (\mu\text{g/mL}) = -1.93 A_{646} + 11.93 A_{663}$$

$$\text{Chlorophyll b } (\mu\text{g/mL}) = 20.36 A_{646} - 5.50 A_{663}$$

$$\text{Total chlorophyll } (\mu\text{g/mL}) = 6.43 A_{663} + 18.43 A_{646}$$

$$B. \text{ Chlorophyll a } (\mu\text{g/mL}) = 12.7 A_{663} - 2.69 A_{645}$$

$$\text{Chlorophyll b } (\mu\text{g/mL}) = 22.9 A_{645} - 4.68 A_{663}$$

$$\text{Total chlorophyll } (\mu\text{g/mL}) = 20.2 A_{645} + 8.0 A_{663}$$

The concentration of chlorophyll content was obtained after substitution of absorbance for each wavelength in the equation. The result is expressed in g/mL.

2.6 Statistical analysis

The results were then statistically analyzed using two-way ANOVA and Tukey's HSD posthoc with a 95% of confidence level. A value of <0.05 is considered significantly different. Whereas, a value of p<0.05 is considered highly significant different. Statistical analysis was performed using GraphPad Prism version 9.2.0 (GraphPad software, Inc).

3. Results and Discussion

3.1 The content of chlorophyll a and chlorophyll b in the leaves with the application of SAF (Lichtenthaler's equation)

Based on the results of a two-way ANOVA statistical analysis after calculating the value of chlorophyll content using Lichtenthaler's equations, it was found that the chlorophyll a content in plants with CF, SAF, CF + SAF media was not significantly different from control plants. The value of chlorophyll a content of control plants was 23.42±5.45. Paddy plants that were treated with SAF in the growing media had significantly less chlorophyll a than paddy plants that were treated with CF and CF+SAF. The value of chlorophyll a content of plants treated with SAF was 18.16±3.22, while the value of chlorophyll a content of plants treated with CF and CF+SAF were 26.91±0.21 and 26.98±0.30. Based on Lichtenthaler's equations used in this study, plants treated with CF+SAF had the highest chlorophyll a content values and plants treated with SAF had the lowest chlorophyll a content values. Using the same equation, it was also found that the value of chlorophyll b content in control plants was not significantly different from plants treated with CF and SAF, but significantly different from plants treated with CF+SAF. The value of chlorophyll b content in control plants was 11.35±4.35. Paddy plants treated with CF in the growing media had a lower and significant value of chlorophyll b content than paddy plants treated with CF+SAF but had a higher and significant value for chlorophyll b content than plants treated with SAF. The value of chlorophyll b content of plants treated with CF was 14.74±0.21, while the value of chlorophyll b content of plants treated with CF+SAF and SAF were 27.46±6.20 and 4.72±0.98. Based on Lichtenthaler's equations used in this study, plants treated with CF+SAF had the highest chlorophyll b content and plants treated with SAF had the lowest chlorophyll a content values. The value of chlorophyll a content in plants treated with control, CF, and SAF was always higher

than the value of chlorophyll b content, while plants treated with CF+SAF had chlorophyll b content higher than chlorophyll a content. The content of chlorophyll a and chlorophyll b in paddy leaves based on Lichtenthaler's equations can be seen in Figure 1.

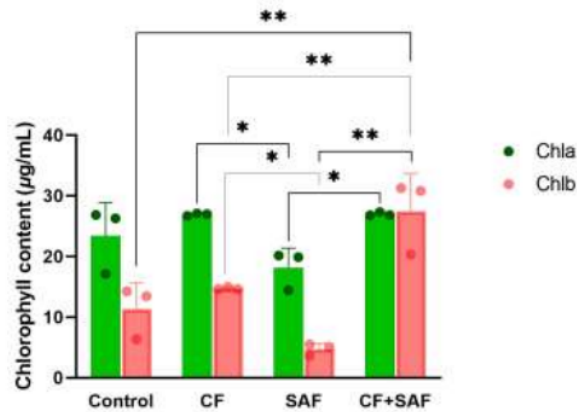


Figure 1. The content of chlorophyll a and chlorophyll b in paddy plants with control treatment, CF, SAF, and CF+SAF calculated by Lichtenthaler's equations, the highest value of chlorophyll a content was obtained in CF+SAF treatment but not significantly different from the control while the highest value of chlorophyll b content obtained in the CF+SAF treatment and significantly different from the control. * indicates significantly different ($p < 0.05$). ** indicates highly significantly different ($p < 0.01$).

3.2 The content of chlorophyll a and chlorophyll b in the leaves with the application of SAF (Arnon's equation)

Based on the results of two-way ANOVA statistical analysis after calculating the value of chlorophyll content using the Arnon equation, it was found that the chlorophyll a content in plants with CF, SAF, CF + SAF media was not significantly different from control plants. The value of chlorophyll a content of control plants was 24.26 ± 5.61 . Paddy plants treated with SAF in the growing media had significantly less chlorophyll a than paddy plants treated with CF and CF+SAF. The value of chlorophyll a content of plants treated with SAF was 18.95 ± 3.37 , while the value of chlorophyll a content of plants treated with CF and CF+SAF were 27.85 ± 0.23 and 27.49 ± 0.53 . Based on the Arnon equation used in this study, plants given CF+SAF had the highest chlorophyll a content and plants given SAF had the lowest chlorophyll a content values. Using the same equation, it was also known that the value of chlorophyll b content in control plants was not significantly different from plants given CF and SAF, but significantly different from plants given CF+SAF. The value of chlorophyll b content in control plants was 15.63 ± 5.40 . Paddy plants that were treated with CF in the growing media had a significantly lower value of chlorophyll b content than paddy plants that were given CF+SAF but had a higher and significant value of chlorophyll b content than plants that were given SAF. The value of chlorophyll b content of plants given CF was 19.55 ± 0.18 , while the value of chlorophyll b content of plants given CF+SAF and SAF was 34.17 ± 7.06 and 7.32 ± 1.35 . Based on the Lichtenthaler's equation used in this study, plants given CF+SAF had the highest chlorophyll b content and plants given SAF had the lowest chlorophyll a content values. The value of chlorophyll a content in plants treated with control, CF, and SAF was always higher than the value of chlorophyll b content, while plants treated with CF+SAF had chlorophyll b content higher than chlorophyll a content. The content of chlorophyll a and chlorophyll b in paddy leaves based on the Arnon's equation can be seen in Figure 2.

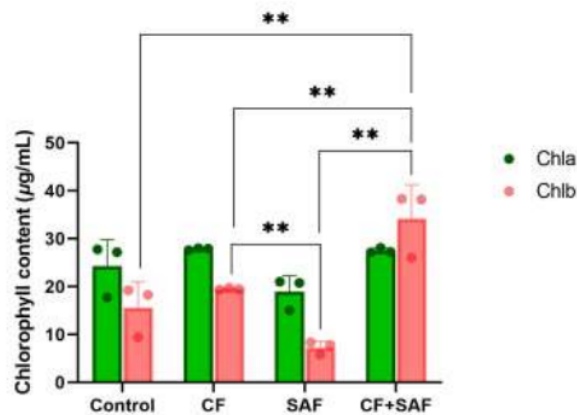


Figure 2. The content of chlorophyll a and chlorophyll b in paddy plants with control treatment, CF, SAF, and CF+SAF calculated by Arnon's equations, the highest value of chlorophyll a content was obtained in CF+SAF treatment but not significantly different from the control while the highest value of chlorophyll b content obtained in the CF+SAF treatment and significantly different from the control. * indicates significantly different ($p < 0.05$). ** indicates highly significantly different ($p < 0.01$).

3.3 Total chlorophyll content in leaves with the application of SAF using the Lichtenthaler's equation and the Arnon's equation methods

The results of the calculation of the total chlorophyll content of leaves using the Lichtenthaler's equation and the Arnon's equation were analyzed by two-way ANOVA statistics. The results of the Lichtenthaler's equation analysis showed that the total chlorophyll content in control plants was not significantly different from plants given CF and SAF, but significantly different from plants in growing media given CF + SAF. The value of total chlorophyll content in control plants was 34.77 ± 9.80 . Paddy plants treated with SAF in growing media had less total chlorophyll than paddy plants treated with CF and CF+SAF. The value of total chlorophyll content of plants treated with SAF was 22.88 ± 4.18 , while the value of total chlorophyll content of plants treated with CF and CF+SAF was 41.65 ± 0.39 and 54.44 ± 5.89 . While the results of the analysis using the Arnon's equation, it was found that the total chlorophyll content in control plants was not significantly different from plants given CF and SAF, but significantly different from plants in growing media given CF + SAF. The value of total chlorophyll content in control plants was 39.89 ± 911.00 . Paddy plants treated with SAF in growing media had less total chlorophyll than paddy plants treated with CF and CF+SAF. The value of total chlorophyll content of plants treated with SAF was 26.27 ± 4.70 , while the value of total chlorophyll content of plants treated with CF and CF+SAF were 47.41 ± 0.35 and 61.66 ± 6.53 . The results of the calculation of the total chlorophyll content using the Arnon's equation method were always higher in all treatments than the results of calculations using the Lichtenthaler's equation. The content of chlorophyll total in paddy leaves based on the Lichtenthaler's equation and Arnon's equation can be seen in Figure 3.

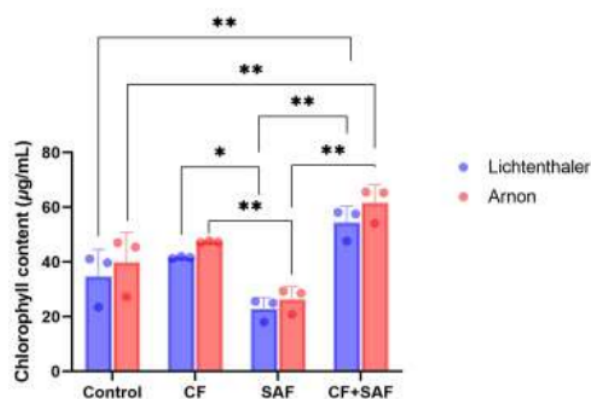


Figure 3. The total chlorophyll content in paddy leaves based on the Lichtenthaler equation and the Arnon equation can be seen in Figure 3. * indicates significantly different ($p < 0.05$). ** indicates highly significantly different ($p < 0.01$).

3.4 Leaf color is equivalent to leaf chlorophyll content

The phenotypic color of the leaves of paddy plants also indicates the amount of chlorophyll contained in the leaves. Paddy plants with a combination of CF and SAF growing media with a ratio of 50%:50% produced the darkest green color. After being tested by spectrophotometry, it was also found that paddy plants with CF+SAF growing media had the highest chlorophyll content. This phenomenon shows that the darker the leaf color indicates the more chlorophyll content in the leaf [17]. This also shows that SAF can increase leaf chlorophyll chlorophyll when combined with CF. The phenotypic color of the leaves in various treatments can be seen in Figure 4.

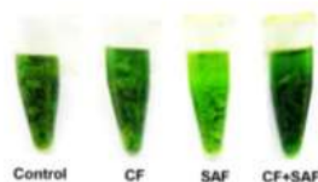


Figure 4. Leaf color is equivalent to leaf chlorophyll content, the darker the leaf color, the more chlorophyll content.

4. Conclusion

The current results suggest that the application of AF to paddy crops can contribute to the development of agricultural sustainability by reducing the use of CF. This is because the administration of SAF mixed with CF can increase the chlorophyll content in the leaves, in particularly chlorophyll b content. Furthermore, leaf color also shows the total leaf chlorophyll content where the darker the leaf color, the higher the total chlorophyll content in the leaf.

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