Effect of Biofertilizer and Salinity on Growth and Chlorophyll Content of *Amaranthus tricolor* L.

D U Siswanti and N Umah

1Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

E-mail: dwiumi@ugm.ac.id

Abstract. *Amaranth* (*Amaranthus tricolor* L.) is one of the most popular crop and vegetable in Indonesia. Their growth can decrease due to abiotic stress on agricultural land. Salinity is one of abiotic stress affects the production of cultivated crops and major problem for agricultural land. Application of biofertilizer can increase availability of nutrients and help plant growth in stress conditions. This research was to analyze the growth and chlorophyll content of *A. tricolor* L. after application of biofertilizer under salinity stress conditions and to determine the optimum dose of biofertilizer needed for optimum growth of *A. tricolor* L. under salinity stress condition. The biofertilizer dose used was 10 l/ha, 20 l/ha, and 30 l/ha, each combined with NaCl; 2500, 5000, 7500, and 10000 ppm. The parameters measured were plants' height, number of leaves, and chlorophyll content. The results showed that salinity treatment reduced plant height, number of leaves, and chlorophyll content. Application of 20 l/ha of biofertilizer increased plant height and number of leaves while application of 10 l/ha of biofertilizer increased chlorophyll content. Generally, it was conducted that biofertilizer can be used to increasing plant growth and chlorophyll content of *A. tricolor* L. under salinity stress condition.

1. Introduction

*Amaranth* (*Amaranthus tricolor* L.) is one of the most popular crops and vegetables in Indonesia. *A. tricolor* L. contains many nutrition such as protein, carbohydrate, vitamin A, and minerals. As a food, amaranth has many health benefits such as improving digestion, its root can be used for the treatment of dysentery and accelerating cell growth [11] and they become a source of economy also quick income for farmers. In Indonesia, amaranth production has increased since 2018 especially in the Special Region of Yogyakarta amaranth production in 2018 is approximately 26,000 tons. However, amaranth production can decrease due to abiotic stress on agricultural land. Salinity is one of the main factors of abiotic stress that affects the production of cultivated crops and is a major problem for agricultural land in the world. Indonesia has a saline land of 440,300 ha while the saline area along the coast reaches 400,000 ha. Salinity on agricultural land is expected to increase which will cause land damage and decreased productivity of cultivated plants [10,14].

The accumulation of salt in agricultural land will have adverse effects on plants, including morphological, physiological, biochemical, and molecular changes. This will affect on plant growth and development by creating osmotic stress conditions, causing specific ion toxicity (Na⁺ and Cl⁻),

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1 Corresponding author. Tel: +62 85228633207
Email addresses: dwiumi@ugm.ac.id (D. U. Siswanti)
stomatal closure, and reducing the rate of photosynthesis [9]. Stress condition will result in decrease of CO₂ fixation which results in decrease of carbon reduction in the Calvin cycle and NADP⁺ oxidation which serves as the last electron receptor in photosynthesis. The increase of salinity also triggers decrease in the rate of transpiration through stomatal closure which can reduce the rate of photosynthesis [4]. In this condition, the availability of N, P, and K elements also becomes difficult for plants to absorb. So that, it is necessary to use appropriate cultivation techniques such as applied a fertilizer that can increase availability of nutrients and help plant growth in stressful condition. Biofertilizer is a fertilizer that contains living microorganisms to stimulate plant growth by increasing nutrient availability and stimulating plant growth. According to Babaei et al. [2] application of biofertilizer increased wheat productivity Triticum aestivum L., chlorophyll content, proline levels, and antioxidant activity under saline conditions. A lot of research has been done about the effect of salinity stress on plant growth. However, research about effect of applying biofertilizer as a biological fertilizer which can increase the availability of nutrients and stimulate the growth of amaranth (Amaranthus tricolor L.) in salinity stress not much reported. Thus, the major objective of this research was to analyze the growth and chlorophyll content of amaranth (Amaranthus tricolor L.) after application of biofertilizer under salinity stress conditions and to determine the optimum dosage of biofertilizer needed for optimum growth of amaranth (Amaranthus tricolor L.) under salinity stress conditions.

2. Material and methods
2.1. Plant materials and treatments
The research was conducted from August – October 2020 in Greenhouse and Plant Physiology Laboratory, Faculty of Biology, Universitas Gadjah Mada. Temperatures ranged from 29°C to 30°C at 10 am. Materials and tools used include Amaranthus tricolor L., soil, NPK fertilizer, biofertilizer, NaCl, filter paper, 80% acetone, polybag, hand sprayer, digital scales, mortar, test tubes, and spectrophotometers. The soil media used is soil which is mixed first with rice husk with a ratio 1:1 and 100 kg/ha of NPK fertilizer. Two seedlings of A. tricolor L. were planted in each polybag. Biofertilizer is made according to Siswanti [12] formula by mixing sheep urine and microbial starter with ratio 49:1 and then made a solution with dosages 10 l/ha, 20 l/ha, and 30 l/ha. Biofertilizer nutritional content includes sheep urine and microbes such as Bacillus sp., Lactobacillus sp., Saccharomyces sp., Streptomyces sp., Azospirillum sp., Pseudomonas sp., Azotobacter sp., Rhizobium sp., and IAA hormone-producing bacteria. That treatment was applied every 5 days after seedling by splashing 0,36 ml for biofertilizer 10 l/ha; 0,72 ml for biofertilizer 20 l/ha; and 1,08 ml for biofertilizer 30 l/ha in each application on the soil surface. The NaCl stock solution is made by dissolving 20 g of NaCl into 1 liter of distilled water and then made a solution with concentrations 2500, 5000, 7500, and 10000 ppm. The treatment of NaCl was applied on day 7 and 14 after seedling by splashing 10 ml of a solution on the soil surface. Watering was carried out once a day, in the morning. If extreme environmental conditions (hot) watering was carried out twice a day, morning and evening. Weeding is done manually by pulling weeds that grow in a polybag.

2.2. Plant growth measurement
Plant height and number of leaves were measured after 2 weeks of treatments. Plant height was measured from the base of the stem to the top of the plant. The number of leaves counted is the leaves that have opened perfectly and are still attached to the stem at the time of observation.

2.3. Chlorophyll content
Chlorophyll a, b, and total chlorophyll were determined using Arnon method (1949) as described in Kumari et al. [7] 0,1 g of finely cut fresh leaves were taken and ground with 10 ml of 80% acetone. The absorbance of the extract was measured at 645 nm and 663 nm using spectrophotometer and an estimate of chlorophyll a, chlorophyll b, and total chlorophyll contents were calculated using the following equation:
Chlorophyll a (mg/g) = \((12.7(A663) - 2.69(A645)) \times V) / (1000 \times W)\)
Chlorophyll b (mg/g) = \((22.9(A645) - 4.68(A663)) \times V) / (1000 \times W)\)
Total Chlorophyll (mg/g) = \((20.2(A645) + 8.02(A663)) \times V) / (1000 \times W)\)

\(W = \) fresh weight of the sample (g)
\(V = \) volume of the sample (ml)

3. Results and discussion

Analysis of variance showed a significant effect between biofertilizer and salinity treatment on plant height, number of leaves, chlorophyll a, b, and total chlorophyll content (Table 1). The application of biofertilizer significantly affected plant height (Figure 1) under salinity treatment. Chlorophyll content was affected by salinity treatment and application of biofertilizer (Figure 3, 4, 5).

Table 1. Effect of biofertilizer and salinity stress (NaCl) on plant height, number of leaves, chlorophyll a, b, and total chlorophyll content of A. tricolor L.

| NaCl Treatment | Control | P1 (10 liters/ha) | P2 (20 liters/ha) | P3 (30 liters/ha) |
|----------------|---------|------------------|------------------|------------------|
| **Plant height (cm)** |         |                  |                  |                  |
| Control        | 17,89 ± 2.95abc | 17,04 ± 1.49ab  | 21,28 ± 2.37de  | 16,74 ± 1.83ab  |
| N1 (2500 ppm)  | 16,23 ± 2.06ab  | 16,71 ± 2.87a   | 21,62 ± 3.97de  | 17,31 ± 1.86abc |
| N2 (5000 ppm)  | 17,49 ± 2.82ab  | 15,05 ± 2.43ab  | 22,66 ± 2.19e   | 19,59 ± 2.78bcde|
| N3 (7500 ppm)  | 16,87 ± 1.78ab  | 16,51 ± 3.29ab  | 22,21 ± 2.05e   | 18,68 ± 1.95bcde|
| N4 (10000 ppm) | 15,11 ± 2.42a   | 16,96 ± 3.72ab  | 22,38 ± 2.89e   | 20,74 ± 4.21cdde|
| **Numb. of leaves** |       |                  |                  |                  |
| Control        | 8,00 ± 0.95bcd | 8,00 ± 0.53bcd  | 8,00 ± 0.79bcd  | 8,00 ± 0.75bcd  |
| N1 (2500 ppm)  | 8,00 ± 0.95bcd | 8,00 ± 0.95bc   | 9,00 ± 0.38d    | 8,00 ± 0.97bcd  |
| N2 (5000 ppm)  | 8,00 ± 0.78bcde| 8,00 ± 1.11abc  | 8,00 ± 0.95cd   | 9,00 ± 0.69d    |
| N3 (7500 ppm)  | 7,00 ± 0.95abc | 8,00 ± 0.95abc  | 8,00 ± 0.53bcd  | 7,00 ± 0.53bcd  |
| N4 (10000 ppm) | 8,00 ± 0.69abcd | 7,00 ± 1.07a   | 8,00 ± 0.97bcd  | 8,00 ± 0.90ab   |
| **Chlorophyll a (mg/g)** |         |                  |                  |                  |
| Control        | 0.41 ± 0.05abc | 0.56 ± 0.06e    | 0.45 ± 0.08abde | 0.42 ± 0.06bcd  |
| N1 (2500 ppm)  | 0.47 ± 0.10bcde| 0.45 ± 0.01abde | 0.45 ± 0.03abde | 0.43 ± 0.07abde |
| N2 (5000 ppm)  | 0.44 ± 0.07abde| 0.49 ± 0.12abde| 0.34 ± 0.08a    | 0.40 ± 0.05abc  |
| N3 (7500 ppm)  | 0.37 ± 0.02ab  | 0.51 ± 0.04cde  | 0.47 ± 0.08abde | 0.45 ± 0.07abde |
| N4 (10000 ppm) | 0.43 ± 0.05abde| 0.55 ± 0.07de   | 0.40 ± 0.07abc  | 0.43 ± 0.03abde |
| **Chlorophyll b (mg/g)** |         |                  |                  |                  |
| Control        | 0.23 ± 0.04abcd | 0.32 ± 0.05d   | 0.24 ± 0.04abde | 0.22 ± 0.03ab   |
Salinity treatment with NaCl induced inhibition of growth in *A. tricolor* L. especially plant height. Data presented in Figure 1 shows that plant height of *A. tricolor* L. without application of biofertilizer (P0) is lower (17.89 cm; 16.23 cm; 17.49 cm; 16.87 cm; 15.11 cm) than after application of biofertilizer dose 10 l/ha (17.04 cm; 16.71 cm; 15.05 cm; 16.51 cm; 16.96 cm), biofertilizer dose 20 l/ha (21.28 cm; 21.62 cm; 22.66 cm; 22.21 cm; 22.38 cm), and biofertilizer dose 30 l/ha (16.74 cm; 17.31 cm; 19.59 cm; 18.68 cm; 20.74 cm). This shows that NaCl treatment is stress in plants. Salinity stress will suppress plant growth processes with effects in inhibiting cell enlargement and division, protein production, and the addition of plant biomass. Plants in this condition generally suppressed growth and gradual change. This is because of the high concentration of dissolved salts causes a decrease in soil potential so that plants lack water. This condition interferes with the process of water absorption and disturbs the plant in maintaining turgor pressure. The presence of specific ions (Na+ and Cl−) will be toxic to plants which can interfere with the integrity and function of the diffusion, disturb the balance of solutes and absorption of nutrients. The accumulation of Na+ and Cl− ions will accumulate in the root tissue, which will cause the roots to have osmotic pressure and the root system is disrupted because cells cannot divide and expand normally [8][9].

![Figure 1. Effect of biofertilizer on plant height of *A. tricolor* L. under salinity conditions](image-url)
Figure 2. Effect of biofertilizer on number of leaves of *A. tricolor* L. under salinity conditions

Salinity (NaCl) also effect on leaf growth, both before the biofertilizer treatment and after the biofertilizer treatment. In unfavourable environmental, plants perform various adaptations to survive in this condition. Plants will reduce number of leaves and decrease leaf size to maintain turgor. In the treatment of biological fertilizers given to plants under saline stress did not show significant leaf growth results. However, providing biological fertilizer at a dose of 20 l/ha gives higher yields than other doses. Biofertilizer doses of 20 l/ha also showed significant results on plants treated with NaCl with concentrations of 2500, 5000, 7500, and 10000 ppm. The addition of biofertilizer can increase plant growth by providing nutrients. The combination of several types of microorganisms in a biofertilizer is more effective than just one type of microorganism. Many combinations of microorganisms increase the supply of phytohormones that can increase plant growth [3]. The combination of types of microorganisms used includes *Bacillus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Pseudomonas* sp., *Azospirillum* sp., *Azotobacter* sp., *Rhizobium* sp., and IAA hormone-producing bacteria. These microorganisms are N-fixing microorganisms, P solvents, decomposers of organic matter, and producing plant growth regulators. Nitrogen (N) is useful for spurring plant growth, especially in the vegetative phase, and plays a role in the formation of chlorophyll, amino acids, fats, and enzymes. Nitrogen also affects the formation of plant roots. The examples of nitrogen fixing microorganisms include *Azotobacter* sp., *Azospirillum* sp., and *Rhizobium* sp. Phosphorus (P) is useful for helping the formation of protein and minerals, stimulating root growth and development, accelerating conception, and fertilization. The groups of phosphate solubilizing microorganisms include *Bacillus* sp., *Pseudomonas* sp., and *Aspergillus* sp. [5]. According to Utomo et al. [16], decomposer microbes are microbes that can remodel organic material using enzymes so that the content in the organic material changes form into energy released in the form of heat and which is readily absorbed by plants. The examples of organic material decomposers include *Lactobacillus* sp. and *Saccharomyces* sp. The application of biofertilizer 10 l/ha and 30 l/ha not significant on plant growth of *A. tricolor* L. The application of biofertilizer at low doses has no effect and is not enough to provide nutrient requirements for amaranth growth, while high biofertilizer doses will cause competition for microorganisms contained in the biofertilizer. This competition will cause competition for nutrients in the soil, so that the level of nutrient availability needed for plant growth decreases so that plant growth is inhibited [13].

The chlorophyll a, b, and total chlorophyll content decreased under salinity treatment. In salinity conditions, reduction of chlorophyll and other pigments finally resulted in the decrease in the
efficiency of photosynthesis. Low chlorophyll content under salinity stress was reported as a result of lower chlorophyll synthesis, destroy the PSII reaction center, inhibit carbonic anhydrase and nitrate reductase activities, an imbalance in the ion flux inside plants, affect membrane stability index, and reduce RWC [2]. Furthermore, the negative effect of abiotic stress on photosynthetic pigments could be due to inhibition of chlorophyll biosynthesis or increase of its degradation by chlorophyllase enzyme, which is more active under stress conditions. Oxidative stress could happen due to salt and water stress leading to deterioration in chloroplast structure and consequently decrease in chlorophyll content [7]. As data presented in the figure 3, 4, and 5, application biofertilizer with dose 10 l/ha increased chlorophyll a (0.56 mg/g; 0.45 mg/g; 0.49 mg/g; 0.51 mg/g; 0.55 mg/g), chlorophyll b (0.32 mg/g; 0.25 mg/g; 0.27 mg/g; 0.29 mg/g; 0.31 mg/g), and total chlorophyll content (0.88 mg/g; 0.70 mg/g; 0.76 mg/g; 0.80 mg/g; 0.86 mg/g) of A. tricolor L. under salinity treatment of NaCl with concentration of 2500, 5000, 7500, and 10000 ppm. The combination of several types of microorganisms in a biofertilizer is more effective. These microorganisms are N-fixing microorganisms, P solvents, decomposers of organic matter, and producing plant growth regulators. Nitrogen (N) element was the most favourable variant for leaf chlorophyll content. It is a structural element of chlorophyll and protein molecules and affects formation of chloroplast and accumulation of chlorophyll. The influence of nitrogen on formation of green pigments in the leaf depends primarily on its concentration. Biofertilizer can increase on chlorophyll content may be due to the improvement of chlorophyll formation and photochemical efficiency of leaf. They maintenance of cell form through improving permeability of plasma membrane due to the increase of anti-oxidative enzymes [1][5]. Biofertilizer dose of 10 l/ha is the optimum dose that can increase the chlorophyll content of Amaranthus tricolor L.

Figure 3. Effect of biofertilizer on chlorophyll an of A. tricolor L. under salinity conditions
Figure 4. Effect of biofertilizer on chlorophyll b of *A. tricolor* L. under salinity conditions

Figure 5. Effect of biofertilizer on total chlorophyll of *A. tricolor* L. under salinity conditions

The application of biofertilizer doses 20 l/ha and 30 l/ha, not significant results. This is presumably because the application of biofertilizer at high biofertilizer doses will cause competition of microorganisms contained in the biofertilizer. This competition will cause competition for nutrients in the soil, so that the microorganisms will use their energy to fight for nutrients. The remaining energy from these is not sufficient to break down inorganic materials in the soil that are ready for uptake for plant so that the availability of nutrients needed for plant growth decreases so that plant growth is inhibited [13].
Figure 6. Light intensity during growing season and measurement

Figure 7. pH value during growing season and measurement
Biofertilizer at a dose of 10 l/ha was more effective in increasing plant chlorophyll content, while that doses do not have an increasing effect on plant height under saline stress conditions. In contrast, a biofertilizer dose of 20 l/ha was more effective in increasing plant height than increasing the chlorophyll content of plants under saline stress conditions. This can occur due to the influence of external factors during growth. External factors that affect plant growth include temperature, soil, pH value, and light intensity. Figure 6 shows the light intensity in each plot (N_P0, N_P1, N_P2, and N_P3) during growing season and measurement. The light intensity on the N_P1 plot is higher than the N_P2 plot, on the other hand the light intensity on the N_P2 plot is lower than the N_P1 plot. Low light intensity on the N_P2 plot will cause plant growth in an etiolated state so that the plants grow taller but are not sturdy due to high water content, the plants look weak, and the leaves are small and pale [15].

4. Conclusion
Our result shows that salinity stress (NaCl) caused a number of physiological and biochemical changes in *Amaranthus tricolor* L. including decreased plant height, number of leaves, and chlorophyll content. Application of biofertilizer dose 20 l/ha improved plant height and number of leaves under salinity condition. Also, the application biofertilizer dose of 10 l/ha increased chlorophyll content under salinity stress conditions.

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