pISSN 2302-1616, eISSN 2580-2909
Vol 9, No. 2, December 2021, pp. 146-155
Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v9i2.21629

Effect of biofertilizer and salinity stress on productivity and vitamin C levels of *Amaranthus tricolor* L.

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ABSTRACT. Abiotic stress is one of the elements that affect plant crop output due to its productivity and environmental factors. Salinity as abiotic stressors can impair plant growth, becoming a concern in the agriculture field in recent years. Biofertilizers are reported to be capable of overcoming salinity stress. Hence, it contains microbial elements in it, play a role for the nitrogen cycle in soil, which can result in increased plant growth. Therefore, this study aimed to analyze the effect of biofertilizer and NaCl as a salinity stress factor on the growth of *Amaranthus tricolor* L. The biofertilizer doses utilized in this study were 10 L/ha, 20 L/ha and 30 L /ha, each in combination with basic manure fertilizer. For the salinity factor, NaCl concentrations of 2500 ppm, 5000 ppm, 7500 ppm, and 10.000 ppm were employed. Environmental characteristics, plant height, number of leaves, root length, plant dry mass, chlorophyll and carotenoid content, as well as vitamin C, were all measured. The parameters were determined quantitatively. The chlorophyll and carotenoid contents were determined using a UV-vis spectrophotometer, while vitamin C levels were determined using iodometric titration. At a 95% level of confidence, the results were examined using the one-way ANOVA approach. The results indicate that a 30 L/ha dose of biofertilizer has an effect on the chlorophyll content and root length of plants, whereas a 10 L/ha dose has an effect on the carotenoid content. The highest amaranth growth was observed when 0 L/ha biofertilizer was combined with a 7500 ppm NaCl treatment, whereas the largest number of leaves was shown when 10 L/ha biofertilizer was combined with a 2500 ppm NaCl treatment. It could be concluded that while biofertilizer has no effect on plant growth parameters, it does increase plant productivity by raising chlorophyll and carotenoid levels.

Keywords: biofertilizer doses; chlorophyll and carotenoid content; growth of amaranth; NaCl concentration; UV-vis spectrophotometer

Article History: Received 25 June 2021; Received in revised form 4 August 2021; Accepted 14 November 2021; Available online 30 December 2021
How to Cite This Article: Khairunnisa NA, Siswanti DU. 2021. Effect of biofertilizer and salinity stress on productivity and vitamin C levels of *Amaranthus tricolor* L. *Biogenesis: Jurnal Ilmiah Biologi*. vol 9(2): 146–155. doi: https://doi.org/10.24252/bio.v9i2.21629.

INTRODUCTION

Food is a fundamental human necessity. To preserve health, humans must meet their body's nutritional requirements. The majority of the world's food is derived from plants. Indonesia is an agricultural country, making agriculture a critical component of the economy. Horticultural crops, one of which is amaranth (*Amaranthus tricolor* L.), have a significant economic impact in Indonesia (Dewi & Fariyanti, 2015; Kementerian Pertanian, 2020). Amaranth is a well-known vegetable in the general society, which has a short vegetative life cycle and as good source of nourishment (Shiyam & Binang, 2011; Mondal *et al*., 2019), making it extremely helpful for farmers.

Amaranthus is a cosmopolitan genus with 70 species, including cosmopolitan weed, or cultivated plant, that is widely distributed in tropical and subtropical regions, such as Southeast Asia (Alegbejo, 2013; Stetter & Schmid, 2017). *A. tricolor* is a common crop in Southeast Asia, as a daily leaf vegetable and one of the most important crops in aspect of economics. However, the market demand for *Amaranthus* plants did not match the amount of production, necessitating improvements (Badan Pusat Statistik, 2020). *A. tricolor* is a cultivated amaranth that frequently used as a vegetable due of its protein content, where the growth is affected by environmental factors, including temperature and soil pH (Andini *et al*., 2013; Jimoh *et al*., 2018; Agil *et al*., 2019).

Biofertilizer is a fertilizer that contains a mixture of free nitrogen fixing bacteria, phosphate solvents, and nutrient solubilizing fungi with a growth-promoting formula and microbial elements needed by plants (Gupta *et al*., 2015; Kalayu, 2019). Since biofertilizers contain microorganisms that deliver nitrogen to plants and nutrients to the soil, farmers can employ these products to create sustainable

farming systems. Our previous studies has established that using a biofertilizer dose of 10 L/ha is capable of increasing the growth of rice plants (Siswanti & Rachmawati, 2013). This study is in line with Elpawati *et al*. (2015), which discovered the biofertilizer to increase corn productivity. Plant growth promoting rhizobacteria (PGPR)-based biofertilizer affects plants by releasing metabolites (usually secondary), root-colonizing bacteria thriving in the plant rhizosphere, bulk soil, and providing nutrients for plant growth (Ibiene *et al*., 2012; Basu *et al*., 2021).

Salt accumulation occurs in the soil as a result of salinity stress. Na^+ and Cl^- are the salt ions that affect plant growth and act as a stress factor in plants, causing ionic imbalances and physiological changes that result in irregular and disproportionate water absorption (James *et al*., 2011; Tavakkoli *et al*., 2011). Salt buildup disrupts metabolism, impairing critical stages of plant growth such as germination, seed growth, vegetative phase, flowering, and development of fruit (Da Silva *et al*., 2016). The content of chlorophyll in a plant defines its condition, which has an effect on its metabolism. This study aims to examine the effect on the growth of *A. tricolor* L. of various biofertilizer doses and NaCl as a salinity stress factor. Increased carotenoids and chlorophyll concentrations in leaf tissue may preserve the photosynthetic apparatus from severe salt stress. *A. tricolor* L. production, which continues to grow despite salinity stress, is critical for Indonesia's efficient utilization of saline area.

MATERIALS AND METHODS

The research was conducted at Laboratory of Plant Physiology, Faculty of Biology, Universitas Gadjah Mada in September-November 2020. This study utilized the formula approach for providing a biofertilizer treatment as our previous studies (Siswanti & Khairunnisa, 2021). Plants were planted in polybags and arranged using RCBD field design methods. Media used for this research were basic manure, soil, and rice husk in 1:1:1 ratio. Biofertilizers and salinity levels were used in this study as treatments. The biofertilizer used was made with the mixture of the cow's urine and a starter of a microbial in the ratio of 49:1 following our previous studies (Siswanti, 2015). Salinity used were 2500, 5000, 7500, and 10000 ppm of NaCl, while biofertilizer doses used were 10 L/ha, 20 L/ha, and 30 L/ha. Treatments were replicated three times. In this study, the parameters measured were plant height, number of leaves, root length, plant dry mass, and vitamin C levels using iodimetric titration, carotenoid content were measured using 480 nm wavelength, and total chlorophyll were measured content using 645 and 663 nm wavelength of UV-vis spectrophotometer. Calculation of vitamin C (ascorbic acid) levels was carried out by the iodimetric titration method, also carotenoid and chlorophyll content as follows (Arnon, 1949; Bruinsma, 1963; Moran & Porath, 1980; Jacobs, 2018):

$$\% \text{ Vitamin C levels} = \frac{\text{Vol Iod} \times \text{N Iod} \times 0.88 \times \text{FP} \times 100}{\text{berat sampel (g)}}$$

$$\text{Carotenoid content (mg/g)} = \frac{[A480+(0.114 \times A663)-(0.638 \times \text{A645})] \times \text{V}}{1000 \times \text{W}}$$

$$\text{Total chlorophyll (mg/g)} = \frac{[(20.2 \times \text{A645})-(8.02 \times \text{A663})] \times \text{V}}{(1000 \times \text{W})}$$

Data analysis. The data was performed using SPSS ver. 20 with one-way ANOVA at 95% of confidence level (α= 0.05%). The results then were proceed using the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The growth parameters and productivity of *Amaranthus tricolor* L. in this study were plant height, the number of leaves, root length, root wet weight, root dry weight, shoot wet weight, shoot dry weight, chlorophyll content, carotenoid content, and ascorbic acid levels. Plant growth parameters included plant height measured from the soil surface to the tip of the highest leaf, the number of fully opened leaves, and root length measured from the root neck to the root tip. The productivity parameters used in this study included measurements of the crown's wet weight and dry weight, chlorophyll content, carotenoid content, and ascorbic acid

content, which indicated the productivity of amaranth plants.

Table 1. Environmental parameters for *Amaranthus tricolor* L. growth.

Biofertilizer plot (L/ha)	Environmental parameter		
	Temp. (℃)	pH	Light intensity
0	29	6	20.198
10	29	6	14.587
20	30	6	14.670
30	30	6	16.258

According to the data from the measurements of environmental condition parameters in Table 1, *A. tricolor* L. thrives in soil conditions that enable optimal growth. *A. tricolor* L. grows at 29°C-30°C with a soil pH of 6. A lux meter indicates a maximum light intensity of 20.198 lux. In line with Rangkuti *et al.* (2017), *A. tricolor* L. productivity can be boosted by providing appropriate nutrients in a pH range of 6-7 and seeding with organic materials such as fertilizer.

Table 2. Effect of variation of NaCl (salinity stress) on growth of *Amaranthus tricolor* L. plant height with biofertilizer doses treatment.

NaCl conc. (ppm)	Biofertilizer (L/ha)			
	0	10	20	30
0	32.24 ± 7.15a	28.44 ± 5.01a	28.23 ± 1.96a	32.84 ± 6.09a
2500	37.74 ± 4.13ab	28.04 ± 4.03a	28.71 ± 3.70a	31.20 ± 4.95a
5000	31.89 ± 6.35a	29.27 ± 2.80a	30.64 ± 4.28a	34.39 ± 4.71a
7500	**40.59 ± 5.09b**	30.34 ± 4.62a	30.14 ± 2.07a	33.53 ± 7.07a
10000	36.66 ± 5.81ab	30.11 ± 3.80a	29.36 ± 3.39a	35.04 ± 6.80a

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

The effect of differences in salinity stress on the height of *A. tricolor* L. is demonstrated using biofertilizer doses of 10 L/ha, 20 L/ha, and 30 L/ha (Table 2). The effect of applying a dose of biofertilizer on the NaCl concentration on the plant height of *A. tricolor* L. was significant when compared to the control treatment or when no biofertilizer was applied. Plant height was not significantly affected by biofertilizer application doses of 10 L/ha, 20 L/ha, or 30 L/ha. *A. tricolor* L. according to Sarker *et al.* (2018), is a plant that can tolerate biotic and abiotic stress conditions, including salinity stress. It retains their ability to develop under salt stress, allowing them to grow optimally. The application of biofertilizer at doses of 10 L/ha, 20 L/ha, and 30 L/ha had no significant effect on the salinity stress treatment of fluctuations in NaCl content. Kumar *et al.* (2017) define biofertilizer as a biological fertilizer whose application results in increased soil quality over time.

Table 3. Effect of dose of biofertilizer 10-30 L/ha on growth of *Amaranthus tricolor* L. height under salinity stress treatment of NaCl.

NaCl conc. (ppm)	*Biofertilizer* (L/ha)			
	0	10	20	30
0	32.24 ± 7.15a	28.44 ± 5.01a	28.23 ± 1.96a	32.84 ± 6.09a
2500	**37.74 ± 4.13b**	28.04 ± 4.03a	28.71 ± 3.70a	31.20 ± 4.95a
5000	31.89 ± 6.35a	29.27 ± 2.80a	30.64 ± 4.28a	34.39 ± 4.71a
7500	**40.59 ± 5.09b**	30.34 ± 4.62a	30.14 ± 2.07a	33.53 ± 7.07a
10000	**36.66 ± 5.81b**	30.11 ± 3.80a	29.36 ± 3.39a	35.04 ± 6.80ab

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

The effect of biofertilizers 10 L/ha, 20 L/ha, and 30 L/ha on the yield of *A. tricolor* L. plant height when treated with NaCl at salinity stress concentrations of 2500 ppm, 5000 ppm, 7500 ppm, and 10000 ppm is presented in Table 3. The treatment of 10000 ppm NaCl with 30 L/ha biofertilizer were considerably different (Table 5). Even under severe NaCl salinity stress, the application of biofertilizer at dose of 30 L/ha was able to promote the growth of *A. tricolor* L. According to the findings presented, the more the amount of biofertilizer applied to *A. tricolor* L., the higher the plants' salinity stress condition. Hoang *et al*. (2020) stated that excessive salinity stress concentrations have a detrimental effect on plant growth due to their unbalanced osmotic and ionic effects.

Table 4. Effect of variation of NaCl (salinity stress) on growth of *Amaranthus tricolor* L. number of leaves with biofertilizer dose treatment.

NaCl conc. (ppm)	Biofertilizer (L/ha)			
	0	10	20	30
0	11 ± 1.13a	12 ± 2.34a	11 ± 1.07a	12 ± 1.41a
2500	14 ± 2.16bc	11 ± 0.69a	11 ± 1.38a	12 ± 0.95a
5000	12 ± 1.25ab	12 ± 0.76a	11 ± 0.90a	13 ± 1.15a
7500	**15 ± 2.29c**	11 ± 0.98a	11 ± 0.90a	12 ± 1.89a
10000	14 ± 3.04bc	11 ± 1.27a	12 ± 1.25a	13 ± 2.48a

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

Table 4 presents results from a calculation of the number of leaves on the *A. tricolor* L. based on the effect of NaCl treatment as salinity stress at 2500 ppm, 5000 ppm, 7500 ppm, and 10000 ppm in the application of 0-30 L/ha biofertilizer. The effect of varying the NaCl concentration with biofertilizer doses of 10 L/ha, 20 L/ha, and 30 L/ha had no discernible effect (Table 6). The effect of modifications in NaCl concentration on the treatment dose of 0 L/ha biofertilizer was significantly different than the effect of 0 ppm NaCl (control).

Table 5. Effect of biofertilizer doses 10-30 L/ha on growth of *Amaranthus tricolor* L. number of leaves under salinity stress of NaCl.

NaCl conc. (ppm)	Biofertilizer (L/ha)			
	0	10	20	30
0	11 ± 1.13a	12 ± 2.34a	11 ± 1.07a	12 ± 1.41a
2500	**14 ± 2.16b**	11 ± 0.69a	11 ± 1.38a	12 ± 0.95a
5000	12 ± 1.25ab	12 ± 0.76a	11 ± 0.90a	13 ± 1.15a
7500	**15 ± 2.29b**	11 ± 0.98a	11 ± 0.90a	12 ± 1.89a
10000	**14 ± 3.04b**	11 ± 1.27a	12 ± 1.25a	13 ± 2.48a

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

Table 5 summarizes the results of a computation of the number of leaves on the *A. tricolor* L. in response to biofertilizer doses of 0 L/ha, 10 L/ha, 20 L/ha, and 30 L/ha. At the 95% level DMRT, there were significant differences in the dose of 0 ppm biofertilizer at NaCl concentrations of 2500 ppm, 7500 ppm, and 10000 ppm (Table 7). Under salinity stress, treatment without a dose of biofertilizer resulted in a higher number of leaves than salinity stress treatment. The effect of light intensity was significant for the treatment without biofertilizer, which achieved the highest average light intensity of 20.198 lux, as shown in Table 3.

Table 6. Effect of variation of NaCl (salinity stress) on growth of *Amaranthus tricolor* L. root length with biofertilizer dose treatment.

NaCl conc. (ppm)	Biofertilizer (L/ha)			
	0	10	20	30
0	11.63 ± 0.92a	11.57 ± 3.22a	12.40 ± 2.91a	11.57 ± 6.00a
2500	13.83 ± 3.72a	9.57 ± 0.70a	10.60 ± 1.74a	11.17 ± 2.69a
5000	10.83 ± 3.81a	8.43 ± 0.93a	8.93 ± 2.14a	11.07 ± 3.56a
7500	10.43 ± 1.46a	11.10 ± 3.61a	13.23 ± 3.73a	**14.10 ± 4.77a**
10000	9.6 ± 0.56a	10.60 ± 1.42a	10.63 ± 3.53a	**14.07 ± 0.45a**

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

Table 6 illustrates the root length measurement of the *A. tricolor* plant L. as a response of NaCl variation. The effect of NaCl variation on the dose of biofertilizer 0-30 L/ha was not significant in the DMRT at the 95%. The results were not significant for each biofertilizer treatment against salinity stress, showing that the root length yielded the maximum yield in the 7500 ppm NaCl treatment with 30 L/ha biofertilizer treatment, compared to other NaCl concentration variations. In comparison to salinity stress treatments at lower concentrations, root length was optimal between 7500 and 10000 ppm NaCl. Biofertilizers rely on the microorganisms contained within them to colonize the rhizosphere on the surface of plants or soil and promote plant development by delivering nutrients such as root elongation. The active elements in biological fertilizers are often nitrogen-fixing microbes, phosphorus solvents, and growth regulators. *Azotobacter*

chroococcum and *Rhizobium leguminosarum* are nitrogen-fixing microorganisms, while *Aspergillus niger* and *Bacillus cereus* are phosphate-solubilizing microorganisms (Kartikawati *et al*., 2017). This is consistent with our study, which utilized *Bacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Azospirillum* sp., *Pseudomonas* sp., *Azotobacter* sp., *Rhizobium* sp., and IAA-producing bacteria as starters.

Table 7. Effect of biofertilizer doses 10-30 L/ha on growth of *Amaranthus tricolor* L. root length under salinity stress of NaCl.

NaCl conc. (ppm)	Biofertilizer (L/ha)			
	0	10	20	30
0	11.63 ± 0.92a	11.57 ± 3.22a	12.40 ± 2.91a	11.57 ± 6.00a
2500	13.83 ± 3.72a	9.57 ± 0.70a	10.60 ± 1.74a	11.17 ± 2.69a
5000	10.83 ± 3.81a	8.43 ± 0.93a	8.93 ± 2.14a	11.07 ± 3.56a
7500	10.43 ± 1.46a	11.10 ± 3.61a	13.23 ± 3.73a	**14.10 ± 4.77a**
10000	9.6 ± 0.56a	10.60 ± 1.42a	10.63 ± 3.53a	**14.07 ± 0.45b**

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

The effect of a 30 L/ha biofertilizer dose produced a 95% difference in the DMRT test under conditions of 10000 ppm salinity stress compared to the control treatment (Table 7). Under conditions of 10000 ppm salinity stress, a biofertilizer dose of 30 L/ha had a positive effect on *A. tricolor* L. root length. Root length grew with each incremental dose of 10 L/ha, 20 L/ha, and 30 L/ha of 7500 ppm NaCl, demonstrating that the biofertilizer at a higher dose was capable of increasing root length growth at a 7500 ppm NaCl concentration. The *Azotobacter* strain utilized in this study is involved in the production of growth hormones such as IAA hormones, cytokinins, gibberellins, and abscisic acid. IAA hormone or endogenous auxin produced by these bacteria is utilized to limit lateral shoot growth, promote abscission, aid in the production of xylem and phloem tissues, and affect root development and elongation (Aly *et al*., 2012; Kurepin *et al*., 2014; Jnawali *et al*., 2015).

Table 8. Effect of variation of NaCl (salinity stress) on growth of *Amaranthus tricolor* L. dry mass with biofertilizer dose treatment.

NaCl conc. (ppm)	Biofertilizer (L/ha)			
	0	10	20	30
Root dry weight (g)				
0	0.13 ± 0.08ab	0.07 ± 0.04a	0.15 ± 0.05a	0.11 ± 0.03a
2500	0.20 ± 0.12ab	0.06 ± 0.04a	0.09 ± 0.06a	0.12 ± 0.06a
5000	0.07 ± 0.05a	0.09 ± 0.03a	0.06 ± 0.02a	**0.16 ± 0.04a**
7500	**0.21 ± 0.03b**	0.11 ± 0.05ab	0.15 ± 0.10a	**0.16 ± 0.04a**
10000	0.14 ± 0.03ab	**0.18 ± 0.06b**	0.08 ± 0.01a	0.13 ± 0.03a
Head dry weight (g)				
0	0.76 ± 0.43a	0.39 ± 0.16a	0.87 ± 0.33a	0.68± 0.24a
2500	1.12 ± 0.63a	0.40 ± 0.04a	0.64 ± 0.19a	**0.76 ± 0.11a**
5000	0.67 ± 0.18a	0.58 ± 0.07ab	0.47 ± 0.08a	**0.81 ± 0.30a**
7500	**1.27 ± 0.48a**	0.70 ± 0.23ab	0.57 ± 0.39a	0.71 ± 0.08a
10000	0.73 ± 0.16a	**0.74 ± 0.23b**	0.48 ± 0.09a	0.73 ± 0.34a

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

The effect of NaCl variation on the dose of 10 L/ha biofertilizer in the DMRT is 95% higher than the control treatment at a NaCl 10000 ppm (Table 8). The 7500 ppm NaCl treatment without the addition of biofertilizer resulted in the highest root dry weight and head weight. Productivity is influenced by the growth of head and root dry weight. Because roots move nutrients and water from the soil and support development, root expansion will effect head growth. The more nutrients obtained from the soil and the greater the root reach, the more favourable the canopy growth.

Table 9. Effect of biofertilizer doses 10-30 L/ha on growth of *Amaranthus tricolor* L. dry mass under salinity stress of NaCl.

NaCl conc. (ppm)	Biofertilizer (L/ha) 0	10	20	30
Root dry weight (g)				
0	0.13 ± 0.08a	0.07 ± 0.04a	0.15 ± 0.05a	0.11 ± 0.03a
2500	0.20 ± 0.12a	0.06 ± 0.04a	0.09 ± 0.06a	0.12 ± 0.06a
5000	0.07 ± 0.05a	0.09 ± 0.03a	0.06 ± 0.02a	**0.16 ± 0.04b**
7500	**0.21 ± 0.03a**	0.11 ± 0.05a	0.15 ± 0.10a	0.16 ± 0.04a
10000	0.14 ± 0.03ab	**0.18 ± 0.06b**	0.08 ± 0.01a	0.13 ± 0.03ab
Head dry weight (g)				
0	0.76 ± 0.43a	0.39 ± 0.16a	0.87 ± 0.33a	0.68± 0.24a
2500	1.12 ± 0.63a	0.40 ± 0.04a	0.64 ± 0.19ab	**0.76 ± 0.11ab**
5000	0.67 ± 0.18a	0.58 ± 0.07a	0.47 ± 0.08a	**0.81 ± 0.30a**
7500	**1.27 ± 0.48b**	0.70 ± 0.23ab	0.57 ± 0.39a	0.71 ± 0.08ab
10000	0.73 ± 0.16a	0.74 ± 0.23a	0.48 ± 0.09a	0.73 ± 0.34a

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

The data in Table 9 pertain to the dry weight measurements of *A. tricolor* L. roots and shoots in relation to the effect of biofertilizer dose administration. The dose of biofertilizer had a significant effect on root dry weight in the DMRT test when applied at a dose of 30 L/ha with a NaCl 5000 ppm. The treatment without biofertilizer and the delivery of 7500 ppm NaCl resulted in the maximum dry weight of the shoots. In comparison to other treatments, this one produced significant results.

The effect of biofertilizer dose was significant at 30 L/ha with no NaCl concentration (control) and 5000 ppm NaCl concentration (95%) in the DMRT test (Tabel 10). The maximum value for total chlorophyll content was 1.32±0.27 mg/g in the treatment without NaCl and biofertilizer 30 L/ha, showing that salinity stress conditions had an effect on the assessment of these parameters. When applied at a dose e of 30 L/ha, NaCl 7500 ppm enhanced chlorophyll levels marginally, while the total chlorophyll content dropped when treated at 10000 ppm.

Table 10. Effect of variation of NaCl (salinity stress) on growth of *Amaranthus tricolor* L. chlorophyll content with biofertilizer dose treatment.

NaCl conc. (ppm)	Biofertilizer (L/ha) 0	10	20	30
0	0.94 ± 0.18a	0.88 ± 0.10a	0.99 ± 0.02a	**1.32 ± 0.27b**
2500	0.88 ± 0.04a	0.87 ± 0.09a	0.87 ± 0.07a	1.01 ± 0.15ab
5000	0.96 ± 0.34a	0.95 ± 0.12a	1.07 ± 0.27a	0.84 ± 0.23a
7500	0.78 ± 0.26a	0.91 ± 0.08a	1.13 ± 0.22a	1.07 ± 0.21ab
10000	1.00 ± 0.12a	1.06 ± 0.18a	0.88 ± 0.12a	0.95 ± 0.30ab

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

Sarker & Oba (2020) state that Na^+ and K^+ ions will bind to and impede the synthesis of metabolic enzymes and proteins, and that a buildup of NaCl in the soil will diminish the water potential, resulting in osmotic pressure. Concentrations of high salinity stress can diminish stomatal conductivity, decreasing the quantity of CO_2 in the leaves.

Table 11. Effect of biofertilizer doses 10-30 L/ha on growth of *Amaranthus tricolor* L. chlorophyll content under salinity stress of NaCl.

NaCl conc.(ppm)	Biofertilizer (L/ha) 0	10	20	30
0	0.94 ± 0.18a	0.88 ± 0.10a	0.99 ± 0.02a	**1.32 ± 0.27b**
2500	0.88 ± 0.04a	0.87 ± 0.09a	0.87 ± 0.07a	1.01 ± 0.15a
5000	0.96 ± 0.34a	0.95 ± 0.12a	1.07 ± 0.27a	0.84 ± 0.23a
7500	0.78 ± 0.26a	0.91 ± 0.08a	1.13 ± 0.22a	1.07 ± 0.21a
10000	1.00 ± 0.12a	1.06 ± 0.18a	0.88 ± 0.12a	0.95 ± 0.30a

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

The dose of biofertilizer had a significant effect on the DMRT 95% at 30 L/ha with no NaCl concentration (control) (Table 11). As in our earlier study (Siswanti & Umah, 2021), 10 L/ha of biofertilizer increased *A. tricolor* L. chlorophyll content. Apart from *Azotobacter* sp., *Rhizobium* sp., and *Azospirillum* sp., the nitrogen-fixing microorganisms present in the composition of the biofertilizer employed in

this study are nitrogen-fixing microorganisms that benefit leaves by delivering nitrogen (N) elements. Amaranth leaves' enhanced chlorophyll concentration ensures a high rate of photosynthesis.

Table 12. Effect of variation of NaCl (salinity stress) on growth of *Amaranthus tricolor* L. carotenoid content with biofertilizer dose treatment.

NaCl conc. (ppm)	Biofertilizer (L/ha)			
	0	10	20	30
0	0.028 ± 0.004a	0.024 ± 0.005a	0.027 ± 0.002a	0.032 ± 0.004a
2500	0.024 ± 0.002a	0.025 ± 0.004a	0.026 ± 0.006a	0.027 ± 0.005a
5000	0.028 ± 0.011a	0.026 ± 0.003a	0.029 ± 0.006a	0.023 ± 0.006a
7500	0.023 ± 0.004a	0.027 ± 0.003a	0.030 ± 0.007a	0.030 ± 0.003a
10000	0.029 ± 0.002a	**0.036 ± 0.008b**	0.024 ± 0.003a	0.028 ± 0.009a

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

Table 12 summarizes findings from measurements of carotenoid levels in *A. tricolor* L. leaves in response to fluctuations in the NaCl content. The effect of the biofertilizer dose was significant in the DMRT 95 % with 10000 ppm NaCl and 10 L/ha biofertilizer dose, compared to the control treatment with NaCl and 10 L/ha biofertilizer dose. Carotenoid levels were highest at 10000 ppm NaCl, followed by 2500 ppm, 5000 ppm, and 7500 ppm. This could imply that the biofertilizer dose of 10 L/ha at 10000 ppm is capable of maintaining salinity conditions by boosting photosynthetic carotenoid pigments. Carotenoids are natural dyes produced by amaranth that are beneficial as pigments in flowers and fruit, as well as color pigments involved in photosynthesis.

The treatment without NaCl (control) and biofertilizer at a dose of 10 L/ha and 30 L/ha significantly difference the DMRT 95% as compared to the control (Table 13).

Table 13. Effect of biofertilizer doses 10-30 L/ha on growth of *Amaranthus tricolor* L. carotenoid content under salinity stress of NaCl.

NaCl conc. (ppm)	Biofertilizer (L/ha)			
	0	10	20	30
0	0.028 ± 0.004ab	**0.024 ± 0.005a**	0.027 ± 0.002ab	**0.032 ± 0.004b**
2500	0.024 ± 0.002a	0.025 ± 0.004a	0.026 ± 0.006a	0.027 ± 0.005a
5000	0.028 ± 0.011a	0.026 ± 0.003a	0.029 ± 0.006a	0.023 ± 0.006a
7500	0.023 ± 0.004a	0.027 ± 0.003a	0.030 ± 0.007a	0.030 ± 0.003a
10000	0.029 ± 0.002ab	0.036 ± 0.008b	0.024 ± 0.003a	0.028 ± 0.009ab

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

There is no significant difference between the biofertilizer control and the biofertilizer doses of 10 L/ha, 20 L/ha, and 30 L/ha when compared to the respective biofertilizer control treatment (Table 15). Significant differences in the NaCl control between the 10 L/ha and 30 L/ha biofertilizer doses revealed that the 10 L/ha biofertilizer application had the lowest carotenoid levels and the 30 L/ha biofertilizer application had the highest carotenoid levels.

Table 14. Effect of variation of NaCl (salinity stress) on growth of *Amaranthus tricolor* L. vitamin C levels with biofertilizer dose treatment.

NaCl conc. (ppm)	Biofertilizer (L/ha)			
	0	10	20	30
0	**12.52 ± 1.47a**	12.71 ± 1.47a	13.10 ± 6.85a	**12.51 ± 3.01a**
2500	16.23 ± 7.99a	13.49 ± 2.12a	14.47 ± 3.77a	13.69 ± 4.44a
5000	**17.99 ± 10.44a**	12.52 ± 4.44a	13.10 ± 5.32a	15.26 ± 1.02a
7500	14.27± 4.16a	**12.12 ± 4.24a**	**12.51 ± 2.44a**	14.67 ± 2.69a
10000	17.21 ± 6.10a	**16.04 ± 1.47a**	14.08 ± 1.17a	13.10 ± 4.44a

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

Vitamin C levels were determined by observing the effect of salinity stress with varied NaCl concentrations in the biofertilizer treatment (0-30 L/ha) and comparing them to the control, which showed no significant difference at the 95% confidence level (Table 14). This suggests that there is no discernible difference between salinity stress treatments with varying biofertilizer dosages. The highest levels of vitamin C were obtained with a salinity stress of 5000 ppm and the application of biofertilizer 0 L/ha, yielding 17.99±10.44, while the lowest levels of vitamin C were obtained with a salinity stress treatment of 7500 ppm with the biofertilizer 10 L/ha, yielding 12.12±4.24.

Table 15. Effect of biofertilizer doses 10-30 L/ha on growth of *Amaranthus tricolor* L. vitamin C levels under salinity stress of NaCl.

NaCl conc. (ppm)	Biofertilizer (L/ha)			
	0	10	20	30
0	**12.52 ± 1.47a**	12.71 ± 1.47a	13.10 ± 6.85a	**12.51 ± 3.01a**
2500	16.23 ± 7.99a	13.49 ± 2.12a	14.47 ± 3.77a	13.69 ± 4.44a
5000	**17.99 ± 10.44a**	12.52 ± 4.44a	13.10 ± 5.32a	15.26 ± 1.02a
7500	14.27± 4.16a	**12.12 ± 4.24a**	**12.51 ± 2.44a**	14.67 ± 2.69a
10000	17.21 ± 6.10a	**16.04 ± 1.47a**	14.08 ± 1.17a	13.10 ± 4,.44a

Note: The similarity of the letters behind the numbers in the same column shows that there is no significant difference at the level = 5% of the DMRT test.

A dose of 30 L/ha biofertilizer was able to maintain plant metabolism under salt stress conditions of 7500 ppm (Table 15). The application of biofertilizer to amaranth plants was able to maintain the plants' condition when they were exposed to salinity stress, suppress the creation of reactive oxygen species (ROS) by the plants, and decrease the levels of activated antioxidants, in this case, vitamin C. By supplementing with a biofertilizer containing *Bacillus cereus*, the production of ascorbic acid peroxidase can be increased (Khan *et al.*, 2020), hence enhancing the cellular level of enzyme substrates and increasing resistance to salt stress in the environment. According to Qin *et al.* (2013), amaranth is a resistant plant to salinity stress and still productive when the NaCl concentration is less than 5000 parts per million. We emphasize in our study that increasing amaranth's production and quality as an edible vegetable are critical, as the community's demand has not been adequately met. Green vegetable production centers that are environmentally friendly and capable of withstanding salt stress condition are a new hope for Indonesia.

CONCLUSION

Amaranthus tricolor L. growth and production were influenced by biofertilizer treatment and fluctuations in NaCl content, hence maintaining salinity stress conditions. Without using biofertilizers, NaCl treatment of salinity stress improved vitamin C levels to 5000 ppm. Biofertilizer doses of 10 L/ha and 30 L/ha have the optimal effect on *A. tricolor* L. growth and productivity.

ACKNOWLEDGEMENTS

The authors would like to thank Nafiatul Umah and Okky Shavira Riesty for the cooperation during the research. This study was supported by RTA grant UGM No. 2488/UNI.P.III/DIT-LIT/PT/2020.

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