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Response of root anatomy and vitamin C content of *Brassica juncea* L. on biofertilizer application in a saline environment

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ABSTRACT. Indian mustard (*Brassica juncea* L.) is part of the family Brassicaceae which has an annual-herbaceous habitus and is counted as a popular vegetable commodity in Indonesia so it has a high-demand market. To fulfill the demand market can be done by hydroponic cultivation and biofertilizer application. Application of biofertilizer which contains inoculant microorganisms can support plant growth with increased availability of nutrients in planting medium and neutralized salinity stress effect. Salinity stress is indicated by the high concentration of Na^+ and Cl^- ions in plants resulting in toxicity and osmotic stress which will inhibit plant growth. This study aimed to determine the effect of various dosages of biofertilizer on plant growth and productivity with plant height, leaf number, cortex thickness, metaxylem diameter, and ascorbic acid levels as parameters. This research used cow urine base biofertilizer containing nitrogen (N), phosphate (P), kalium (K), and calcium (Ca). There are a few treatments: negative control, positive control, biofertilizer treatment 40 mL, 80 mL, and 120 mL dosage, and salinity treatment by applying NaCl 5,000 ppm 10 mL/system. The diameter of root cortex and metaxylem was determined by making embedding preparations across the *Brassica* roots and the vitamin C content measured by iodometric titration. As the result showed on 120 mL biofertilizer dosage has the highest mean of plant height and leaves number, and 80 ml biofertilizer dosage treatment has the highest average of cortex thickness and metaxylem diameter, but the various dosage of biofertilizer treatment did not have a significant effect on ascorbic acid levels increase. Growth in plants given biofertilizer is higher than the plant growth that grew in the control treatment. It can be concluded that the application of biofertilizer can support the growth of *B. juncea* L. in the hydroponic system under salinity stress.

Keywords: biofertilizer; *Brassica juncea* L.; NaCl, salinity stress; wick system hydroponic

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INTRODUCTION

Brassica juncea L. which can be called Indian Mustard, Chinese mustard, or brown mustard is a part of the family Brassicaceae and is widely cultivated in Asian regions which has a subtropical climate. *B. juncea* has an annual-herbaceous habitus, erected branched stem, and obovate leaves with serrated edges (*mt.gov*, 2012; Rai *et al*., 2022). Indian mustard has a medium level tolerance of to salinity stress. Salinity stress affects various metabolic and physiological processes in plants which are determined by the concentration levels, duration, and severity of stress (Gupta & Huang, 2014). Salinity stress suppresses plant growth through osmotic stress, followed by ion toxicity caused by a high accumulation of Na^+ and Cl^- ions. That will reduce water absorption due to the osmotic effect on roots, oxidative stress due to excess production of reactive oxygen species (ROS), and deficient K^+ (Mahjoor *et al*., 2016; Bhattarai *et al.*, 2020). Reactive oxygen species (ROS) that are formed in plants can bring damage to nucleic acid, so the plants will synthesize several enzyme and non-enzyme compounds such as antioxidants, such as ascorbic acid to maintain the optimal conditions of plants (Kumar *et al*., 2021; Paciolla *et al*., 2019). Ascorbic acid can reduce ROS production through electron donors in the ascorbate-glutathione pathway (Al Farisy & Jadid, 2018).

Hydroponic is one of the planting methods that use a medium other than soil to provide mechanical support for roots to anchor and absorb nutrients that dissolved in a water medium. In this method, plants are grown in a state where the roots are suspended in a water medium containing nutrients. The hydroponic method has several advantages including the availability of water, temperature, and humidity can be regulated relatively, and fewer pests such as weeds and insects to be found (Sharma *et al*., 2018). Generally, AB Mix is used in hydroponic as a nutrient solution. It's composed of stock A containing macronutrients and stock B containing micronutrients. However, it has disadvantages such as being made from synthetic chemicals, being difficult to obtain, and being expensive (Hidayanti & Kartika, 2019) so an alternative is needed, like using organic fertilizer or biofertilizer.

Biofertilizers can be described as microorganism inoculants containing nitrogen-fixing bacteria, phosphate solvent bacteria, and nutrient-solving fungi which can provide plant growth (Elpawati & Dasumiati, 2015; Alami *et al*., 2017). Biofertilizers increase plant growth and productivity by the colonization of microorganisms in the rhizosphere which will provide the nutrient for the plant (Kartikawati *et al*., 2017). In the previous study by (Siswanti & Umah, 2021), the application of biofertilizer on *Amaranthus* sp. resulted in an increase in the length of the stem and the number of leaves. Siswanti *et al* (2019) showed the significant effect of biofertilizer application in the root's anatomical structure on root metaxylem diameter and root pericarp thickness as well as increasing capsaicin content in the fruit of *Capsicum annuum* L. The high concentration of carbon and nitrogen contained in biofertilizers accelerates plant growth. Research conducted by Arif et al (2019), *Brassica napus* which growth under salinity stress experienced damage to its leaves and flowers. Apart from that, there were changes in root structure and morphology, there was an increase in the length of the third-order lateral root and increased in the length and density of root hair as well as root surface area, whereas the diameter decreased. Therefore, this research was conducted to determine the effect of various dosages of biofertilizer on Indian Mustard growth (*B. juncea*) grown in a hydroponic system.

MATERIALS AND METHODS

This research was conducted at Green House Sawitsari, Laboratory of Plant Physiology, and Laboratory of Plant Structure and Development, Faculty of Biology, Universitas Gadjah Mada in March-September 2022.

Plant treatment. This research was initiated by planting *B. juncea* seeds in the hydroponic wick system using rock wool and roasted husk as root anchor medium. Then the 5 best-similar individuals' plants will be selected to observe their growth. The biofertilizer is made from cow urine mixed with bacteria inoculant (*Bacillus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Azospirillum* sp., *Pseudomonas* sp., *Azotobacter* sp., dan *Rhizobium* sp.) and IAA (*Indole3-Acetic Acid*) producing bacteria (Khairunnisa & Siswanti, 2021; Siswanti & Umah, 2021). The negative treatment plant growth in water and the biofertilizer treatment is applied by dissolving biofertilizer in water with a dose of 1:100 for water with various dosages of 40 mL, 80 mL, and 120 mL for each system. Salinity stress is given by adding 5,000 ppm NaCl solution 10 mL/system which is applied after 7 days after planting once a week. The application of biofertilizer and NaCl solution was repeated 5 times. During the growth period, measurement of growth parameters including plant height and the number of leaves is carried out, followed by measuring the environmental parameter including the temperature of air and medium and pH medium.

Vitamin C level measurement. Vitamin C levels measured with the iodometric titration method referring to the procedure (Ernest *et al*., 2017) begin with sample preparation which is then titrated with I_2 standard solution (0.01 N) until it forms a blue complex after adding starch 1%. Vitamin C levels are calculated following AOAC (1995) in Tandikurra *et al* (2019).

$$Vit.C\left(\frac{\text{mg}}{100}g\right) = \frac{\text{V iod} \times 0{,}88 \times \text{Fp}}{\text{Sample weight (g)}} \times 100$$

Roots anatomy observation. Observation of root anatomy begins with preparing embedding preparations. The plant roots sample were processed through fixation, dehydration and dealcoholization, infiltration, enveloping, slicing, coloring, and sealing (Sutikno, 2006). The preparations were then observed with a light microscope connected to Optilab. Observations are focused on observing cortex tissue and the metaxylem of roots. The results of observations were captured and stored for cortex thickness and metaxylem diameter measurement using Image Raster 3 software.

Data analysis. The obtained data measurement was analyzed using SPSS ver. 27, carried out with ANOVA test, then continued with DMRT test using 95% (α=0,05) of significant level when the result showed a significant level effect of treatment.

RESULTS AND DISCUSSION

The measured plant growth and productivity parameters in this study are plant height, leaf number, cortex thickness, metaxylem diameter, and vitamin C level. Height of the plant is measured with measurement tape, starting from the surface of the planting media to the tip of plant leaves and continued with counting the number of leaves. Cortex thickness and metaxylem diameter measurements were carried out by observing the cross-sectional preparation of plant roots. It was measured from the picture was taken with Optilab which then measured using Image Raster 3. Vitamin C levels were measured by iodometric titration on the sample filtrate. The result of the measurements that have been analyzed are presented in tabular form below:

Table 1. Effect of various dosages of biofertilizer application on plant height, number of leaves, cortex thickness, metaxylem diameter, and vitamin C levels of Indian Mustard (*B. juncea*) in saline environment hydroponic system

Biofertilizer treatment	Parameter Height of plant (cm)	Number of leaves	Cortical thickness (μm)	Metaxylem diameter (μm)	Vitamin C levels (mg/100 g)
Control	16.67±5.84 d	3.80±0.84 b	92.95±19.78 **b**	43.59±9.30 **b**	**0.019±0.005 a**
AB Mix	36.67±3.26 a	6.80±0.84 a	185.05±19.78 a	65.62±13.16 a	0.017±0.005 ab
40 ml/system	20.08±3.67 cd	3.40±0.55 b	114.35±33.30 **b**	46.16±5.51 **b**	0.013±0.001 ab
80 ml/system	24.67±3.88 bc	3.80±0.84 b	**174.32±30.20 a**	**47.96±8.68 b**	0.011±0.001 b
120 ml/system	**27.10±2.60 b**	**4.00±0.71 b**	136.68±15.40 **ab**	39.64±2.95 **b**	0.012±0.004 ab

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

Growth of plant. Based on Table 1. can be seen that the application of various dosages of biofertilizer showing a significant effect ($p<0.05$) on the plant height parameter compared to the negative control treatment while on leaves number parameter showed that the biofertilizer treatment did not show significant effect compared to the negative control treatment. Biofertilizer treatment with 120 mL of dosage showed the highest average for plant height and the number of leaves which are 27.10±2.60 cm and 4.00±0.71 leave blade. Based on the result, can be seen that increasing the dose of biofertilizer application causes an increase in plant growth. These results are following Siswanti & Umah (2021), which showed that the application of biofertilizer on *Amaranthus* sp. resulting an increase in stem length and the number of leaves of the plant. Biofertilizer has a role in providing nutrient that is ready to absorb for the plant to increase the plant's growth. Inoculants of nitrogen-fixing bacteria, phosphate-solving bacteria, and nutrient solvent fungi contained in biofertilizers can mobilize soil nutrients to ready-to-absorb nutrients for plants through a biological process (Elpawati & Dasumiati, 2015; Alami *et.al*., 2017; Herlinawati *et al*., 2019). Nitrogen-fixing bacteria including *Azotobacter* sp., *Azospirilum* sp., and *Rhizobium* sp. while phosphate-solving bacteria *Aspergillus niger, Bacillus* sp., and *Pseudomonas* sp. (Kartikawati *et al*., 2017).

B. juncea which grows under salinity stress experiences leaf discoloration or chlorosis. Plants under control treatment have green-yellow leaf color, plants in 40 mL and 80 mL biofertilizer treatment have bright green leaves and some leaves have green-yellow color, and in the 120 mL biofertilizer treatment plant has fresh green color leaves (Figure 1). Chlorosis is caused by nutrient deficiencies due to decreased water potential, such as N and M elements which play important role in chlorophyll and photosynthetic pigment formation. Nutrient deficiencies cause a decrease in pigment synthetics and affect the ability of leaves to absorb sunlight so that the process of photosynthesis is disrupted and results in inhibited plant growth and biomass production (Okon, 2019). *B. juncea* that given biofertilizer treatment has greener leaf color due to a higher concentration of dissolved nutrients in the growth medium. It is caused by the colony of bacteria in biofertilizers that can increase the binding and conversion of N which is the main component of chlorophyll. The nitrogen-fixing bacteria include *Bacillus* sp., *Pseudomonas* sp., *Azotobacter* sp., dan *Rhizobium* sp. (Purwaningsih *et al*., 2021; Radhakrishnan *et al*., 2017; Sah *et al*., 2021; Sumbul *et al*., 2020). Therefore, the levels of chlorophyll are increased so the color of *B. juncea* leaves became greener and more optimal in absorbing light and photosynthetic process.

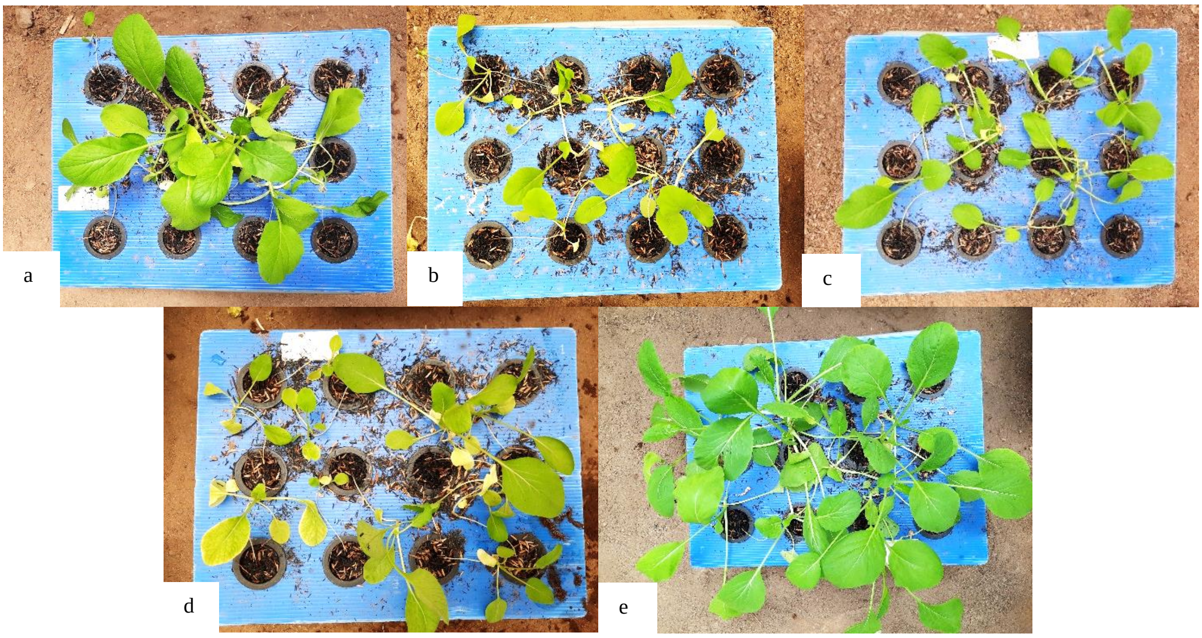

Fig. 1. Mustard Plants (*B. juncea*) in the sixth week of age: a. Biofertilizer 40 mL/system; b. Biofertilizer 80 mL/system; c. Biofertilizer 120 mL/system; d. Control; e. AB Mix

Roots anatomy. From the result in Table 1, the biofertilizer treatment did not show a significant difference ($p>0.05$) in metaxylem diameter and cortical thickness compared to the negative control treatment. The 80 mL dosage of biofertilizer treatment has the highest average metaxylem diameter 47.96±8.68 µm and cortical root thickness is 174.32±30.20 µm. Based on the result, the NaCl which is given as salinity stress can reduce the diameter of the metaxylem and the thickness of the cortex in the *B. juncea* It is caused by inhibiting cell growth due to abnormal cell growth (Karjunita *et al*., 2019).

Salinity stress is caused by a high accumulation of Na^+ and Cl^- ions and for a long time will trigger roots to rearrange their anatomical characteristics to adapt to the environment with salinity stress (Gupta & Huang, 2014; Kheloufi & Mansouri, 2019). NaCl accumulation induces reduction or inhibition of root cell elongation, so the growth of the root cell is unbalanced. This condition will affect in effectiveness and efficiency of roots in absorbing water and nutrients (Karjunita *et al*., 2019). The anatomical structure changes included epidermal cells thickening, endoderm cells thickening, decreased number of vascular bundles, and the protoxylem and metaxylem cells becoming disorganized and deformed (Younis *et al*., 2013; Hoque *et al*., 2018).

The xylem in roots plays an important role in the transport of water and nutrients absorbed by the roots to the leaves. Based on the results in Table 1. the addition of NaCl as salinity stress can reduce the diameter of the root metaxylem and did not show a significant difference between root metaxylem diameter on plants treated with biofertilizer and plants in the control treatment. The decrease in metaxylem diameter is due to the accumulation of NaCl which induces reduced in cells elongation, so the growth of root cells is unbalanced and results in decreasing the roots' ability to water absorb (Gupta & Huang, 2014; Karjunita *et al.,* 2019). Therefore, it is known that increasing the dose of biofertilizer in green mustard (*B. juncea*) has not been able to support the root's metaxylem growth under salinity stress.

Cortical thickness is related to plants' defense mechanism to prevent water loss of plants under stress conditions (Kheloufi & Mansouri, 2019). The decrease in the thickness of the root's cortex is a form of adaptation mechanism in *B. juncea* due to the osmotic stress and ionic imbalance. This will affect cell division, cell expansion, and decreased size in parenchyma cells. It is also caused by insufficient nutrient supply to parenchymal cells, so the cell turgidity is decreased (Setiawati *et al*., 2018; Younis *et al*., 2013). Based on the result, the biofertilizer application to *B. juncea* maintains the optimal condition of the root cortex. This condition is possible due to the addition of external IAA from biofertilizers which has a role in cell division and root expansion (Bhatla & Lal, 2018). This hormone can be synthetic by bacteria such as *Rhizobium*, *Bradyrhizobium*, *Bukholderia*, *Azotobacter*, *Bacillus*, dan *Paenabacillus polymixy* (Fatimah et al., 2022).

Based on the results, *B. juncea* which treated with 120 ml biofertilizer/system had higher growth compared to the plants treated with 80 ml/system biofertilizer but it had a thicker cortex and smaller diameter. Giving high doses of biofertilizer causes high competition between microorganisms in meeting nutrient needs which causes the nutrient needs of microorganisms to not be met so that the performance of microorganisms is not optimal so that the supply of nutrients that are ready to be absorbed by plants is hampered (Siswanti *et. al* 2018; Siswanti & Umah, 2021). Apart from that, this could be because in the application of biofertilizer it is necessary to pay attention to the suitability of the availability of nutrients and the plant's need for nutrients. Providing inappropriate nutrients can cause nutrient deficiencies so that plant growth is hampered (Nuryani *et al*., 2019).

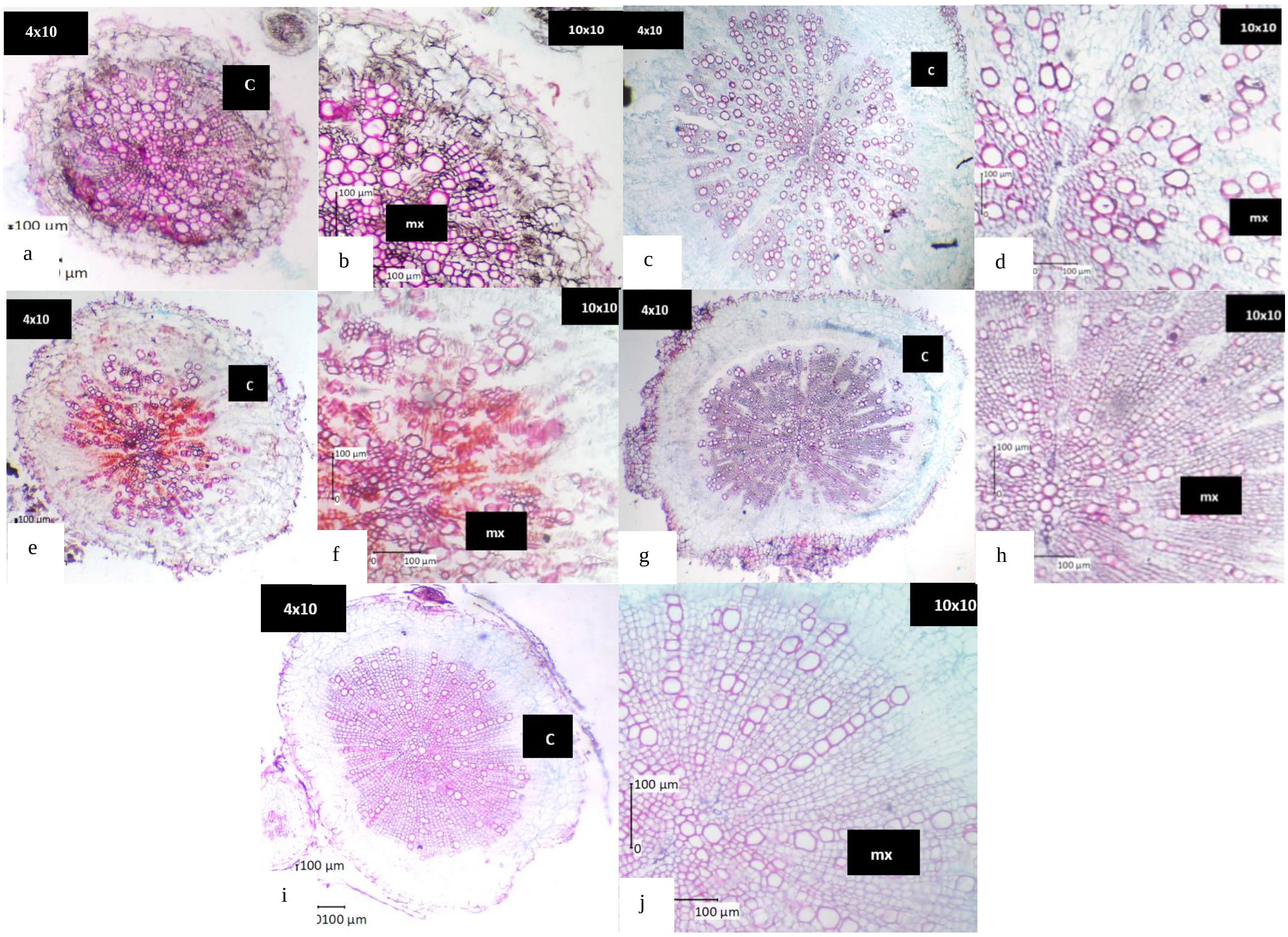

Fig. 2. Cross section of *B. juncea* roots at six weeks of age: a-b. Control; c-d. AB Mix; e-f. Biofertilizer 40 mL/system; g-h. Biofertilizer 80 mL/system; i-j. Biofertilizer 120 mL/system

Vitamin C levels. Based on the result of Table 1. the application of various dosages of biofertilizer did not show a significant difference ($p>0,05$) in vitamin C (ascorbic acid) levels compared with vitamin C levels in negative control plant treatment. The highest results were shown in the control treatment plant 0.019±0.005 mg/100 while the lowest vitamin C levels were shown in the 80 mL biofertilizer treatment 0.011±0.001 mg/100 g. The highest vitamin C level in control treatment due to the plant in control treatment can maintain its condition to remain stable due to salinity stress which increases the reactive oxygen species (ROS) production and antioxidant synthesis, such as vitamin C, to overcome the toxic effect of ROS accumulation by increasing the active vitamin C levels. Vitamin C can reduce ROS production by electron donors in the ascorbate-glutathione pathway. Ascorbate-glutathione or Foyer-Halliwell-Asada pathway is a metabolic pathway for antioxidant defense, especially in the detoxification of H_2O_2 which is produced as a by-product of plant metabolism (Hasanuzzaman *et al*., 2019).

Difference levels of vitamin C in *B. juncea* can be influenced by the environmental factors and abiotic stresses such as salinity stress. It was related to difference plants sensitivity in responding stress. Plant stress tolerance is correlates with the increased biosynthetic of antioxidant, both enzymatic antioxidant and non-enzymatic antioxidant. The activity of enzymatic antioxidant such as APX, MDHAR, and DHAR are increased significantly in plants which grown under salinity stress (Rao *et al.*, 2019). Therefore, in this study, the application of biofertilizer help to maintain the plant condition grown in a saline environment, and reduces the production of ROS, so the vitamin C levels are low-activated. The lower vitamin C levels in *B. juncea* grown with biofertilizer treatment was caused by the bacteria contained in biofertilizer released metabolites, enzymes and phytohormones that increase plants resistance to tolerance the stress. Bacteria that play role in increasing plant tolerance to salinity stress include *Pseudomonas* sp., *Bacillus* sp. dan *Azospirillum* sp. (Bensidhoum & Nabti, 2019). This result is following Khairunnisa & Siswanti (2021), the application of biofertilizer and varying salinity levels in *Amaranthus tricolor* L. did not show a significant difference in vitamin C levels. Biofertilizers increase plant resistance to salinity by suppressing ROS production and reducing the levels of active antioxidants.

CONCLUSION

Brassica juncea L. growth and production were influenced by biofertilizer treatment (40 ml, 80 ml, and 120 ml) under salinity stress. The application of biofertilizer supports plant growth and maintains the anatomical plant structure in quite optimal conditions. The various dosages of biofertilizer did not have a significant effect on increasing vitamin C levels.

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