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Effect of biofertilizer on growth and metaxylem diameter of *Amaranthus tricolor* L. in salinity stress condition

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ABSTRACT. Throughout history, agricultural sector in Indonesia has faced a shortage of land. As a result, we must take use of land that is still available, even if the conditions are unsuitable for plant growth, one of which is salinity-stressed land. Amaranth is a frequently cultivated plant in Indonesia (*Amaranthus tricolor* L.). This vegetable plant is commonly consumed as food due to its nutritional content and numerous health benefits. To cultivate amaranth on salinity-stressed land, additional nutrients are required to ensure that the plants continue to thrive. One of them is the provision of biofertilizers, a type of organic fertilizer that contains beneficial bacteria for plant growth. This study aimed to determinate the effect of biofertilizer application on the growth and diameter of the stem metaxylem of amaranth plants growing in a salinity-stressed environment. As a salinity stress treatment, NaCl was applied at doses of 0, 2500, 5000, 7500, and 10000 ppm. The diameter of the metaxylem was determined by making fresh preparations across amaranth stems. The biofertilizer application does not affect the height and number of leaves of the plant. However, as the dose of biofertilizer was increased, the value of the metaxylem diameter of the stem increased.

Keywords: anatomical observation; biofertilizer doses; metaxylem of amaranth; plant nutrient; salinity land

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INTRODUCTION

Amaranth (*Amaranthus tricolor* L.) is a vegetable plant belonging to the Amaranthaceae family that is frequently consumed, has a large number of widely spread species, and contribute to economic impact in Indonesia (Dewi & Fariyanti, 2015; Kementerian Pertanian, 2020). This plant is commonly cultivated due to its numerous health benefits, associated with its function in a variety of pharmacological activities, including anticancer, hepatoprotective properties, maintaining cardiovascular health, and resolving blood pressure issues due to its composition (Jovanovski *et al*., 2015; Pradana *et al*., 2016; Putri *et al*., 2017; Hidayanti *et al*., 2021). Apart from fiber, vitamin, protein, and carbohydrate, amaranth contains flavonoids, saponins, and tannins with antioxidant pigment, as well as lutein, which may help reduce vision loss associated with aging and protect the skin from sun exposure (Latha *et al*., 2013; Han & Xu, 2014; Moilati *et al*., 2020; Sarker & Oba, 2020).

Environmental factors are one of the important factors for plant growth. Several regions in Indonesia are known to have coastal lands that have good potential for agriculture if they can be managed optimally, especially regarding the level of salinity (Sudrajat, 2013; Karolinoerita & Yusuf, 2020). Soil under salinity stress contains a high concentration of dissolved salts as a result of the accumulation of various dissolved salts in the water, which, when present in sufficient concentrations, can stress plants, including NaCl, Na_2SO_4, and $MgCl_2$ (Mindari *et al*., 2011; Parihar *et al*., 2015). This situation can limit plant growth due to abiotic pressure. High levels of dissolved salts in the soil can also affect the occurrence of osmotic stress, disruption of ion transport membranes, induce oxidative stress, and hormonal imbalance (Farooq *et al*., 2015; Volkov & Beilby, 2017).

Fertilizers can be used to aid in the growth of plants that thrive in a saline environment. However, instances of rising fertilizer prices have recently resurfaced (Kementerian Pertanian, 2021). Therefore, it is necessary to find a solution that does not interfere with the agricultural sector's operation, one of which is through the use of organic fertilizers manufactured from waste such as livestock waste and by optimizing biofertilizer use. Biofertilizer contain microorganisms that are added to the basic ingredients of livestock urine, which has a number of beneficial properties, including enhancing soil fertility, soil health, and organic matter biodegradation in the soil (Radha & Rao, 2014; Zhang *et al*., 2018). There are biofertilizers that are created with formula compositions that include certain *Trichoderma* sp. that can help stimulate plant growth (Shida *et al*., 2020). *Rhizobium* sp., *Azospirillum* sp., *Azotobacter* sp., *Azomonas* sp., *Pseudomonas* sp., *Bacillus* sp., *Aspergillus* sp., and *Penicillium* sp. are among the microorganisms found in the biofertilizer (Selvi *et al*., 2017; Atieno *et al*., 2020), involved in nitrogen fixation, phosphate dissolution, and the production of IAA phytohormones, which can assist stimulate growth and create a variety of hormones, including auxins, gibberellins, and cytokinins (Mohammadi & Sohrabi, 2012; Hassan & Bano, 2015; Soumare *et al*., 2020). The microbes contained in the biofertilizer may stimulate colonization of the rhizosphere, hence increasing soil fertility through increased plant nutrients and beneficial synergy amongst microbes (Malusá *et al*., 2012; Kartikawati *et al*., 2017). In line with our previous studies that applying rice plants (*Oryza sativa* L.) 10 L/ha of biofertilizer resulted in an increase in soil nutrient content necessary for optimal plant growth (Siswanti & Rachmawati, 2013).

It is necessary to learn more about the effect of biofertilizers and the optimal dose for increasing the growth of amaranth in a salinity-stressed environment. Additionally, no research has been conducted previously on the observation of stem metaxylem diameter of amaranth in relation to salinity stress or the impact of fertilizer application. This study aims to determine the effect of biofertilizer application on the growth and diameter of amaranth stem metaxylem in a salinity-stressed environment. Thus, it is envisaged that in the future, as agricultural land becomes increasingly scarce, amaranth planting can also be accomplished on land subject to salinity stress with the use of biofertilizer applications.

MATERIALS AND METHODS

The research was conducted at the Green House for plant planting and care and Laboratory of Plant Physiology, Faculty of Biology, Universitas Gadjah Mada in July-November 2020.

Biofertilizer treatment. This research was initiated by planting amaranth (*Amaranthus tricolor* L.) seeds in polybags with a plant medium in the form of a mixture of soil, roasted husks, and manure as basic fertilizer. Each polybag is planted with five seeds each, then when they grow, the best two seeds will be selected for observation. A biofertilizer was prepared which was made with the origin of several microbes including *Bacillus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Azospirillum* sp., *Pseudomonas* sp., *Azotobacter* sp., *Rhizobium* sp., and IAA-producing bacteria, and livestock urine based on the formula following our previous studies (Siswanti & Khairunnisa, 2021), and also prepared NaCl as salinity stress. The doses of biofertilizer given in this study as treatment were 0, 10, 20, and 30 L/ha. Meanwhile, NaCl solution as salinity stress was made in various concentrations of 0, 2500, 5000, and 10000 ppm. Biofertilizer treatment was treated to plants when the plants were two weeks old after the seeds are planted, with routine administration once in five days. Meanwhile, salinity stress treatment of NaCl was given on the 7^{th} day and 14^{th} day at two weeks after the seedling period. Plants are maintained with regular watering every morning. During the growth period, observations and measurements of plant height and number of leaves on each treatment were carried out as growth parameters, and each observation was followed by measurements of environmental parameters. After approximately one month and the plants are ready to be harvested, the plants are removed from the soil for anatomical observations of the metaxylem on the plant stems.

Anatomical observations. Anatomical observations began with making fresh plant preparations. The stems of the plant were cut and cleaned with water. The stem was cut crosswise as thinly as possible. The cut results were then placed on a clean object glass and then dripped with distilled water. The preparations were covered with a cover glass and immediately observed under a microscope that had been connected to an optiLab. Observations focused on the transport bundle network in plant stems, especially the metaxylem. The observations were captured and stored for further measurement of the metaxylem diameter of the stem using Image Raster software (Siswanti *et al*., 2019).

Data analysis. The data obtained in this study were analyzed using SPSS ver. 25, with the ANOVA test at a 95% confidence level ($\alpha = 0.05$), and continued with the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Plant height. Measurement of *Amaranthus tricolor* L. height was carried out using a ruler during the growth period. The results of measuring plant height with various doses of biofertilizer treatment and with NaCl as salinity stress are presented in Table 1.

Table 1. Plant height (cm) of amaranth (*Amaranthus tricolor* L.) with various doses of biofertilizer in salinity concentration 0-10000 ppm.

NaCl conc.	Biofertilizer doses (L/ha)				
(ppm)	0 (B0)	10 (B1)	20 (B2)	30 (B3)	Average
0 (A0)	19.21 ± 5.31^{a}	18.24 ± 3.83^{a}	20.31 ± 1.98^{a}	19.00 ± 4.14^{a}	19.19 ± 1.57^{b}
2500 (A1)	24.31 ± 3.12^{a}	16.90 ± 3.10^{b}	19.87 ± 2.50^{b}	19.27 ± 3.20^{b}	20.09 ± 1.33^{ab}
5000 (A2)	19.51 ± 4.73^{a}	18.75 ± 1.73^{a}	20.99 ± 3.00^{a}	21.73 ± 3.59^{a}	20.24 ± 1.61^{ab}
7500 (A3)	26.44 ± 2.67^{a}	18.92 ± 3.76^{b}	21.32 ± 1.49^{b}	20.60 ± 4.68^{b}	21.82 ± 1.17^{a}
10000 (A4)	23.58 ± 3.26^{a}	19.93 ± 1.98^{a}	20.41 ± 2.29^{a}	22.67 ± 4.97^{a}	21.65 ± 1.74^{a}
Average	22.61 ± 2.15^{a}	18.55 ± 1.48^{c}	20.58 ± 1.31^{b}	20.65 ± 1.09^{b}	

Notes: a-c= the means on the same column or row with different superscripts are significantly different ($P<0.05$).

According to Table 1, the average value of *A. tricolor* L. height under salinity stress and with biofertilizer application was 26.44 cm with a biofertilizer dose of 0 L/ha and a salinity concentration of 7500 ppm, while the lowest average value was 16.90 cm in plants with a biofertilizer dose of 10 L/ha and a salinity concentration of 2500 ppm. The majority of *A. tricolor* L. in 0 L/ha biofertilizer had the highest plant height, as demonstrated by the highest average value when compared to other biofertilizer dose treatments. This is because other factors outside biofertilizers may have a greater effect on plant height growth in this study, and the dose of biofertilizer used may not be sufficient to meet plant requirements. When the Duncan test results for the average value of plant height between biofertilizer treatments are compared, it is clear that the average value of plant height increases as the dose of biofertilizer is increased, but the treatment given biofertilizer at a dose of 0 L/ha has the highest average value, with all mean values being significantly different, except for the treatments given biofertilizer at doses of 20 and 30 L/ha. The correlation between the increase in plant height and the increase in biofertilizer dose indicates that biofertilizer can assist in maintaining and increasing plant growth, particularly plant height, in conditions with salinity stress. However, it can be shown that the control plants had the highest mean value. This is apparently because environmental conditions, particularly light intensity, exerted a stronger effect in this study than the effect of the applied biofertilizer. Additionally, it is probable that the dose of biofertilizer used in this study was insufficient to meet the plant's nutritional requirements. Alternatively, it could be that the biofertilizers content is insufficient to promote plant development. The composition of the biofertilizer, might also influence the outcome of the application, considering that microbes stimulates and increases plant development in a special way. Furthermore, mixed organic liquids other than urine, such as tofu waste water, coconut water waste, microalgae, or vermiwash formula, can be utilized as supplementary compositions for biofertilizers that must be adapted to the nutrient requirements of the plants (de Siqueira Castro *et al.*, 2020). Meanwhile, it is known that plant height increases with increasing salinity stress concentrations, as indicated by plant height at salinity concentrations of 7500 and 10000 ppm, which was significantly different from control plants and salinity treatments of 2500 and 5000 ppm.

Number of leaves. The number of leaves of *A. tricolor* L. on each treatment of was counted together with the measurement of plant height. The results of calculating the average number of leaves are presented in Table 2.

Table 2. Number of leaves of amaranth (*Amaranthus tricolor* L.) with various doses of biofertilizer in salinity concentration 0-10000 ppm.

NaCl conc.	Biofertilizer doses (L/ha)				
(ppm)	0 (B0)	10 (B1)	20 (B2)	30 (B3)	Average
0 (A0)	7.86 ± 1.57[a]	8.29 ± 1.25[a]	8.43 ± 0.79[a]	7.57 ± 0.98[a]	7.86 ± 0.38[c]
2500 (A1)	9.86 ± 1.07[a]	7.57 ± 1.13[b]	8.57 ± 1.13[b]	8.00 ± 1.00[b]	8.71 ± 0.49[ab]
5000 (A2)	8.71 ± 1.11[a]	8.14 ± 0.69[a]	8.57 ± 0.98[a]	8.43 ± 0.98[a]	8.57 ± 0.54[b]
7500 (A3)	9.86 ± 0.69[a]	8.00 ± 0.82[c]	8.71 ± 0.49[bc]	8.29 ± 1.11[bc]	9.00 ± 0.00[ab]
10000 (A4)	9.57 ± 0.79[a]	8.57 ± 0.79[b]	9.00 ± 0.58[ab]	8.57 ± 1.27[b]	9.14 ± 0.38[a]
Average	9.14 ± 0.90[a]	8.14 ± 0.38[b]	8.57 ± 0.54[ab]	8.14 ± 0.38[b]	

Notes: a-c= The means on the same column or row with different supercripts are significantly different ($P<0.05$).

A. tricolor L. with the most leaves are those treated with 0 L/ha biofertilizer at salinity stress levels of 2500 and 7500 ppm, respectively, with a value of 9.86 (Table 2). Meanwhile, the lowest value, 7.57, was seen in plants treated with 30 L/ha biofertilizer at 0 ppm salinity stress. Similar to the results of measuring plant height, the results of measuring the average number of leaves revealed that the majority of plants treated with 0 L/ha biofertilizer had a higher value than plants treated with other treatments, as evidenced by the fact that the average value for 0 L/ha biofertilizer dose treatment is the highest. The average number of leaves tends to decrease as the dose of biofertilizer is increased. According to the average results between biofertilizer treatments in Table 2, the optimal biofertilizer dose to assist in increasing the number of leaves in plants living under salinity stress is 20 L/ha. The reason for the decline in the value of the 30 L/ha biofertilizer dose is because the dose of biofertilizer is too high. As a result, the biofertilizer's microbial content increases significantly. The abundance of microbes can result in intense competition between microbes in the rhizosphere area, which can have an effect on nutrient uptake inhibition. The Duncan test revealed that the average number of leaves in all treatments was substantially different from one another, except for the treatment in which biofertilizer concentrations of 10 and 30 L/ha were applied. In contrast to the average number of leaves between biofertilizer treatments, which tends to decrease as the dose of the given biofertilizer increases, when the average number of leaves between salinity treatments is observed, it can be concluded that the number of leaves tends to increase as the salinity stress concentration increases, with which was not significantly different in stress treatments of 2500 and 7500 ppm, but significantly different with and between.

The increase in plant height and the number of leaves are two examples of characteristics that indicate plant growth. Growth is an irreversible process defined by a gain in height, length, stem diameter, tissue volume, and fresh weight, among other characteristics, which is characterized by a variety of elements, both internal and external (Lastdrager *et al.*, 2014; Gardiner *et al.*, 2016). External factors are those that originate external to the plant, such as environmental influences. External influences can be further classified into natural variables such as sunlight, soil moisture, soil pH, and soil respiration as well as artificial factors or elements that are purposely provided by humans (Barron-Gafford *et al.*, 2011; Prasad *et al.*, 2017), such as the biofertilizer provided to aid plant development in this study. Additionally, soil fertility can be increased, resulting in an increase in the productivity of plants, through the application of biofertilizers (Adekayode & Ogunkoya, 2011). However, the results of this study indicated that plants grown without biofertilizer had the highest average plant height and leaf number values when compared to other treated plants.

Our previous studies (Siswanti & Umah, 2021), used same plant species and biofertilizer formula, but with NPK fertilizer as the basic fertilizer, the results indicated that a biofertilizer dose of 20 L/ha was capable of increasing plant height and leaf number on plants exposed to salinity stress. These findings contrast with those obtained in this study, in which control plants not treated with biofertilizers had the highest growth values, presumably due to other environmental factors in addition to the effect of the biofertilizer. Intensity of sunlight, as light is the primary factor, impacts plant physiology and biochemistry, as well as having the potential to limit plant growth and production (Chen *et al*., 2021; Shafiq *et al*., 2021). It was determined that the B0 plant plot had the highest average light intensity value compared to the other treatment plant plots. In contrast to our previous studies (Siswanti & Umah, 2021) that the light intensity in the control plant plot was extremely low, the light intensity in this research was significantly higher than in other treatments. Thus, even though no biofertilizer was used in this treatment, the plants grew effectively due to the strong impact of sunlight. Sufficient light intensity can boost plant growth and productivity by increasing numerous growth indices such as plant height, leaf number, and leaf area. Additionally, it can have an effect on the germination process of seeds. Besides that, the light impact can be enhanced by incorporating a combination of light sources (Chen *et al*., 2015; Ren *et al*., 2018). This study used manure as a basic fertilizer, which can help in plant growth in addition to the biofertilizer treatment. Manure contains a high concentration of organic matter, which is used as a food source by soil microorganisms and changed into a form that may be used by plants as nutrients to aid in growth via the absorption process by soil microbes (Zhang *et al*., 2012). Additional elements such as the availability of nutrients from manure must be considered because they can assist in activating the biofertilizer's microbial activity (Subowo *et al*., 2013). As previously stated, competition between bacteria in the rhizosphere can impede nutrient uptake.

The value of the biofertilizer application treatment increased with a dose of 20 L/ha and subsequently reduced with a dose of 30 L/ha, which may also indicate that a dose of 20 L/ha biofertilizer is an appropriate dose for amaranth to increase their growth in a salinity-stressed environment. However, it is possible that different doses, either higher or lower, are better compatible with the amaranth requirements. In this study, the number of leaves and plant height had an effect on one another. This is demonstrated by the fact that amaranth with the most leaves also have the greatest height in comparison to other plants. The high number of leaves on plants enables the plants to grow more rapidly and effectively. This is connected to the CO_2 assimilation process occurring in leaves, which utilizes CO_2, H_2O, and photon energy collected by chlorophyll (Hikosaka & Noda, 2019). However, having a high number of leaves does not always imply improved growth metrics, as demonstrated in Wulandari *et al*. (2017) on the effect of leaf number of *Citrus aurantifolia* on cutting growth. In this study, plants with two leaves grew faster than plants with four leaves, owing to the fact that other factors influenced the outcomes.

Meanwhile, plants treated with B1 (10 L/ha) biofertilizer had the lowest average growth value. Amaranth receiving the shortest dose of biofertilizer had an average value for both average plant height and leaf number, indicating that the plants receiving the treatment lacked sufficient nutrients to grow effectively. Because, in addition to receiving a little amount of biofertilizer, plants are subjected to stress in salinity. Unlike the amaranth with B0, which similarly thrive in salinity-stressed environments, these plants receive no biofertilizer, but their plots receive abundant light. Conversely, the light intensity in the plots of plants treated with B1 was not excessive, indicating that the plants received just a limited amount of growth support. Additionally, plants treated with biofertilizer at a dose of 30 L/ha at a salinity concentration of 0 ppm had a low mean number of leaves, despite the fact that this treatment plant got the highest amount of biofertilizer. The data collection is achievable because the physiological variables of the plant or the dosage of biofertilizer are still insufficient to meet the plant's requirements. Each plant has unique nutritional requirements depending on variety of parameters, including the spread of the plant's roots and size.

Metaxylem diameter. Observation of stem metaxylem diameter was measured by observing the fresh cross section of amaranth stem for each treatment under a microscope. Diameter length was measured by image of the preparation taken with OptiLab and then measured by Image Raster. The results of measuring the metaxylem diameter of spinach stems in this study are listed in Table 3.

Table 3. Stem metaxylem diameter (µm) of amaranth (*Amaranthus tricolor* L.) with various doses of biofertilizer in salinity concentration 0-10000 ppm.

NaCl conc. (ppm)	Biofertilizer doses (L/ha)				
	0 (B0)	10 (B1)	20 (B2)	30 (B3)	Average
0 (A0)	30.53 ± 3.67^{a}	32.48 ± 4.89^{a}	33.19 ± 4.03^{a}	35.63 ± 6.11^{a}	32.96 ± 1.77^{ab}
2.500 (A1)	27.21 ± 1.99^{b}	31.44 ± 4.67^{ab}	30.41 ± 4.53^{ab}	36.51 ± 2.01^{a}	31.39 ± 0.38^{b}
5.000 (A2)	37.80 ± 5.09^{a}	33.74 ± 3.82^{a}	37.76 ± 2.80^{a}	38.92 ± 7.88^{a}	37.06 ± 2.18^{a}
7.500 (A3)	33.17 ± 9.58^{a}	32.89 ± 3.50^{a}	39.14 ± 2.94^{a}	39.38 ± 9.67^{a}	36.15 ± 3.29^{a}
10.000 (A4)	32.21 ± 2.60^{ab}	34.71 ± 1.71^{ab}	35.45 ± 3.56^{a}	29.88 ± 2.38^{b}	33.07 ± 2.33^{ab}
Average	32.18 ± 1.61^{c}	33.05 ± 0.59^{bc}	35.19 ± 0.87^{ab}	36.06 ± 1.98^{a}	

Notes: a-c= the means on the same column or row with different superscripts are significantly different ($P<0.05$).

The highest diameter of metaxylem on the stems of amaranth is 39.38 µm treated with 30 L/ha biofertilizer at a salinity of 7500 ppm, while the smallest diameter of metaxylem is 27.21 µm treated with 0 L/ha biofertilizer at a salinity of 2500 ppm (Table 3). When the average value is observed, the value of the metaxylem diameter of the stem increases in proportion to the amount of biofertilizer applied. This demonstrates that the administration of biofertilizer can assist the metaxylem diameter of plants growing in a salinity-stressed environment, and that a higher dose of biofertilizer can further enhance the stem's metaxylem diameter. The Duncan test revealed that the average value of stem metaxylem diameter was substantially different in all treatments between biofertilizer dosages. However, when the average value of metaxylem diameter is compared to other salinity treatments, there was an erratic increase and decrease in the value of metaxylem diameter, with plants under stress 5000 ppm having the highest average, with salinity treatments of 0 ppm being not significantly different from 10000 ppm, and 5000 ppm treatments being not significantly different from 7500 ppm, but stress at 2500 ppm being significantly different. In salinity-stressed environment, the diameter of the carrier beam, particularly the xylem, decreases as the stress concentration increases, with the goal of speeding up the process of water ascending from the soil to the leaves through capillarity. However, based on the average of the salinity treatments, it was discovered that despite the application of biofertilizer, the value of metaxylem diameter decreased at stress concentrations of 7500 and 10000 ppm. This is feasible because plants are already extremely stressed at this degree of stress, and even if a biofertilizer is provided to promote growth, the plant will shorten its metaxylem diameter in order to tolerate it. Salinity tolerance occurs for a variety of reasons, some of which are determined by ion transport. It also involves complicated reactions at the molecular, cellular, metabolic, physiological, and amaranth levels, including plant species that are very tolerant of drought and heat (Gupta & Huang, 2014; Idris *et al*., 2020). Another possible cause is that the formula or composition of the biofertilizer is not appropriate for the amaranth nutritional requirements. Variations in the type, formula, or concentration of biofertilizers applied to plants could be the factors that contribute to plant development (Xu *et al*., 2020). On the basis of these results, it may be concluded that stress has no discernible effect on amaranth metaxylem diameter. The Fig. 2 are the results of observations of fresh preparations cross-sectional of *A. tricolor* L. stems observed with a light microscope.

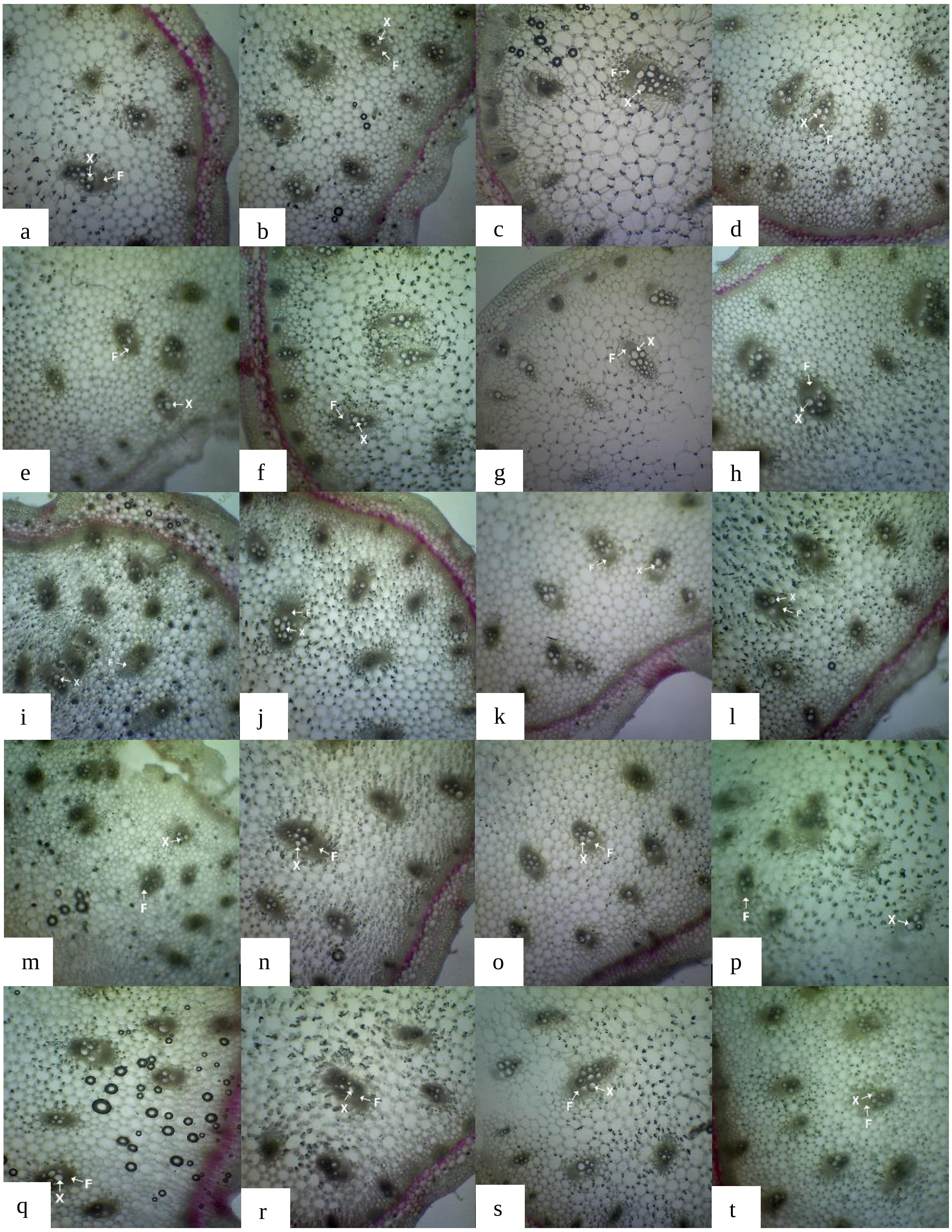

Fig. 2. Cross section of stems of *Amaranthus tricolor* L. at various salinity stress treatment with biofertilizer treatment: a-d= 0 ppm salinity stress treatment; e-h= 2500 ppm salinity stress treatment; i-l= 5000 ppm salinity stress treatment; m-p= 7500 ppm salinity stress treatment; q-t= 10000 ppm salinity stress treatment; a/e/i/m/q= 0 L/ha dose of biofertilizer; b/f/j/n/r= 10 L/ha dose of biofertilizer; c/g/k/o/s= 20 L/ha dose of biofertilizer; d/h/l/p/t= 30 L/ha dose of biofertilizer; X= xylem; F= phloem.

Salinity, which may occur due to climate change, excessive use of groundwater near the coast, or irrigation with low-quality water, is one of the factors that significantly affect crop productivity in terms of growth and growth rate. High salinity can be stressful and have a negative effect on plant productivity, as salt disrupts plant nutrition and increases soil osmotic pressure. NaCl as one of the compounds whose presence in the soil can increase salinity, its accumulation can also cause osmotic stress in plants (Gupta & Huang, 2014; Machado & Serralheiro, 2017). Plants respond to salinity stress in a variety of ways, ranging from molecular to physiological, and these responses can affect physiology, morphology, and metabolism. Salinity stress can cause the xylem vessels to shrink and embolism to occur, which is an event in which gas bubbles in the form of water vapor become trapped in the xylem and can obstruct the passage of water from the roots to the top of the plant for metabolic processes, thereby inhibiting the plant's growth process (Gupta & Huang, 2014; Rajput *et al*., 2015; Patel *et al*., 2020). Reduced area or diameter of the vascular bundle is an adaptive response of plants to stress (Atabayeva *et al*., 2013). Additional nutrients and minerals supplied to plants, one of which can be accomplished through the application of fertilizers, have been shown to influence the plants' ability to adapt to harsh circumstances. The provision of fertilizers, such as biofertilizers, is designed to assist plants in continuing to grow and accelerating their growth, allowing plants to thrive in the face of challenges that could threaten their survival. By providing a biofertilizer, the bacteria contained therein can encourage plant development by functioning as an inoculant and providing nutrients to the plants. Moreover, there are additional roles for bacteria in assisting plants in surviving in stressful environments, depending on the microbe, the stress, and the plant (Waraich *et al*., 2012; Al-Amri, 2021).

Measuring the diameter of the metaxylem in this study revealed that the higher the dose of biofertilizer applied to the plant, the larger the diameter of the metaxylem detected in amaranth. The provision of biofertilizer is undoubtedly a driving force that contributes to the growth of amaranth exposed to salinity stress. Hence metaxylem is created after primary growth begins, biofertilizer administration can assist in expanding the diameter of metaxylem by promoting plant development even under adverse situations. In contrast to the growth observations, the diameter of the metaxylem biofertilizer appears to be more influential. This is because, as a transport system, the process begins in the soil, where the roots collect nutrients and minerals, which are subsequently transported to various parts of the plant via the xylem. The biofertilizer used in this study was also applied to the soil, allowing plants whose soil received a larger dose of biofertilizer to grow and develop normally, one of which was the growth and development of stem organs and associated systems, such as xylem. Thus, plants exposed to a high dose of biofertilizer had a larger xylem diameter than plants exposed to a low dose of biofertilizer. We underline in our study the crucial importance of expanding amaranth output and quality as an edible vegetable, as community demand has not been addressed properly. Green vegetable production facilities that are both environmentally friendly and salt-tolerant are a new hope for Indonesia.

CONCLUSION

The biofertilizer application did not affect the increased amaranth (*Amaranthus tricolor* L.) growth in this study, particularly in plant height and leaf number under salinity stress conditions, but it did affect the metaxylem diameter of plant stems. The higher the dose of biofertilizer applied, the larger the metaxylem diameter on the stem.

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REFERENCES

Adekayode FO, Ogunkoya MO. 2011. Comparative effects of organic compost and NPK fertilizer on soil fertility, yield and quality of amaranth in southwest Nigeria. *International Journal of Biological and Chemical Sciences*. vol 5(2): 490–499. doi: https://doi.org/10.4314/ijbcs.v5i2.72087.

Al-Amri SM. 2021. Application of bio-fertilizers for enhancing growth and yield of common bean plants grown under water stress conditions. *Saudi Journal of Biological Sciences*. vol 28(7): 3901–3908. doi https://doi.org/10.1016/j.sjbs.2021.03.064.

Atabayeva S, Nurmahanova A, Minocha S, Ahmetova A, Kenzhebayeva S, Aidosova S, Nurzhanova A, Zhardamalieva A, Asrandina S, Alybayeva R, Li T. 2013. The effect of salinity on growth and anatomical attributes of barley seedling (*Hordeum vulgare* L.). *African Journal of Biotechnology*. vol 12(18): 2366–2377. doi: https://doi.org/10.5897/AJB2013.12162.

Atieno M, Herrmann L, Nguyen HT, Phan HT, Nguyen NK, Srean P, Than MM, Zhiyong R, Tittabutr P, Shutsrirung A, Bräu L. 2020. Assessment of biofertilizer use for sustainable agriculture in the Great Mekong Region. *Journal of Environmental Management.* vol 275: 1–9. doi: https://doi.org/10.1016/j.jenvman.2020.111300.

Barron-Gafford GA, Scott RL, Jenerette GD, Huxman TE. 2011. The relative controls of temperature, soil moisture, and plant functional group on soil CO2 efflux at diel, seasonal, and annual scales. *Journal of Geophysical Research: Biogeosciences.* vol 116(1): 1–16. doi: https://doi.org/10.1029/2010JG001442.

Chen G, Wang S, Huang X, Hong J, Du L, Zhang L, Ye L. 2015. Environmental factors affecting growth and development of Banlangen (*Radix isatidis*) in China. *African Journal of Plant Science*. vol 9(11): 421–426. doi: https://doi.org/10.5897/AJPS2015.%201266.

Chen M, Zeng X, Liu Y, Zhang H, Hu Q. 2021. An orthogonal design of light factors to optimize growth, photosynthetic capability and metabolite accumulation of *Anoectochilus roxburghii* (Wall.) Lindl. *Scientia Horticulturae*. vol 288: 1–9. doi: https://doi.org/10.1016/j.scienta.2021.110272.

de Siqueira Castro J, Calijuri ML, Ferreira J, Assemany PP, Ribeiro VJ. 2020. Microalgae based biofertilizer: A life cycle approach. *Science of the Total Environment*. vol 724: 1–10. doi: https://doi.org/10.1016/j.scitotenv.2020.138138.

Dewi P, Fariyanti A. 2015. Pendapatan usahatani bayam di Desa Ciaruteun Ilir Kecamatan Cibungbulang Kabupaten Bogor Jawa Barat. *Forum Agribisnis*. vol 5(2): 159–174.

Farooq M, Hussain M, Wakeel A, Siddique KHM. 2015. Salt stress in maize: effects, resistance mechanisms, and management. A review. *Agronomy for Sustainable Development.* vol 35(2): 461–481. doi: https://doi.org/10.1007/s13593-015-0287-0.

Gardiner B, Berry P, Moulia B. 2016. Wind impacts on plant growth, mechanics and damage. *Plant Science*. vol 245: 94–118. doi: https://doi.org/10.1016/j.plantsci.2016.01.006.

Gupta B, Huang B. 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International Journal of Genomics*. vol 2014: 1–19. doi: https://doi.org/10.1155/2014/701596.

Han S, Xu B. 2014. Bioactive components of leafy vegetable edible amaranth (*Amaranthus mangostanus* L.) as affected by home cooking manners. *American Journal of Food Science and Technology*. vol 2(4): 122–127. doi: https://doi.org/10.12691/ajfst-2-4-3.

Hassan TU, Bano A. 2015. Role of carrier-based biofertilizer in reclamation of saline soil and wheat growth. *Archives of Agronomy and Soil Science*. vol 61(12): 1719–1731. doi: https://doi.org/10.1080/03650340.2015.1036045.

Hidayanti R, Rusmini H, Fitriani D, Ulfa AM. 2021. Pengaruh pemberian ekstrak daun bayam merah (*Amaranthus tricolor* L.) terhadap kadar ldl dan hdl pada tikus putih (*Rattus norvegicus*) galur wistar jantan yang diberi diet tinggi lemak. *JKM (Jurnal Kebidanan Malahayati)*. vol 7(4): 809–817. doi: http://dx.doi.org/10.33024/jkm.v7i4.4619.

Hikosaka K, Noda HM. 2019. Modeling leaf CO_2 assimilation and Photosystem II photochemistry from chlorophyll fluorescence and the photochemical reflectance index. *Plant, Cell & Environment*. vol 42(2): 730–739. doi: https://doi.org/10.1111/pce.13461.

Idris LM, Nulit R, Zaman FQ, Arifin FKM. 2020. Hydrotime analysis of *Amaranthus* spp. seed germination under salinity condition. *Journal of Applied Research on Medicinal and Aromatic Plants*. vol 17: 1–6. doi: https://doi.org/10.1016/j.jarmap.2020.100249.

Ji S, Liu Z, Liu B, Wang Y, Wang J. 2020. The effect of Trichoderma biofertilizer on the quality of flowering Chinese cabbage and the soil environment. *Scientia Horticulturae*. vol 262: 1–8. doi: https://doi.org/10.1016/j.scienta.2019.109069.

Jovanovski E, Bosco L, Khan K, Au-Yeung F, Ho H, Zurbau A, Jenkins AL, Vuksan V. 2015. Effect of spinach, a high dietary nitrate source, on arterial stiffness and related hemodynamic measures: a randomized, controlled trial in healthy adults. *Clinical Nutrition Research*. vol 4(3): 160–167. doi: https://doi.org/10.7762/cnr.2015.4.3.160.

Karolinoerita V, Yusuf WA. 2020. Salinisasi Lahan dan Permasalahannya di Indonesia. *Jurnal Sumberdaya Lahan*. vol 14(2): 91–99. doi: http://dx.doi.org/10.21082/jsdl.v14n2.2020.91-99.

Kartikawati A, Trisilawati O, Darwati I. 2017. Pemanfaatan pupuk hayati (biofertilizer) pada tanaman rempah dan obat/Biofertilizer utilization on spices and medicinal plants. *Perspektif*. vol 16(1): 33–43. doi: http://dx.doi.org/10.21082/psp.v16n1.2017.33-43.

Kementerian Pertanian. 2020. Pacu volume ekspor, Kementan latih 32 eksportir benih. Jakarta: Kementerian Pertanian Republik Indonesia.

https://www.pertanian.go.id/.

Kementerian Pertanian. 2021. Kunjungi Petrokimia Gresik, Kementan jamin stok pupuk bersubsidi untuk petani aman. Jakarta: Kementerian Pertanian Republik Indonesia. https://www.pertanian.go.id/.

Lastdrager J, Hanson J, Smeekens S. Sugar signals and the control of plant growth and development. *Journal of Experimental Botany*. vol 65(3): 799–807. doi: https://doi.org/10.1093/jxb/ert474.

Latha P, Sudhakar P, Krisna MBM, Begam CR, Krishna TG. 2013. Quantification of lutein and β carotene in underutilized green leafy vegetables using high performance liquid chromatography. *Vegetable Science*. vol 40(1): 109–110.

Machado RMA, Serralheiro RP. 2017. Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. *Horticulturae*. vol 3(2): 1–13. doi: https://doi.org/10.3390/horticulturae3020030.

Malusá E, Sas-Paszt L, Ciesielska J. 2012. Technologies for beneficial microorganisms inocula used as biofertilizers. *The Scientific World Journal*. vol 2012: 1–13. doi: https://doi.org/10.1100/2012/491206.

Mindari W. Maroeto M, Syekhfani S. 2011. Maize tolerance to salinity of irrigation water. *Journal of Tropical Soils*. vol 16(3): 211–218. doi: http://dx.doi.org/10.5400/jts.2011.v16i3.211-218.

Mohammadi K, Sohrabi Y. 2012. Bacterial biofertilizers for sustainable crop production: a review. *ARPN Journal of Agricultural and Biological Science*. vol 7(5): 307–316.

Moilati VO, Yamlean PVY, Rundengan G. 2020. Formulasi sediaan krim ekstrak etanol daun bayam merah (*Amaranthus tricolor* L.) dan uji aktivitas antioksidan menggunakan metode DPPH (1.1-diphenyl-2-picrylhydrazyl). *Pharmacon*. vol 9(3): 372–380. doi: https://doi.org/10.35799/pha.9.2020.30021.

Parihar P, Singh S, Singh R, Singh VP, Prasad SM. 2015. Effect of salinity stress on plants and its tolerance strategies: a review. *Environmental Science and Pollution Research*. vol (6): 4056–4075. doi: https://doi.org/10.1007/s11356-014-3739-1.

Patel D, Jayswal S, Solanki H, Maitreya B. 2020. Effect of salinity on different vegetable crops – a review. *International Journal of Recent Scientific Research*. vol 11(2): 37418–37422. doi: http://dx.doi.org/10.24327/ijrsr.2020.1102.5104.

Pradana DA, Rahmah FS, Setyaningrum TR. 2016. Potensi antihiperlipidemia ekstrak etanolik daun bayam merah (*Amaranthus tricolor* L.) terstandar secara in vivo berdasarkan parameter LDL (*Low Density Lipoprotein*). *Jurnal Sains Farmasi & Klinis*. vol 2(2): 122–128. doi: http://dx.doi.org/10.29208/jsfk.2016.2.2.72.

Prasad H, Sajwan P, Kumari M, Solanki SPS. 2017. Effect of organic manures and biofertilizer on plant growth, yield and quality of horticultural crop: A review. *International Journal of Chemical Studies*. vol 5(1): 217–221.

Putri CA, Pradana DA, Susanto Q. 2017. Efek ekstrak etanolik daun bayam merah (*Amaranthus tricolor* L.) terstandar terhadap indeks massa tubuh dan kadar glukosa darah pada tikus sprague dawley yang diberikan diet tinggi lemak sebagai upaya preventif obesitas. *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*. vol 13(2): 150–161. doi: http://dx.doi.org/10.30595/pji.v13i02.1251.

Radha TK, Rao DL. 2014. Plant growth promoting bacteria from cow dung based biodynamic preparations. *Indian Journal of Microbiology*. vol 54(4): 413–418. doi: https://doi.org/10.1007/s12088-014-0468-6.

Rajput VD, Chen Y, Ayup M. 2015. Effects of high salinity on physiological and anatomical indices in the early stages of Populus euphratica growth. *Russian Journal of Plant Physiology*. vol 62(2): 229–236. doi: https://doi.org/10.1134/S1021443715020168.

Ren X, Liu Y, Jeong HK, Jeong BR. 2018. Supplementary light source affects the growth and development of *Codonopsis lanceolata* seedlings. *International Journal of Molecular Sciences*. vol 19(10): 1–19. doi: https://doi.org/10.3390/ijms19103074.

Sarker U, Oba S. 2020. Leaf pigmentation, its profiles and radical scavenging activity in selected *Amaranthus tricolor* leafy vegetables. *Scientific Reports*. vol 10(1): 1–10. doi: https://doi.org/10.1038/s41598-020-66376-0.

Selvi KB, Paul JJA, Vijaya V, Saraswathi K. 2017. Analyzing the efficacy of phosphate solubilizing microorganisms by enrichment culture techniques. *Biochemistry and Molecular Biology Journal*. vol 3(1): 1–7. doi: https://doi.org/10.21767/2471-8084.100027.

Shafiq I, Hussain S, Raza MA, Iqbal N, Asghar MA, Raza A, Fan YF, Mumtaz M, Shoaib M, Ansar M, Manaf A, Yang WY, Yang F. 2021. Crop photosynthetic response to light quality and light intensity. *Journal of Integrative Agriculture*. vol 20(1): 4–23. doi: https://doi.org/10.1016/S2095-3119(20)63227-0.

Siswanti DU, Rachmawati D. 2013. Pertumbuhan tiga kultivar padi (*Oryza sativa* L.) terhadap aplikasi pupuk bio cair dan kondisi tanah pertanian pasca erupsi Merapi 2010. *Biogenesis: Jurnal Ilmiah Biologi*. vol 1(2): 110–115. doi: https://doi.org/10.24252/bio.v1i2.456.

Siswanti DU, Utaminingsih U, Pangestuti NH. 2019. Capsaicin level and anatomy response of curly red chili (*Capsicum annuum* L.) to bio fertilizer and sludge biogas application. Proceeding of International Conference on Science and Technology. May 2-3, 2019. Makassar: FST Universitas Islam Negeri Alauddin Makassar. pp 1–8. doi: http://dx.doi.org/10.4108/eai.2-5-2019.2284700.

Siswanti DU, Khairunnisa NA. 2021. The effect of biofertilizer and salinity stress on *Amaranthus tricolor* L. growth and total leaf chlorophyll content. *BIO Web of Conferences*. vol 33: 1–8. doi: https://doi.org/10.1051/bioconf/20213302004.

Siswanti DU, Umah N. 2021. Effect of biofertilizer and salinity on growth and chlorophyll content of *Amaranthus tricolor* L. *IOP Conference Series: Earth and Environmental Science*. vol 662: 1–11. doi: https://doi.org/10.1088/1755-1315/662/1/012019.

Soumare A, Diedhiou AG, Thuita M, Hafidi M, Ouhdouch Y, Gopalakrishnan S, Kouisni L. 2020. Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. *Plants.* vol 9(8): 1–22. doi: https://doi.org/10.3390/plants9081011.

Subowo S, Purwani J, Rochayati S. 2013. Prospek dan tantangan pengembangan Biofertilizer untuk perbaikan kesuburan tanah. *Jurnal Sumberdaya Lahan*. vol 7(1): 15–26. doi: http://dx.doi.org/10.21082/jsdl.v7n1.2013.%25p.

Sudrajat J. 2013. Potensi dan problematika pembangunan wilayah pesisir di Kalimantan Barat. *Jurnal Social Economic of Agriculture.* vol 2(1): 29–41.

Volkov V, Beilby MJ. 2017. Salinity tolerance in plants: Mechanisms and regulation of ion transport. *Frontiers in Plant Science.* vol 8: 1–4. doi: https://doi.org/10.3389/fpls.2017.01795.

Waraich EA, Ahmad R, Halim A, Aziz T. 2012. Alleviation of temperature stress by nutrient management in crop plants: a review. *Journal of Soil Science and Plant Nutrition*. vol 12(2): 221–244. doi: http://dx.doi.org/10.4067/S0718-95162012000200003 .

Wulandari F, Astiningrum M, Tujiyanta T. 2017. Pengaruh jumlah daun dan macam media tanam pada pertumbuhan stek jeruk nipis (*Citrus aurantifolia* Swingle). *Vigor: Jurnal Ilmu Pertanian Tropika dan Subtropika*. vol 2(2): 48–51. doi: http://dx.doi.org/10.31002/vigor.v2i2.487.

Xu F, Chu C, Xu Z. 2020. Effects of different fertilizer formulas on the growth of loquat rootstocks and stem lignification. *Scientific Reports*. vol 10(1): 1–11. doi: https://doi.org/10.1038/s41598-019-57270-5.

Zhang F, Huo Y, Cobb AB, Luo G, Zhou J, Yang G, Wilson GWT, Zhang Y. 2018. Trichoderma biofertilizer links to altered soil chemistry, altered microbial communities, and improved grassland biomass. *Frontiers in Microbiology*. vol 9: 1–11. doi: https://doi.org/10.3389/fmicb.2018.00848.

Zhang QC, Shamsi IH, Xu DT, Wang GH, Lin XY, Jilani G, Hussain N, Chaudhry AN. 2012. Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. *Applied Soil Ecology*. vol 57: 1–8. doi: https://doi.org/10.1016/j.apsoil.2012.02.012.