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Growth response, chlorophyll content, and nitrate reductase activity of mustard greens (*Brassica rapa* L.) to salinity stress post application of biofertilizer in hydroponic system

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ABSTRACT. Mustard greens (*Brassica rapa* L.) is a herbaceous plant from the Cruciferae or Brassicaceae family which has 3700 species. *B. rapa* has a taproot system, short stems and segmented and winged leaves and long stems that are flat in shape and are tolerant of salinity stress. Giving biofertilizer is one way to help increase crop production and quality. This research aimed to determine the activity of nitrate reductase and the growth response of mustard greens (*B. rapa*) in salinity stress with a hydroponic system. Parameters measured were plant height, number of leaves, wet weight, dry weight, leaf chlorophyll content, and nitrate reductase activity. Mustard greens are grown in a hydroponic system at the Sawitsari greenhouse, Faculty of Biology, UGM. Media in the form of clean water is added with biofertilizer in various doses. The biofertilizer doses were graded from 0, 10 mL/L, 20 mL/L, and 30 mL/L. This research used 5000 ppm salinity stress application. The resulting data was then analyzed by ANOVA (Analysis of Variance) using SPSS version 26 and tested by DMRT (Duncan Multiple Range Test). The results showed that biofertilizer at a dose of 30 mL/L was able to increase the growth of plant height (20.28 cm) and number of leaves (8.6 strands) and productivity of wet weight (82.67 g) and dry weight (5.76 g) compared to controls negative. At a dose of 20 mL/L biofertilizer showed an increase in Nitrate Reductase Activity with a value of 4.66 mol NO_2^-/gram leaf wet weight/hour of incubation) with 5000 ppm salinity stress application.

Keywords: *Brassica rapa* L., biofertilizer doses, growth response, hydroponic system, nitrate reductase activity

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INTRODUCTION

Vegetables are food ingredients that are needed daily for human nutrition. Mustard green is one of the horticultural crop commodities in the form of vegetables that is much favored by the community because of its very diverse nutrient content and beneficial for the body (Harahap *et al*., 2019). Mustard green or *B. rapa* is a plant from the Brassicaceae family. *B. rapa* is a widely distributed genus of herbaceous plants from the family Cruciferae or Brassicaceae, estimated to consist of 3700 species (Cheng *et al*., 2014). The mustard used is a type of mustard green that has the characteristics of a short stem, and the leaves are whitish green (Amer *et al*., 2019; Anjum *et al*., 2012). The leaves have a round or oval shape that is wide and narrow, hairless, some are wavy, light green to dark green. The petiole is long or short, white to green, strong, and smooth. The midrib opens and wraps around the younger one. Generally, the veins are pinnate and branched (Vanktesh *et al*., 2021). The content in mustard greens that are beneficial to the body include calories, protein, fat, carbohydrates, fiber, Ca, P, Fe, Vitamin A, Vitamin B, Vitamin C, and many more. Mustard green is also a plant that is known as an accumulator of heavy metals (Anjum *et al*., 2012). The advantages that exist of mustard greens, such as their delicious taste, affordable price, and most importantly, the high nutritional content of mustard greens, make the plant much in demand by the public and increase market demand for mustard greens. This makes farmers have to increase their production (Istarofah & Salamah, 2017).

In Indonesia, the production of mustard greens from 2016 to 2019 continued to increase from 601,204 tons to 652,727 tons. Unlike the case with mustard production in D.I.Yogyakarta, which is from 2016 to 2019 decreased. In 2016 the production of mustard greens was 3,910 tons to 3,094 tons (Badan Pusat Statistik, 2021). Of course, this is influenced by many factors. Inorganic fertilizers that

are used continuously can cause the effect of these fertilizers to be ineffective, so plant growth is inhibited (Muhadiansyah *et al*., 2016). To increase the production of mustard greens, one of the efforts often carried out by farmers is the method of planting and providing additional fertilizer for plants. Hydroponics is an alternative that can be done because it is a cultivation of plants without soil and only requires a nutrient solution/material containing nutrients such as coconut fiber, sand, husk charcoal, etc. (Susilawati, 2019). Many types of fertilizers are often or can be used to help the plant growth process, including compost, manure, chemical fertilizers such as NPK fertilizers, and fertilizers that are produced with several types of microbes in them such as biofertilizers (Mulqan *et al*, 2017).

Biofertilizers are known as a promising, cost-effective and environmentally friendly renewable resource of plant nutrients to supplement chemical fertilizers as well as being useful for remediation of contaminated soil. Microbial compost is a vital part of proper agricultural practices (Pandey & Singh, 2019). Biofertilizer or biological fertilizer is a fertilizer that already 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 (Elpawati *et al*., 2015). Based on research has established that using a biofertilizer dose of 10 L/ha is capable of increasing the growth of rice plants (Siswanti & Rachmawati, 2013).

Salinity is an abiotic pressure on salt levels in the water that can limit plant growth and productivity. Salinity suppresses plant growth in the form of osmotic stress which will be followed by ionic toxicity caused by the accumulation of $Na+$ and Cl^- ions, reducing water absorption and availability (Mahjoor *et al*., 2016).The level of salinity affects various metabolic and physiological processes of plants depending on the duration of stress on saline and the severity which can ultimately inhibit plant production (Gupta & Huang, 2014). Chlorophyll content has a close relationship with nitrate reductase activity. However, some types of mustard greens, such as *B. rapa*, have a tolerance to salinity stress that is quite high, reaching 3000 ppm (Mulyadi & Abror, 2021).

The results of Ramlawati's (2016) research showed that *B. juncea*, which were treated with various nutrient concentrations of hydroponic solutions made from NPK, Gandasil D, KCl, and water with a wick system, were able to have a very significant effect on the increase in the number of leaves, leaf width, and wet weight, as well as a significant effect on leaf length and root length. Another study conducted by Khairunnisa and Siswanti (2021) showed that biofertilizer doses of 10 L/ha and 30 L/ha were able to provide optimum growth and productivity increases under a salinity stress of 5000 ppm.

This study was conducted to determine the activity of nitrate reductase, chlorophyll content, and the growth response of mustard green (*B. rapa*) in 5000 ppm salinity stress application with a hydroponic system. under conditions of 5000 ppm salinity stress using a hydroponic system, namely a wick system with husk charcoal as growing media.

MATERIALS AND METHODS

The research was conducted at Laboratory of Plant Physiology, Faculty of Biology, Universitas Gadjah Mada in June 2021-Mei 2022. This study utilized the formula approach for providing a biofertilizer treatment as in our previous studies (Siswanti & Khairunnisa, 2021). Plants were planted in a hydroponic system with husk chorcoal and arranged by wick system. 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. A series of 5 tubs was prepared as a positive control (given AB mix solution), negative control (only water), first treatment (10 mL/L biofertilizer), second treatment (20 mL/L biofertilizer), and third treatment (30 mL/L biofertilizer).The biofertilizer used was made with the mixture of the cow's urine and a starter of a microbial (*Bacillus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Azospirillum* sp., *Pseudomonas* sp., *Azotobacter* sp., *Rhizobium* sp. and IAA (Indole-3-Acetic Acid) producing bacteria.) in the ratio of 49:1 following our previous studies (Siswanti, 2015). Salinity used were 5000 ppm while biofertilizer doses used were 10 ml/L, 20 ml/L, and 30 ml/L. NaCl used as much as 500 mg dissolved in 100 ml of water. In this research

also used AB-Mix as a positive control and 0 ml/L biofertilizers for negative control. Treatments were replicated five times.

In this study, the parameters were plant height, number of leaves, plant dry mass, wet plant mass, total chlorophyll and Nitrate Reductase Activity. Plant height data was obtained by measuring the height of the mustard plant from the base of the stem to the top of the plant. Measurements were carried out every 2 times a week until harvest time. Data on the number of leaves was obtained by counting the leaves that had fully opened and were still attached to the stem of the plant. The number of leaves was measured twice a week until harvest. The wet weight of mustard plants was carried out by removing each sample plant from each treatment combination. Samples were weighed using an analytical balance. The dry weight of the mustard plant was obtained by removing the moisture content of the mustard plant. Mustard plants are air-dried first for 1-2 days, then in the oven for 1-2 days. Every 3 days the samples were weighed dry until constant results were obtained. The weighing was carried out 3 times in a row. Observations were done when the plants were 10 DAT (days after transplanting), with observation intervals of 4 days to 5 WAT (weeks after transplanting).

Total chlorophyll were measured content using 645 and 663 nm wavelength of UV-vis spectrophotometer. Calculation of chlorophyll content and Nitrate Reductase Activity is as follows (Khaleghi, 2012; Susanti, 2014).

$$\text{Chlorophyll a (mg/g)} = \frac{[(12{,}7 \times 663) - (2{,}69 \times 645)] \times V}{(1000 \times W)}$$

$$\text{Chlorophyll b (mg/g)} = \frac{[(22{,}9 \times 663) - (4{,}68 \times 645)] \times V}{(1000 \times W)}$$

$$\text{Total Chlorophyll (mg/g)} = \frac{[(20{,}2 \times 663) - (8{,}02 \times 645)] \times V}{(1000 \times W)}$$

Notes:
W = Weight of leaves (100 mg)
V = Extract volume (10 ml)

$$\text{ANR} = NO_2^- \text{ content (µmol)} \times \frac{5\ ml}{0{,}1\ ml} \times \frac{1000\ mg}{200\ mg} \times \frac{60\ minutes}{incubation\ time\ (minutes)}$$

Unit of Nitrat Reductase Activity = …µmol NO_2/g weight of leaves/incubation time

Data analysis. The data was performed using SPSS ver. 21 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

Mustard green (*B. rapa*) can grow well in the highlands and lowlands. Areas suitable for mustard greens to grow at an altitude of 5–1200 m above sea level. However, this plant usually grows in areas with an altitude of 100–500 meters above sea level. This plant can be planted throughout the year, both in the dry season and the rainy season (Anggraini, 2020). The optimum degree of soil acidity (pH) for growth ranges from 6-7 (Gentili *et al*., 2018). Table 1 shows the values of environmental parameters during the growth of mustard greens (*B. rapa*), which were measured in the range of 13.00–16.00.

pH is an important parameter in growth. pH is a parameter that measures the acidity or alkalinity of a solution. The magnitude of the pH value is an indicator that can determine the fertility of a plant because the availability of nutrients is closely related to the pH value of the nutrients dissolved in the hydroponic system (Setiawati *et al*., 2019). Based on the table above, mustard plants grow in a normal range. This can support the growth of mustard plants well. The increase in pH with the addition of biofertilizer compared to the positive control was because the biofertilizer contains N which will cause the formation of ammonia. The pH will increase if the ratio of ammonia to ammonium increases. This is because nitrogen will react with water and produce ammonium and OH^- ions so that

the pH value of the water also increases. The process of photosynthesis by phytoplankton which reduces the acid content in the water causes the pH to increase. Photosynthetic activity, temperature, and the presence of cations and anions are several factors that can affect the degree of acidity in a water (Damayanti, 2015).

Table 1. Environmental parameters for *B. rapa* growth in 13.00-16.00

Plot	Environmental parameter	
	pH	Temp. (°C)
KN	6.94	30
KP	6.35	30
P1	6.68	30
P2	6.60	30
P3	6.53	30

Note:
KN : Variation of *Biofertilizer* 0 mL/L + 5000 ppm salinity stress concentration of NaCl
KP : Variation of *Biofertilizer* 0 mL/L (AB Mix) + 5000 ppm salinity stress concentration of NaCl
P1 : Variation of *Biofertilizer* 10 mL/L + 5000 ppm salinity stress concentration of NaCl
P2 : Variation of *Biofertilizer* 20 mL/L + 5000 ppm salinity stress concentration of NaCl
P3 : Variation of *Biofertilizer* 30 mL/L + 5000 ppm salinity stress concentration of NaCl

Temperature is an environmental factor that influences growth and development. The effect of temperature on plant growth is known as the cardinal temperature which includes the optimum temperature (at this condition the plant will grow well), the minimum temperature (at temperatures below this the plant will not grow), and the maximum temperature (above this temperature the plant will not grow) (Rai, 2014). In this study, the temperature parameter showed a result of 30 ° C, meaning that the environmental temperature was relatively good because according to Telaumbauna (2014) hydroponic cultivation of mustard (*B. rapa*) in a greenhouse showed optimal results at a temperature of 35°C.

Table 2 shows that the combination of organic biofertilizer treatments gave different effects on the height of the mustard plant. Table 2. showed the results of the DMRT test showed that the plant heights of P1, P2, and P3 were significantly different compared to the negative control of the biofertilizer dose treatment, meaning that the biofertilizer was able to reduce salinity stress. The results of measuring the height of the mustard plant are presented in Table 2.

Table 2. The effect of variations in the concentration of biofertilizer 0-30 ml/L on the height of mustard plants under salinity (NaCl) stress application with a concentration of 5000 ppm at intervals of 5 weeks.

Treatment	Plant height (cm)
KN (0 ml/L)	$9{,}04 \pm 0{,}76^{a}$
KP (AB-Mix)	$22{,}46 \pm 1{,}15^{d}$
P1 (10 ml/L)	$17{,}12 \pm 3{,}08^{b}$
P2 (20 ml/L)	$19{,}20 \pm 3{,}51^{bc}$
P3 (30 ml/L)	$20{,}28 \pm 0{,}79^{cd}$
Average	$17{,}62 \pm 5{,}13$

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.

According to Semary *et al*. (2020) different microbial treatment, mechanisms can reduce salinity stress. Recent studies have revealed that halotolerant rhizobacterials are able to reduce salinity stress by increasing root surface area through the regulation of phytohormones and gene expression when auxin increases and abscisic acid decreases. Halotolerant rhizobacterials are bacteria that have the ability to live in a salt concentration range of 0–30% NaCl. Several bacteria that are classified as halotolerant bacteria are *Halomonas*, *Staphylococcus*, *Pseudomonas*, and *Bacillus* (Yasmin *et al*., 2020). In this study, biofertilizer containing nine types of microbes, including *Pseudomonas* and *Bacillus* (Siswanti, 2010). The level of salinity, duration of stress, and stage of development affect the response of plants to salinity stress. Plant response to salinity stress can be classified as a tolerance

mechanism as well as an avoidance mechanism. The mechanism of plant tolerance to the influence of salinity stress includes changes in morphology, physiology, and biochemistry. This mechanism is generally effective at low to moderate salinity levels (Kristiono *et al*., 2013). The tolerance mechanism of B. rapa through increasing the length of plant roots is shown in Figure 1.

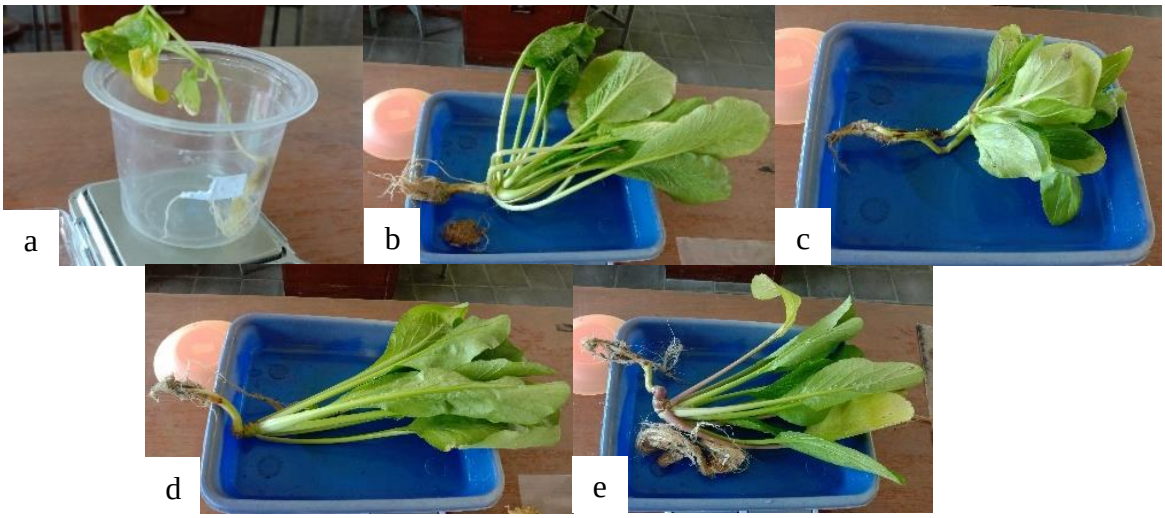

Fig 1. Root length at each treatment: a. KN; b. KP; c. P1; d. P2; e. P3 *B. rapa*

The application of biofertilizer at a dose of 30 mL/L (Fig. 2) was able to increase plant height growth at a salinity (NaCl) stress level of 5000 ppm. This indicates that the biofertilizers dose of 30 ml/L is the optimum dose required for mustard plants to support growth during salinity stress conditions. This is also evidenced by the research of Ataribaba *et al*. (2021) which stated that under normal conditions (without salinity stress), biofertilizer treatment with a dose of 5 mL/L was able to increase leaf area, the number of leaves, plant height, and net weight of mustard greens (*Brassica juncea* L.) when compared to control and fertilizer treatments of urea.

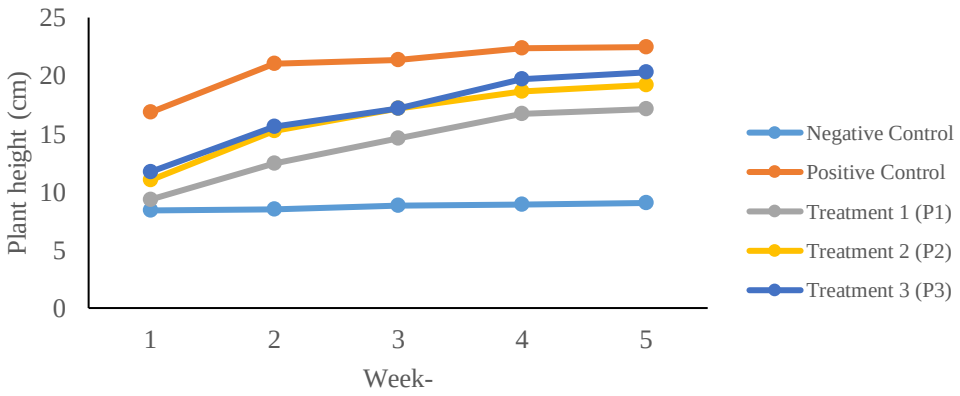

Fig. 2. The effect of variations in biofertilizer doses on the height of mustard (*B. rapa*) given salinity (NaCl) stress with a concentration of 5000 ppm with 5-week intervals

Treatment of salinity can cause stunted plant growth, especially observed from the height of the plant. Based on the data presented in Fig.1. It can be seen that the height of the mustard plant without biofertilizer (KN) was lower (9.04 cm) than the plant height given the biofertilizer with a concentration of 10 mL/L (17.12 cm), a concentration of 20 mL/ L (19.20 cm) and a concentration of 30 mL/L (20.18 cm).

The P3 treatment was not significantly different from the P2 and KP treatments but significantly different from the KN. The KN treatment tends to have conditions that are much more stunted when

compared to the other treatments because the KN treatment does not provide any nutrients, so there is no protection that can increase plant height. These results indicate that the salinity treatment given is stress for plants. Salinity is abiotic stress that can inhibit plant growth and productivity. The inhibition is in the form of osmotic stress which will be followed by ion toxicity due to the accumulation of Na^+ and Cl^- which results in a decrease in the ability to absorb water by the roots. The decrease in the ability to absorb water disrupts the osmotic balance and absorption of plant nutrients. To maintain their internal equilibrium, plants will synthesize organic osmolyte compounds such as proline, polyol, and betaine (Mahjoor *et.al*, 2016; Anosheh *et.al*, 2016; Siswanti & Umah, 2021). Plant growth depends on its root system, which functions to absorb nutrients efficiently. The requirement for a plant to grow normally is that it needs sufficient essential nutrients (Romera *et al*., 2021). The results of measuring the number of leaves of the mustard plant are presented in the Table 3.

Table 3. The effect of variations in the concentration of biofertilizer 0-30 mL/L on the number of mustard leaves on the application of 5000 ppm salinity stress (NaCl) at intervals of 5 weeks

Treatment	The number of leaves
KN (0 ml/L)	$4{,}00\pm16^{a}$
KP (AB-Mix)	11.80 ± 1.1^{c}
P1 (10 ml/L)	8.20 ± 1.79^{b}
P2 (20 ml/L)	8.00 ± 1.87^{b}
P3 (30 ml/L)	8.60 ± 0.89^{b}
Average	8.12±2.83

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 number of leaves P1, P2, and P3 was significantly different compared to the negative control with biofertilizer dose treatment and the positive control with AB-Mix nutrition. The number of leaves is influenced by several factors, one of which is water and nutrients. Sufficient water and nutrient content can affect the appearance of leaves (Shella, 2021). This is following research that shows the higher the concentration of biofertilizer, the higher the plant in maintaining the growth of the number of leaves.

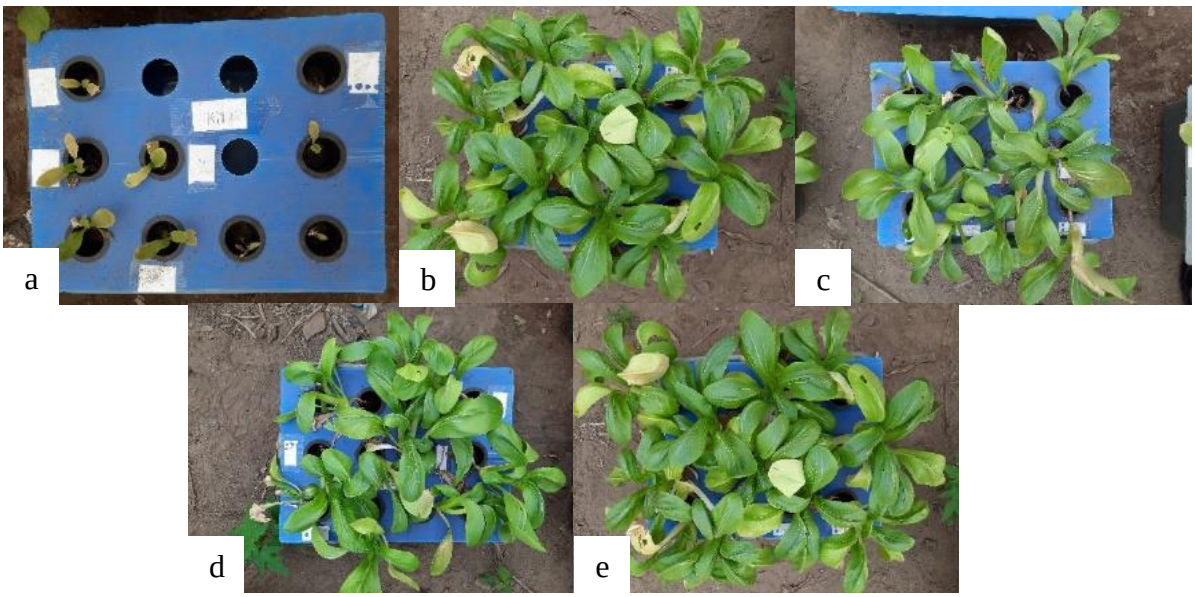

Fig 3. Morphology of *Brassica rapa* leaves in treatments: a. KN; b. KP; c. P1; d. P2; e. P3 week 5

The growth of the number of leaves of mustard greens can affect the level of salinity stress concentration between 2500 ppm-10000 ppm. This condition is caused by the presence of excess salt

which can inhibit the process of cell division. The ability of plants to absorb water will decrease and the cell division process is disrupted and affects the function of the auxin hormone (Shella, 2021). P3 has 8.6 leaves, or if rounded up, 9 leaves. P2 and P1 have 8 leaves. This is consistent with research mention before, which shows that the higher the concentration of biofertilizer, the greater the plant's ability to maintain leaf growth. The number of leaves between treatments is shown in Fig. 3.

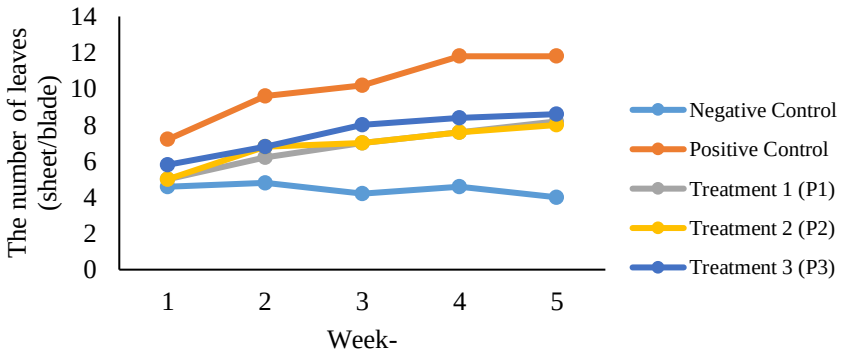

Fig. 4. Effect of biofertilizer dose variation on the number of mustard leaves (*B. rapa*) given salinity stress (NaCl) with a concentration of 5000 ppm at 5-week intervals.

Fig. 4. shows that the application of biofertilizer at a dose of 30 mL/L was able to increase plant growth at a salinity (NaCl) stress level of 5000 ppm. This indicates that the biofertilizer dose of 30 mL/L is the optimum dose required for mustard plants to support growth during salinity stress conditions. Salinity (NaCl) also affects the growth of mustard leaves. In the treatment without the use of a biofertilizer, the number of leaves was less and the size of the leaves was smaller than the plants that were given the biofertilizer. This situation is caused because plants will reduce leaf growth (number and leaf size) to maintain cell turgor pressure (Siswanti & Umah, 2021). Based on the results of the analysis performed, the administration of biofertilizer at various concentrations of 10 mL/L, 20 mL/L, and 30 mL/L had a significant effect on the number of leaves in the negative control (KN) and positive control (KP) treatments. This is because the need for nutrients for the formation of mustard leaves is fulfilled in this treatment. According to Leghari *et al*. (2016), one of the elements that play an important role in the formation of leaves is nitrogen. Nitrogen functions in the formation of green color in leaves and the process of photosynthesis which can improve the quality of vegetables.

This study uses a hydroponic system. The hydroponic system is a method of cultivating plants without using soil media, but by using a nutrient solution of minerals or other materials that contain nutrients, such as broken bricks, sand, sawdust, and others (Mulasari, 2018). The media used in this study was burnt husk. The burnt husk is believed to have light, porous, and clean properties which can be used as an alternative that can be used in hydroponic cultivation (Mishra *et al*., 2017). This is following the character of the mustard plant which is easy to flower and seed naturally and can increase growth in the mustard plant (Amer *et al*., 2019; Anjum *et al*., 2012).

The increase in growth in mustard plants is influenced by the availability of nutrients in the growing medium. The availability of nutrients can be increased by adding a biofertilizer so that it can increase plant growth through the activity of the microorganisms contained in it. In the biofertilizer, there are N-fixing bacteria (*Azotobacter* and *Rhizobium*), P solubilizing bacteria (*Aspergillus* and *Bacillus*), and growth regulators (PGR) producers (Kartikawati *et.al*, 2017). In this study, *Bacillus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Azospirillum* sp., *Pseudomonas* sp., *Azotobacter* sp., *Rhizobium* sp., and bacteria that produce IAA (Indole-3-Acetic Acid). Nitrogen-fixing bacteria can reduce free nitrogen (N_2) inert to ammonia to be absorbed by plants. Nitrogen plays an important role in the preparation of nucleotides, lipid membranes, amino acids, and the formation of chlorophyll. Phosphate solubilizing bacteria play a role in dissolving bound phosphate ($Ca_3(PO_4)^2$, Ca-P, or Ca-Fe) to form $H_2PO_4^-$ and HPO_4^{2-} ions. Phosphate plays a role in energy (ATP)

and signal transduction, respiration, macromolecular biosynthesis, and photosynthesis. (Prasad *et.al*., 2019). Nitrogen and phosphate are macromolecular nutrients and when their availability to plants is met, plant growth will increase. In addition, the microorganisms contained in the biofertilizer used to play a role in providing plant nutrients that are not available to be available. Plants that are adequate in nutrients will stimulate the growth of new leaves and plants that are sufficient or even rich in nitrogen to have greener leaves (Marpaung *et al*., 2021). The results of fresh and dry weight measurements of mustard plants are presented in Table 4.

Table 4. The effect of variations in the concentration of biofertilizer 0-30 mL/L on the wet and dry weight of mustard plants in the application of 5000 ppm salinity stress (NaCl) concentration

Treatment	Wet weight	Dry weight
KN (0 ml/L)	0.76 ± 0.03^{a}	0.017 ± 0.001^{a}
KP (AB-Mix)	102.67 ± 45.01^{d}	7.67 ± 2.31^{b}
P1 (10 ml/L)	33.67 ± 3.06^{ab}	3.33 ± 0.57^{ab}
P2 (20 ml/L)	48.67 ± 16.17^{bc}	5.00 ± 1.00^{b}
P3 (30 ml/L)	82.67 ± 27.06^{cd}	5.76 ± 4.91^{b}
Average	53.69±42.64	4.35±3.39

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 with standard deviation

The wet weight and dry weight of P2 and P3 were significantly different from the negative control. P3 has the highest wet weight value with a value of 82.67 g and the highest dry weight of 5.76 g compared to other biofertilizer dose treatments. Wet weight and dry weight at P3 have the highest value because in the previous results both on plant height and number of leaves, P3 also has the highest value for the biofertilizer treatment. Plant height and the number of leaves will store a lot of water content, which will affect the wet weight and the dry weight of mustard greens (*B. rapa*). Wet weight is also related to the leaf area and root length of a plant. According to Asih *et al*. (2015) salinity stress treatment can affect the wet weight and dry weight of mustard greens. Salinity can affect the absorption of important nutrient ions due to disruption of the nutrient water absorption system in plants. This is also evidenced by the research of Shella (2021) which showed the results that NaCl salinity stress had a significant effect on root and canopy biomass.

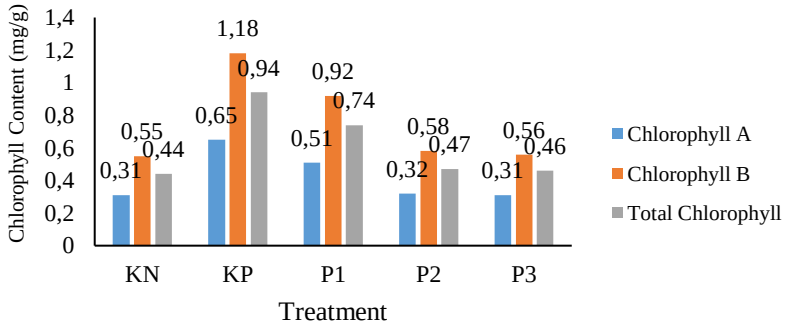

Fig. 5. The effect of variations in the dose of biofertilizer 0-30 mL/L on the levels of chlorophyll a, b, and total (mg/g) of mustard (*B. rapa*) with salinity stress (NaCl) concentration of 5000 ppm at 5th week intervals

In this study, the interaction of the dose of biofertilizer with planting media had a significant effect on wet weight and dry weight. This is because in the 30 mL/L treatment there is a biofertilizer that contains microbes and can provide nutrients (Totong *et al*, 2016). In addition, in this study, husk charcoal was used as a planting medium. The addition of husk charcoal to the growing media can have an effect, especially on the growth of mustard plants. Husk charcoal contains elements of N and K. Elemental N is an element needed in the process of photosynthesis and increases the formation and growth of the vegetative parts of plants (roots, stems, and leaves). Element K has a role as a catalyst for various enzymes (Ramdani *et al*., 2018). In addition, element K plays an important role

in photosynthesis in increasing growth, leaf area index, and increasing CO_2 assimilation (Xu *et al*., 2020). Fig. 5 shows the results of measuring the chlorophyll content of mustard greens (*B. rapa*) based on the effect of biofertilizer application at various concentrations. Based on the data obtained, the effect of the dose of biofertilizer showed that the application of a dose of 10 mL/L with a NaCl concentration of 5000 ppm showed the highest chlorophyll a, b, and total values with successive values of 0.51; 0.92; and 0.74 mg/g. The highest total chlorophyll value in all treatments was in the positive control application (AB-Mix nutrition) with a total chlorophyll value of 0.94 mg/g.

Table 5. The effect of variations in the dose of biofertilizer 0-30 mL/L on the total chlorophyll content (mg/g) of mustard (*B. rapa*) plants under stress treatment of 5000 ppm NaCl concentration at 5 week intervals

Treatment	Total Chlorophyll
KN (0 ml/L)	0.45±0.13[a]
KP (AB-Mix)	0.94±0.44[a]
P1 (10 ml/L)	0.74±0.27[a]
P2 (20 ml/L)	0.47±0.19[a]
P3 (30 ml/L)	0.46±0.24[a]
Average	0.61±0.31

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 Shows the results of measuring the chlorophyll content of mustard (*B. rapa*) based on the effect of biofertilizer application at various concentrations. Based on the data obtained, the effect of the dose of biofertilizer showed insignificant results on the total chlorophyll of mustard plants. The results of the highest chlorophyll levels showed a value of 0.94±0.44 mg/g in the positive control treatment (AB-Mix administration) and 0.74±0.27 mg/g in the 10 mL/L biofertilizer treatment. Biofertilizers with a dose of 10 ml/L is more effective increase total chlorophyll levels than the other treatments.

The solution used in this study is a biofertilizers. Biofertilizers contains several nutrients, one of which is nitrogen. The nitrogen element in the biofertilizer can add nutrients that will be absorbed by plants. Macronutrients, especially nitrogen, will be available in the vegetative phase of plants. Nitrogen element has a role in the growth of leaves and stems so that it can increase plant height and stem diameter. In addition, nitrogen plays a role in the photosynthesis process of plants because nitrogen is a constituent of chlorophyll (Ramdani *et al*., 2018). According to (Afrilandha & Setiawati, 2018) the chlorophyll content will be higher if the Nitrogen content in plants is also high. This of course will speed up the process of photosynthesis.

According to statistical calculations, the administration of biofertilizer at a dose of 10-30 mL/L did not give a significant difference in the chlorophyll content of either chlorophyll a, b, or total. Chlorophyll content does not depend on high conductivity and osmotic pressure. The high conductivity is caused by high nutrient concentrations (Ding *et* al., 2018). The salt concentration is correlated with high solution concentration. This will cause the solution to have a high ability to conduct electricity, but in this study, the concentration of the solution did not affect the chlorophyll content in the leaves. The recommended pH in a hydroponic system ranges from 5.5 to 6.5, if the water has a pH below 5.5 or more then the nutrients will settle and not be absorbed by the roots (Gillespie & Kubota, 2020)

The chlorophyll content decreased due to the high salinity process causing the photosynthesis process to decrease and the turgor pressure to decrease. The response of mustard plants due to the presence of NaCl salinity is to reduce the content of chlorophyll a, b, and total. However, according to Asih *et al*. (2015) not all chlorophyll content of mustard greens decreased with salinity stress treatment. This is because the mustard plant releases secondary metabolites that are used in regulating osmotic pressure so that NaCl sanitation stress does not affect the process of chlorophyll formation. Salinity can affect the amount of pigment complex fluid in the chloroplast structure (Shella, 2021).

Table 6. Effect of variations in the dose of biofertilizer 0-30 ml/L on nitrate reductase activity (µmol NO_2-/gram leaf wet weight/hour of incubation) of a mustard plant (*B. rapa*) under stress treatment of 5000 ppm NaCl concentration at 5-week intervals

Treatment	Nitrate reductase activity
KN (0 ml/L)	2.05±0.17[a]
KP (AB-Mix)	6.05±0.59[c]
P1 (10 ml/L)	1.64±0.37[a]
P2 (20 ml/L)	4.66±0.94[b]
P3 (30 ml/L)	1.47±0.16[a]
Average	3.17±1.96

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 shows data from the results of measurements of Nitrate Reductase Activity in the leaves of mustard greens (*B. rapa*) based on the effect of biofertilizer application at various concentrations. Based on the data obtained, the effect of the dose of biofertilizer showed that the supply of elemental N in doses of 10 and 30 mL/L did not have a significant effect on increasing the nitrate reductase activity of B. rapa This was evidenced by the Nitrate Reductase activity at a dose of 20 mL/L which was significantly different from the negative control and the other two treatments (10 and 30 mL/L). The results of the highest nitrate reductase activity showed a value of 6,05±0,59 µmol NO_2-/gram wet weight of leaf/hour of incubation in the positive control treatment (AB-Mix nutrition) and 4,66±0,94 µmol NO_2^-/gram wet weight of flag leaf/hour of incubation in the 20 mL/L dose of biofertilizer. Biofertilizer with a dose of 20 ml/L was able to effectively increase nitrate reductase activity compared to no biofertilizer application. This shows that the biofertilizer containing urine contains 9 species of microbes (*Bacillus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Azospirillum* sp., *Pseudomonas* sp., *Azotobacter* sp., *Rhizobium* sp. and IAA-producing bacteria (Indole). -3-Acetic Acid) capable of supplying N for *B. rapa* because N is needed in the enzyme nitrate reductase, which functions to reduce nitrate to nitrite (Siswanti, 2010).

Nitrate Reductase Activity is an enzyme that can catalyze nitrate (NO_3^-) to nitrite (NO_2^-) and the availability of nitrate will affect the rate of Nitrate Reductase Activity (Primavani & Zulaika, 2014). Biofertilizer provides groups of N-fixing microbes such as Azotobacter, Azomonas, Azotococcus, and Derxia. Furthermore, N_2 gas will be converted into nitrate or ammonium by nitrifying microbes. Nitrate Reductase will then reduce nitrate to nitrite (Aasfar *et al*., 2021; Hakeem *et al*., 2021). Substrate (nitrate), sunlight, and optimum pH (6.5-7.5) are important components of Nitrate Reductase Activity in reducing nitrate to nitrite (Fageria *et al*., 2014; Moro *et al*., 2017).

Treatment of salinity stress (NaCl) and solution of 10 mL/L and 30 mL/L biofertilizers had lower Nitrate Reductase activity than the 20 mL/L biofertilizer treatment because microbes tended to compete for water. According to Sudadi & Setyawan (2021) a low dose of biofertilizer will cause a low number of microbes so plants will find it difficult to absorb nutrients. High doses of biofertilizer also cause the number of microbes to be large. Many microbes will affect the absorption of plant nutrients. Microbes will affect intensive root growth and some will stick to the roots where there is root exudate (Hindersah *et al*., 2016). Root exudates of higher plants are highly favored by heterotrophic nitrogen-fixing bacteria (Noya *et al*., 2012). In addition, the large number of microbes will lower the pH due to residues of microbial metabolism in the form of CO_2. If the water has a pH below 5.5 or more then the nutrients will settle and not be absorbed by the roots.

CONCLUSION

Biofertilizer treatment can affect the growth and production of *Brassica* sp on the salinity stress condition. Application of biofertilizer at a concentration of 30 mL/L gave the effect of 53.63% increasing plant height and 22,86% number of leaves compared to negative controls and other doses but not so with chlorophyll levels. Application of biofertilizer at a concentration of 20 mL/L gave the effect of 31,43% increasing wet biomass and 30,24% dry biomass compared to negative control and

other doses. Application of biofertilizer with a dose of 10 mL/L gave 12,62% an increase in Nitrate Reductase activity compared to negative control and other doses.

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