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THE EFFECT OF BIOFERTILIZER AND AMELIORANT BRIQUETTES ON N-FIXING ENDOPHYTIC BACTERIA, PHYSIOLOGICAL DAN GROWTH OF RICE SEEDLING IN SALINE SOILS

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ABSTRACT

The growth of rice plants is restricted by saline soil conditions. The use of ameliorant briquettes and the application of biofertilizer (Klebsiella pneumoniae and Pseudomonas stutzeri) are efforts to promote plant growth and reduce the effects of salt stress on rice plants cultivated in saline soil. The combination of PGPR biofertilizer with ameliorant briquettes has been studied to increase plant development, N-fixing bacteria in the rhizosphere and endophytic bacteria, plant N uptake, and dry weight of rice plants on saline soil. The experiment was conducted in the Ciparanje Experimental Greenhouse at the Faculty of Agriculture Universitas Padjadjaran. A Randomized Block Design with 12 treatment combinations and 3 replications was used in this study. The treatment used is the application of biofertilizer, ameliorant briquettes, and their combination on non-saline soil with salinities ranging from 4 to 8 dS.m-1. The results of the experiments revealed that using biofertilizer with ameliorant briquettes affected the population of N-fixing endophytic bacteria, nitrogen content of plant, chlorophyll content and plant height, root length, dry weight of rice plants in the saline soil. The research findings revealed that biofertilizer mixed with ameliorant briquettes on 4 dS.m-1 saline soil performed better in improving all measured parameters than treatment without biofertilizer or ameliorant briquettes at the same salinity level. Biofertilizer treatment combined with ameliorant briquettes on saline soil of 8 dS.m-1 enhances plant height, root length, population of N-fixing endophytic bacteria, and N content compared to treatment without biofertilizer or ameliorant briquettes at the same salinity level.

Keywords: N-fixing endophytic bacteria, Klebsiella pneumoniae, Pseudomonas stutzeri, saline soil.

1. INTRODUCTION

Paddy is a rice-producing food crop that is still the most important food commodity in Indonesia, as most Indonesians consume rice as their staple food. The increase in demand for rice occurs along with the increase in population, while rice production for food consumption has decreased. In 2021, rice production for food consumption was 31.30 million tons, and decreased by 140.73 thousand tons from the previous year which was 31.50 million tons (BPS, 2021).

Based on data from the Central Statistics Agency, in 2021 there was also a decrease in the area of rice harvest land by 2.30% compared to the previous year. The rice harvesting area in

2020 was 10.66 million hectares, while in 2021 it will be 10.41 million. This happened because agricultural land was converted into industry, housing, and infrastructure. The conversion of agricultural land, including paddy fields, caused rice production to decline. One of the efforts that can be made to increase rice production is through extensification of paddy fields by utilizing sub-optimal land, including saline land.

Soil is considered saline if it has an electrical conductivity value (DHL) of 4 dS.m-1 or more (Negacz et al., 2022). Land with salinity problems is generally found in coastal areas, because salts from the sea will easily enter the soil through tides and seawater intrusion (Cahyadi et al., 2017). Indonesia is a maritime country that has a large saline area. This is supported by the statement of Masganti et al. (2022) which states that due to the impact of extreme climate change over the past two decades, it is estimated that the area of saline land in Indonesia is around 600.000 hectares.

The utilization of saline land for crop cultivation has many obstacles. Saline soils contain high levels of soluble salts such as NaCl, Na2CO3, and Na2SO4 that can inhibit growth and reduce crop productivity (Karolinoerita & Yusuf, 2020). NaCl is the dominant type of salt in saline soils and causes low aeration ability and soil permeability because the high NaCl content in saline soils can damage the soil structure (Kusrachdiyanti et al., 2020). In addition, plants growing in saline soils will be stressed due to high osmotic pressure, toxicity, and nutrient imbalance (Shrivastava & Kumar, 2015). Increased osmotic pressure inhibits the absorption of water and nutrients needed by plants, including nitrogen. According to Gondek et al. (2020), nutrient N is hardly available in soils with high salt concentrations.

Nitrogen is one of the macronutrients whose availability greatly affects the growth of rice plants, because plants require N during their growth phase. In the vegetative phase, namely at the beginning of growth until the middle of tiller formation and the initial stage of panicle formation is the phase that requires the most N elements (Dobermann & Fairhurst, 2000). Therefore, nutrient deficiency and soil damage due to salinity stress in saline soils need to be addressed, one of which is fertilization.

Most farmers use inorganic fertilizers for fertilization because they are considered efficient and effective in increasing agricultural production. However, if inorganic fertilizers are applied continuously, it can reduce soil quality. In addition to these problems, fertilization with N fertilizer in rice cultivation has low efficiency because the N element is easily leached and evaporated, so that plants will only absorb about 30-50% of the N fertilizer given (Setiawati et al., 2008). Therefore, it is necessary to add biofertilizer to increase nitrogen availability and reduce the impact of salinity stress on saline soils.

The biofertilizers used in this study include PGPR bacteria Klebsiella pneumoniae, Pseudomonas stutzeri from the rhizosphere of saline soil rice plants, and endophytic bacteria P. stutzeri. Based on the results of research by Khumairah et al. (2022), P. stutzeri and K. pneumoniae are halotolerant bacteria that can promote growth because they can produce IAA, nitrogenase enzymes, P dissolution, ammonia, and siderophores that help rice plants grow under salinity stress. Endophytic bacteria P. stutzeri are N-fixing bacteria that live in rice plant tissues that are in a salinity-stressed environment, therefore these bacteria are able to provide fixed N elements for rice plants, so as to encourage the growth of rice plants in saline soils (Rediers et al., 2003). The results of research by Jha et al. (2011) showed that endophytic bacteria combined with PGPR bacteria in rice plants can protect plants from salinity stress by forming Osmo protectant and antioxidant proteins.

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The application of ameliorants in the form of briquettes can also help in optimizing the use of saline soils. Ameliorants themselves act as materials to improve soil properties and provide nutritional needs for plants (Kusmiyati et al., 2014). Ameliorant materials incorporated into ameliorant briquettes consist of dolomite, biochar, compost, guano, and gypsum. Nutrients bound to the briquettes are stored and released slowly according to plant needs (slow release). Regular application of ameliorant materials into the soil, the ability to produce nutrients in the soil for a long time will remain good.

2.MATERIALS AND METHODS

Seeds and Soil

The rice seeds used in this research is saline-resistant Inpari 35 Agritan from the Subang Rice Plant Research Center. The soil used is Inceptisols soil from Jatinangor. The chemical properties of Jatinangor Inceptisols contain pH 5.63 (acid), C-organic 2.94% (medium), N-total 0,20% (low), total P 60.87 mg/100g (very low), available P 4.20 ppm (very low) and total K 34.67 mg/100g (medium), CEC 26.69 cmol.kg-1 (high), base saturation 44.02% (medium), while the texture is silty clay. The soil used was saline-conditioned according to the treatment (non-saline, 4 dS.m-1, and 8 dS.m-1).

Bioferilizer and Ameliorant Briquettes

Biofertilizer contained a consortium of rhizosphere bacteria Klebsiella pneumoniae, Pseudomonas stutzeri, and endophytic bacteria Pseudomonas stutzeri isolated from saline rice fields in Karawang Regency. Isolates were obtained from the collection of Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. Ameliorant briquettes are used with formulation of materials namely compost, biochar, dolomite, guano, gypsum, and NPK. Inorganic fertilizers are used as base fertilizers according to the recommended doses of Urea 300 kg ha-1, SP-36 50 kg ha-1, and KCl 50 kg ha-1.

Experimental Design

This experiment was conducted using a Randomized Group Design. The treatment used is the soil with different salinity levels combined with biofertilizer at a dose of 10 L ha-1 and ameliorant briquettes at a dose of 16 tons ha-1. There were 12 treatment combinations and 3 replications, so there were 36 experimental units. The details of the treatment combinations are:

Preparation of Planting Media and Planting

The planting medium used was Inceptisols paddy field soil taken from Ciparanje village. 10 kg of soil was added to each pot, then pulverized and mixed with NaCl based on the appropriate dose with salinity levels of 4 and 8 dS.m-1. Soil salinity was measured using EC. Sow rice seeds for 3 weeks, then transplant them into pots as many as 2 rice seedlings per pot.

Treatment Application

The application of biofertilizer and ameliorant briquette treatments was carried out when transplanting. The application of biofertilizers was done by injecting them into the planting media near the plant root area and ameliorant briquettes were given by immersing them in the planting media.

Observation

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The observations consisted of plant height at 7 and 14 DAP, root length, population of N-fixing bacteria and endophytic bacteria, nitrogen content and plant nitrogen uptake, chlorophyll content, and dry weight of rice plants. Bacterial populations were observed using Total Plate Count (TPC) method, nitrogen content using Kjeldahl method, and dry weight of rice plants was done by inserting the crown of rice plants of each treatment in a paper envelope and then heated in an oven with a temperature of 80oC for 48 hours until the dry weight of the rice plant is constant.

Statistical Analysis

Observation data were analyzed by analysis of variance (ANOVA) using SPSS version 25.0. Differences among treatment means were determined by conducting further tests using Duncan's Multiple Range Test at the 5% significant level.

3.RESULTS AND DISCUSSION

Plant Height

The application of biofertilizer and ameliorant briquettes had a significant effect on the height of rice plants in saline soil. The results of the Duncan test showed that the height of plants applied to a combination of biofertilizers and ameliorant briquettes at the age of 14 DAP was better than those given only biofertilizers or ameliorant briquettes singly, both in plants grown in non-saline soil, saline soil 4 and 8 dS.m-1. The addition of consortium biofertilizers in the form of PGPR bacteria (P. stutzeri and K. pneumoniae) can produce plant growth-promoting metabolites and oxidant enzymes that help plants to grow under salinity stress, both isolates can be used as strong bioinoculants to improve rice growth in saline soils (Khumairah et al., 2022).

Table 1. Effect of Biofertilizer and Ameliorant Briquettes on Plant Height at 7 and 14 DAP

Treatments	Plant Height at 7 DAP (cm)	Plant Height at 14 DAP (cm)
A = Without biofertilizer + non saline soil	40.9 b	48.4 d
B = Without biofertilizer + salinity of 4 $dS.m^{-1}$	37.8 ab	39.4 ab
C = Without biofertilizer + salinity of 8 $dS.m^{-1}$	35.2 a	35.8 a
D = Biofertilizer + non saline soil	45.3 c	54.1 e
E = Biofertilizer + salinity of 4 $dS.m^{-1}$	38.7 ab	41.4 bc
F = Biofertilizer + salinity of 8 $dS.m^{-1}$	38.0 ab	38.9 ab
G = Ameliorant briquettes + non saline soil	45.6 c	58.4 f
H = Ameliorant briquettes + salinity of 4 $dS.m^{-1}$	39.8 b	42.1 bc
I = Ameliorant briquettes + salinity of 8 $dS.m^{-1}$	37.7 ab	38.6 ab
J = Biofertilizer + ameliorant briquettes + non saline soil	47.1 c	60.6 f

K = Biofertilizer + ameliorant briquettes + salinity of 4 $dS.m^{-1}$	40.1 b	44.4 c
L = Biofertilizer + ameliorant briquettes + salinity of 8 $dS.m^{-1}$	39.0 ab	40.3 b

Increased growth of rice plants is also caused by the ability of endophytic bacteria to produce growth hormones and can regulate several aspects of plant growth and development such as activity in meristem tissues (Su et al., 2011). The addition of ameliorants can also help in the activity of microbes that have been inoculated in the rhizosphere area as a nutrient provider. It is suspected that ameliorant forming materials such as organic matter can help to improve the physical, chemical, and biological properties of soil that can increase plant height. Plants grown in saline soil of 4 and 8 dS.m-1 had slow growth and the height tended to decrease after a few days after planting compared to plants grown in non-saline soil.

Figure 1. Rice Plants Aged 14 Days After Planting

Plants grown in saline soils of 4 and 8 dS.m-1 had slow growth, and their height tended to decrease after a few days after planting compared to plants grown in non-saline soils. Increasing the level of soil salinity resulted in increasingly inhibited plant growth. This condition can be caused by osmotic stress and inhibits the ability of plants to absorb water and nutrients. In addition, the accumulation of Na+ and Cl- in plant tissues and soil disrupt physiological functions in plants, so that growth is inhibited (James et al. 2011).

Symptoms of rice plants that are stressed by high salt content can be seen from the edges of the leaves that dry, curl, and plant growth becomes stunted so that the plant dies (Rustikawati et al., 2014). When rice plants were grown in saline soil of 8 dS.m-1 aged 14 DAP, the condition of the plant experienced death due to salinity stress (Figure 1). According to Saqib et al. (2012), the high content of sodium (Na+) and chloride (Cl-) in saline soils will result in the accumulation of toxic ions in plants that have an impact on cell membrane damage that can interfere with plant growth and even cause death.

Root Length

The application of biofertilizer and ameliorant briquettes significantly affected root length. The combination treatment of biofertilizer and ameliorant briquettes at salinity of 4 dS.m-1 produced better root length than the treatment of only biofertilizer or ameliorant briquettes and the treatment without biofertilizer or ameliorant briquettes at the same salinity level. While in saline soil of 8 dS.m-1, the treatments of biofertilizer, ameliorant briquettes, and the combination of both were not significantly different, but the three treatments could increase root length by 29.19%, 19.70%, and 35.04% when compared to the treatment without biofertilizer or ameliorant briquettes in soil with the same salinity. The combined treatment of biofertilizer and ameliorant briquettes at 4 dS.m-1 was also better than control. While in saline soil of 8 dS.m-1, the combination treatment showed an effect that was not significantly different from control. This condition indicates that the combination treatment is more effective in suppressing the negative impact of salinity on plant root length.

Table 2. Effect of Biofertilizer and Ameliorant Briquettes on Root Length in Saline Soil

Treatments	Root Length (cm)
A = Without biofertilizer + non saline soil	21.1 de
B = Without biofertilizer + salinity of 4 $dS.m^{-1}$	14.7 ab
C = Without biofertilizer + salinity of 8 $dS.m^{-1}$	13.7 a
D = Biofertilizer + non saline soil	23.7 g
E = Biofertilizer + salinity of 4 $dS.m^{-1}$	19.7 de
F = Biofertilizer + salinity of 8 $dS.m^{-1}$	17.7 cd
G = Ameliorant briquettes + non saline soil	23.3 g
H = Ameliorant briquettes + salinity of 4 $dS.m^{-1}$	18.3 cd
I = Ameliorant briquettes + salinity of 8 $dS.m^{-1}$	16.4 bc
J = Biofertilizer + ameliorant briquettes + non saline soil	25.8 h
K = Biofertilizer + ameliorant briquettes + salinity of 4 $dS.m^{-1}$	21.9 fg
L = Biofertilizer + ameliorant briquettes + salinity of 8 $dS.m^{-1}$	18.5 cd

The applied biofertilizer contains PGPR and endophytic bacteria that can survive in saline conditions, so the activity of these bacteria can still support plant growth. According to Paul et al. (2014), PGPR can neutralize salinity stress in plants and increase plant growth through the production of growth-promoting hormones, namely IAA hormones. PGPR bacteria can also significantly increase root length and root surface area which leads to increased nutrient absorption, so that even under saline stress conditions plant growth remains good (Egamberdieva & Kuscharova, 2009). According to Khan et al. (2020), inoculation of endophytic bacteria can increase the root length of rice plants under salinity stress. In addition, ameliorant briquettes combined with biofertilizer in this treatment have a positive impact on the availability of nutrients resulting in increased growth of rice plants. The organic materials in the ameliorant briquettes can also support bacterial growth as they provide energy for the bacteria in the applied biofertilizer.

Based on the results of this study, there was a decrease in root length in line by increasing salinity levels. It is thought that the high accumulation of salt in the soil causes osmotic stress that can inhibit plant root growth. According to Dachlan et al. (2013), osmotic stress in saline soils is caused by an increased concentration of dissolved salts which results in the inhibition of cell division and expansion at the root tip and causes the root length to decrease.

Population of N-Fixing Endophytic Bacteria

The application of biofertilizer, ameliorant briquettes, and their combination had a significant effect on increasing the population of endophytic bacteria. The combination treatment at 4 dS.m-1 salinity was not significantly different from the combination treatment in non-saline soil and both treatments had endophytic bacterial populations of 21 and 18.5 x 105 CFU g-1 (Table 3). Application of biofertilizer with ameliorant briquettes in 4 dS.m-1 saline soil also showed 115.1% higher yield compared to the control and 172% higher compared to no application at 4 dS.m-1 salinity.

Table 3. Effect of Biofertilizer and Ameliorant Briquettes on the Population of N-Fixing Endophytic Bacteria.

Treatments	Population of N-Fixing Endophytic Bacteria ($\times 10^5$ CFU.g^{-1})
A = Without biofertilizer + non saline soil	8.6 bc
B = Without biofertilizer + salinity of 4 dS.m^{-1}	6.8 ab
C = Without biofertilizer + salinity of 8 dS.m^{-1}	5.1 a
D = Biofertilizer + non saline soil	14.1 e
E = Biofertilizer + salinity of 4 dS.m^{-1}	12.3 de
F = Biofertilizer + salinity of 8 dS.m^{-1}	11.0 cd
G = Ameliorant briquettes + non saline soil	10.3 cd
H = Ameliorant briquettes + salinity of 4 dS.m^{-1}	9.1 bc

I = Ameliorant briquettes + salinity of 8 $dS.m^{-1}$	5.1 a
J = Biofertilizer + ameliorant briquettes + non saline soil	21.0 f
K = Biofertilizer + ameliorant briquettes + salinity of 4 $dS.m^{-1}$	18.5 f
L = Biofertilizer + ameliorant briquettes + salinity of 8 $dS.m^{-1}$	11.3 cde

At 4 dS.m-1 salinity, the application of biofertilizer and briquette ameliorant applied singly can increase the population of endophytic bacteria up to 80.8% and 33.8% compared to without any application at the same salinity. When compared to the control, it has an increase of 43% and 5.8%. The biofertilizer treatment alone showed significantly different results from the control, while the briquette ameliorant treatment alone showed results that were not significantly different from the control.

The addition of ameliorants with biofertilizers can further increase the population of endophytic bacteria compared to only providing biofertilizers alone. This is thought to be because nutritional needs such as carbon and nitrogen for microbe growth are more available from the ameliorant ingredients added. In addition, biochar as one of the ingredients of the ameliorant used is known to be a good place to grow microbes because of its porous nature (Sarwono, 2016).

Soil with a salinity level of 8 dS.m-1 that was applied with biofertilizer alone and its combination with briquette ameliorant had results that were not significantly different. Both treatments were also not significantly different from the control and significantly different from no biofertilizer or briquette application at 8 dS.m-1 salinity. The increase in bacterial population in the biofertilizer treatment and its combination with the ameliorant could reach 27.9% and 31.3% compared to the control, respectively. In contrast, when compared to no application at 8 dS.m-1 salinity, the increase in endophytic population could reach 115.6% and 121.5%. This indicates that the applied biofertilizer bacteria can still survive at a salinity level of 8 dS.m-1.

Endophytic bacteria can fix nitrogen in plants, reduce osmotic pressure, regulate phytohormone production, maintain nutrient balance to reduce excess Na, and increase root length so that plants can absorb more (Ali et al., 2022). Endophytic bacteria have the potential to provide resistance and adaptation in plants to salinity stress. As the results of research from Jha et al. (2011), the combination of biofertilizers from endophytic and rhizosphere bacteria can protect plants from salinity stress.

Nitrogen Content and Plant Nitrogen Uptake

The application of biofertilizer and ameliorant briquettes significantly affects the increase in nitrogen content of rice plants in saline soils. The treatment combination of non-saline soil and treatment combination on saline soil 4 dS.m-1 had results that were not significantly different with total nitrogen content of 2.559% and 2.362%. Both treatments also had an increase in nitrogen levels of 38.6% and 28% compared to the control.

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Table 4. Effect of Biofertilizer and Ameliorant Briquettes on Nitrogen Content of Plant in Saline Soil

Treatments	Nitrogen Content (%)
A = Without biofertilizer + non saline soil	1.84 ef
B = Without biofertilizer + salinity of 4 $dS.m^{-1}$	1.53 cd
C = Without biofertilizer + salinity of 8 $dS.m^{-1}$	1.08 a
D = Biofertilizer + non saline soil	2.25 g
E = Biofertilizer + salinity of 4 $dS.m^{-1}$	2.11 fg
F = Biofertilizer + salinity of 8 $dS.m^{-1}$	1.21 ab
G = Ameliorant briquettes + non saline soil	2.06 efg
H = Ameliorant briquettes + salinity of 4 $dS.m^{-1}$	1.80 de
I = Ameliorant briquettes + salinity of 8 $dS.m^{-1}$	1.35 abc
J = Biofertilizer + ameliorant briquettes + non saline soil	2.55 h
K = Biofertilizer + ameliorant briquettes + salinity of 4 $dS.m^{-1}$	2.36 gh
L = Biofertilizer + ameliorant briquettes + salinity of 8 $dS.m^{-1}$	1.41 bc

The increase in plant nitrogen content is thought to be the effect of the application of the biofertilizer consortium of PGPR and endophytic bacterial isolates that can fix nitrogen. Fixation of N2 from the air can be done by endophytic bacteria with the enzyme nitrogenase converted into NH3 in plant tissues, so that plant nitrogen levels can increase (Miliute et al., 2015). The results of nitrogen fixation by halotolerant bacteria themselves are an important source of nitrogen for plants available in saline soils. The presence of root exudates provides the ability for nitrogen-fixing halotolerant bacteria to colonize the root zone of plants in saline soil conditions, so that microbes can live in intracellular symbiosis with the host plant (Setiawati et al., 2007).

The treatment of biofertilizer and its combination with ameliorant briquettes at 4 dS.m-1 salinity showed results that were not significantly different, with an increase in nitrogen levels of up to 37.9% and 54.2% compared to no application of biofertilizer or ameliorant briquettes at the same salinity level. The treatment of biofertilizer and ameliorant briquettes alone in saline soil of 4 dS.m-1 was not significantly different from the control, with an increase of 14.7% in the application of biofertilizer alone. This indicates that biofertilizer, ameliorant briquettes, and their combination can suppress the negative effects of salinity stress on plant nitrogen levels up to 4 dS.m-1.

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Based on the results of further tests in Table 4, at the salinity level of 8 dS.m-1, the biofertilizer and ameliorant treatments applied singly have values that are not significantly different from the combination treatment. The combination treatment of biofertilizers with ameliorant briquettes produced better nitrogen levels compared to the treatment without biofertilizers or ameliorant briquettes in soils with the same salinity. Biofertilizer and ameliorant briquette treatments given singly at 8 dS.m-1 salinity had increased nitrogen levels, but both treatments were not significantly different from the treatment without biofertilizer or ameliorant at the same salinity level.

The addition of ameliorant briquettes also plays a role in increasing plant nitrogen. Judging from Table 4, the use of biofertilizer added with ameliorant has a higher increase in nitrogen levels when compared to other treatments at each of the same salinity levels. Ameliorant itself can play a role in providing nutrients to support microbe activity, so that the availability of nutrients for plants increases (Setiawati, 2021). Based on the results of research conducted by Hindersah et al. (2022), the use of biofertilizers and organic ameliorants can increase plant nitrogen levels up to 3.11% compared to the control.

Chlorophyll Content

The addition of biofertilizers and ameliorant briquettes that can help in providing nitrogen elements can support the formation of chlorophyll in plants. In Table 5, treatment combination + non saline soil has the highest chlorophyll content up to 7.1 CCI at the age of 10 HST. Treatment combination with salinity of 4 dS.m-1 has a leaf chlorophyll value that is not significantly different from the control, so this treatment has the potential to suppress the impact of salinity stress on leaf chlorophyll content.

Table 5. Effect of Biofertilizer and Ameliorant Briquettes on Chlorophyll Content of Rice Plants in Saline Soil

Treatments	Chlorophyll Content (CCI)
A = Without biofertilizer + non saline soil	4.3 e
B = Without biofertilizer + salinity of 4 $dS.m^{-1}$	2.2 bc
C = Without biofertilizer + salinity of 8 $dS.m^{-1}$	1.1 a
D = Biofertilizer + non saline soil	5.4 f
E = Biofertilizer + salinity of 4 $dS.m^{-1}$	2.9 cd
F = Biofertilizer + salinity of 8 $dS.m^{-1}$	1.5 ab
G = Ameliorant briquettes + non saline soil	4.3 e
H = Ameliorant briquettes + salinity of 4 $dS.m^{-1}$	2.8 cd
I = Ameliorant briquettes + salinity of 8 $dS.m^{-1}$	1.2 ab

J = Biofertilizer + ameliorant briquettes + non saline soil	7.1 g
K = Biofertilizer + ameliorant briquettes + salinity of 4 $dS.m^{-1}$	3.5 de
L = Biofertilizer + ameliorant briquettes + salinity of 8 $dS.m^{-1}$	1.6 ab

Chlorophyll is the main factor that plays a role in the photosynthesis process of plants. One of the important parts in the formation of chlorophyll is nitrogen nutrients so with sufficient nitrogen in the plant, the chlorophyll formed becomes optimal and the photosynthesis process can run. Based on Table 5, in plants grown in non-saline soil, the application of biofertilizer has a higher chlorophyll content than the control and treatment with only an added ameliorant. The results of research by Setiawati et al. (2021) showed that rice seedlings inoculated with endophytic bacteria P. stutzeri can fix nitrogen and have higher chlorophyll compared to the control. The high value of chlorophyll measured indicates plant health, this is related to photosynthetic activity and nitrogen levels in the leaves.

At the salinity level of 4 dS.m-1 treated with biofertilizer and briquette ameliorant alone showed results that were not significantly different (Table 4). Both treatments were also not significantly different from the 4 dS.m-1 treatment without biofertilizer or ameliorant, although there was an increase of up to 31.8% and 27.2%. The combination treatment of biofertilizer with ameliorant briquettes at 4 dS.m-1 salinity increased up to 59.1% and was significantly different from the treatment without application of biofertilizer or ameliorant briquettes at the same salinity level.

The treatment in soil salinity of 8 dS.m-1 chlorophyll content had results that were not significantly different between biofertilizer, and ameliorant treatments applied singly, with combination treatment, or without any application. The biofertilizer and ameliorant treatments were applied singly, and the combination of both increased up to 36.3%, 9.1%, and 45.4% compared to no application of biofertilizer or ameliorant briquettes at the same salinity level.

The lower chlorophyll content along with the higher salinity level shows that the amount of salt content in the soil affects the chlorophyll content of the leaves. Based on research conducted by Ali et al. (2017), salinity stress was found to be negatively correlated with chlorophyll content. The decrease in chlorophyll content under salinity stress is thought to be due to the induction of osmotic pressure that interferes with the absorption of essential minerals. Specific uptake of ions followed by Na accumulation in the leaves is also a possible cause of inhibition of chlorophyll accumulation (Narimani et al., 2020).

Soil pH

The application of biofertilizer and ameliorant briquettes had a significant effect in increasing soil pH. The combined treatment of biofertilizer and ameliorant briquettes in non-saline soils showed the highest soil pH compared to other treatments (Table 6). Meanwhile, the treatment without biofertilizer or ameliorant briquettes in soil with salinity of 8 dS.m-1 had the lowest soil pH than other treatments.

Table 6. Effect of Biofertilizers and Ameliorant Briquettes on Soil pH in Saline Soil

Treatments	Soil pH
A = Without biofertilizer + non saline soil	6.45 cde
B = Without biofertilizer + salinity of 4 dS.m^{-1}	6.35 bcd
C = Without biofertilizer + salinity of 8 dS.m^{-1}	5.87 a
D = Biofertilizer + non saline soil	6.70 def
E = Biofertilizer + salinity of 4 dS.m^{-1}	6.44 cde
F = Biofertilizer + salinity of 8 dS.m^{-1}	6.04 ab
G = Ameliorant briquettes + non saline soil	6.77 ef
H = Ameliorant briquettes + salinity of 4 dS.m^{-1}	6.49 cdef
I = Ameliorant briquettes + salinity of 8 dS.m^{-1}	6.20 abc
J = Biofertilizer + ameliorant briquettes + non saline soil	6.84 f
K = Biofertilizer + ameliorant briquettes + salinity of 4 dS.m^{-1}	6.49 cdef
L = Biofertilizer + ameliorant briquettes + salinity of 8 dS.m^{-1}	6.33 bcd

Soil acidity (pH) in each treatment, including the treatments without biofertilizer and ameliorant briquettes in saline soils of 4 and 8 dS.m-1 increased when compared to the pH at the time of initial soil analysis, which was 5.63. This occurred due to soil inundation. In soil that continues to be inundated, the soil pH will approach neutral due to the reduction reaction. Soil inundation will cause the Fe(OH)3 compound to be reduced to Fe(OH)2, releasing OH- ions (Cyio, 2008). However, the high salinity stress causes the pH value in saline soil to be lower than non-saline soil. This condition is thought to occur because saline soils contain base cations such as Ca2+ and K+ which are low due to the high concentration of Na+ in the soil (Hu and Schmidhalter, 1997). Low Ca2+ and K+ cations in saline soils can cause a decrease in soil pH due to the hydrolysis of Fe and Al cations (Tavakkoli et al., 2015).

Based on Table 6, the combination treatment of biofertilizer with ameliorant briquettes in saline soils of 4 and 8 dS.m-1 resulted in higher soil pH values compared to the treatment of biofertilizers or ameliorant briquettes alone and without both treatments at the same salinity levels. The combination treatment at 4 and 8 dS.m-1 salinity was also not significantly different from the control. This means that the treatment can reduce the negative impact of salinity stress on soil pH.

The increase in soil pH value can be influenced by the process of nutrient absorption in plants. The biofertilizer applied in this experiment is thought to increase nutrient absorption resulting in an increase in soil pH. According to Widyati (2013), when plants absorb nitrogen in the form of nitrate, it will release hydroxyl ions that make the rhizosphere more alkaline. In addition, the provision of organic and inorganic ameliorants is also able to increase soil pH.

Khairuna et al. (2015) stated that compost can adsorb cations, including H+ so that pH tends to increase. Dolomite as an inorganic ameliorant containing Ca and Mg elements can also replace the position of ions that cause acidity in the sorption complex so that it has an impact on increasing pH (Setiawati et al., 2020).

4. CONCLUSION

Salinity is one of the main stresses that has a negative impact on rice plant growth. The use of halotolerant rhizosphere bacteria K. Pneumoniae and P. stutzeri can tolerate high salt concentrations with metabolites produced such as IAA, nitrogenase enzymes, ammonia, and siderophores which help rice plants grow under salinity stress. The endophytic bacteria P. stutzeri is also an N-fixing bacteria that can live in rice plant tissue. Ameliorant briquettes also play a role in helping provide plant nutrients and bacteria added to saline soil.

The research results showed that applying biofertilizer with ameliorant briquettes had an effect on population of N-fixing endophytic bacteria, nitrogen content of plant, chlorophyll content and plant height, root length of rice plants in saline soil. Biofertilizer mixed with ameliorant briquettes on saline soil of 4 dS.m-1 had better performance in improving all measured parameters compared to treatment without biofertilizer or ameliorant briquettes at the same salinity level. Biofertilizer treatment combined with ameliorant briquettes on saline soil of 8 dS.m-1 increased plant height, root length, population of N-fixing endophytic bacteria, as well as nitrogen content compared to treatment without biofertilizer or ameliorant briquettes at the same salinity level.

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Conflict of interest statement

The authors state there is no conflict of interest among them.

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