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Viability test of halotolerant nitrogen-fixing rhizobacterial on different carrier composition and application dosage of nitrogen biofertilizer to increase rice growth on saline ecosystems

Abstract. The use of saline soils as productive agricultural land poses major challenges. The uUtilization of nitrogen biofertilizer with halotolerant N-fixing rhizobacteria as the active material at the right dosage can increase soil productivity and support plant growth. The aim of this study was to obtain the composition of the carrier material that can maintain rhizobacteria viability, water content, and pH of nitrogen biofertilizer and to obtain the right dosage to increase the growth of rice plants in saline ecosystems. The research location was at Microbiology Laboratory of CV Bintang Asri Arthauly, Bandung and greenhouse of Jayamukti Village, Banyusari District, Karawang Regency from February to November 2020 used completely randomized design. The viability test consisted of nine treatments, while the application dosage test consisted of 13 treatments and repeated three times. The rResults showed that the H carrier composition of carrier material H (50% peat + 17,5% compost + 17,5% biochar + 5% dolomite + 5% guano + 5% nutrition) was able to maintain high viability of halotolerant N-fixing rhizobacteria compared to other treatments (10.22 x 10^7 CFU mL^{-1}). Water content (34,50%) and pH level (7.9) in the composition H also meet the quality standard requirements of the biofertilizer, respectively. Nitrogen biofertilizer with Ha carrier composition of carrier material H at a dosage of 1500 g/ha applied to seeds and nursery can increase the height and biomass of rice plants grown under saline conditions. Further research is needed on the application of nitrogen biofertilizers in saline soils that can increase the effectiveness of N fertilization.

Keywords: Carrier · Rhizobacteria · Rice · Saline ecosystem · Viability

Uji viabilitas rhizobakteri penambat nitrogen halotoleran pada komposisi media pembawa yang berbeda dan dosis aplikasi pupuk hayati nitrogen untuk meningkatkan pertumbuhan padi pada ekosistem salin

Sari. Penggunaan tanah salin sebagai lahan pertanian produktif memiliki tantangan yang besar. Pemanfaatan pupuk hayati nitrogen dengan rhizobakteri penambat N halotoleran sebagai bahan aktifnya pada dosis yang tepat dapat meningkatkan produktivitas tanah dan mendukung pertumbuhan tanaman padi. Penelitian bertujuan mendapatkan komposisi bahan pembawa yang dapat mempertahankan viabilitas rhizobakteri, kadar air, dan pH pupuk hayati nitrogen serta mendapatkan dosis yang tepat untuk meningkatkan pertumbuhan tanaman padi pada ekosistem salin. Lokasi penelitian di Laboratorium Mikrobiologi CV Bintang Asri Arthauly Bandung dan rumah kaca Desa Jayamukti, Kecamatan Banyusari, Kabupaten Karawang sejak bulan Februari sampai November 2020. Metode percobaan menggunakan metode eksperimental dengan Rancangan Acak Lengkap. Uji viabilitas terdiri dari sembilan perlakuan, sedangkan uji dosis aplikasi pupuk hayati terdiri dari 13 perlakuan dan masing-masing diulang sebanyak tiga kali. Hasil menunjukkan bahwa komposisi bahan pembawa H (Gambut 50% + kompos 17,5% + biochar 17,5% + dolomit 5% + guano 5% + nutrisi 5%) mampu mempertahankan viabilitas rhizobakteri penambat N halotoleran yang tinggi dibandingkan perlakuan lainnya yaitu sebesar 10,22 x 10^7 CFU/mL. Kadar air dan pH level pada komposisi H juga memenuhi syarat baku mutu pupuk hayati yaitu sebesar 34,50% dan 7,9. Pupuk hayati dengan komposisi bahan pembawa H dengan dosis 1500 g/ha yang diaplikasikan pada benih dan persemaian mampu meningkatkan tinggi dan biomassa tanaman padi yang ditanam pada kondisi salin. Perlu penelitian lebih lanjut mengenai aplikasi pupuk hayati N di tanah salin yang dapat meningkatkan efektivitas pemupukan N.

Kata kunci: Bahan pembawa · Ekosistem salin · Padi · Rhizobakteria · Viabilitas

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Introduction

The use of saline soil in the context of land extensification to overcome the high rate of conversion of agricultural land to non-agricultural areas such as industrial or residential areas can be done but has its own challenges. Saline soil is a soil that contains a high sodium (Na) which isthat is higher above thethan critical or tolerance threshold (Rachman *et al.*, 2018).

In rice plants (Oryza sativa L.), high salinity can be the main cause of decreased productivity (Marwanto *et al.*, 2009). Plants ability to absorb water and nutrients is reduced due to poisoning to old leaves resulted in the early aging of young leaves and a reduction of leaf area which functions in the photosynthetic process (Munns and Tester, 20082). Changes in the nitrogen cycle and N uptake of rice plants also affect the low productivity of rice in saline soils. On the other hand, the N element N plays a very important role in the growth of rice plants, namely to stimulate vegetative growth (stems and leaves), increase the number of tillers, and increase the number of seeds per hill (Damanik *et al.*, 2009).

One of the efforts to deal with this problem is the use of biofertilizers containing halotolerant nitrogen fixing rhizobacteria where these nitrogen-fixing bacteria are non simbyotic, located in plant root areas and tolerant to high salinity, hereinafter we call it as nitrogen biofertilizer. Nitrogen biofertilizer is a biological fertilizer that contains a large amount or a group of specific N-fixing microorganisms which are useful for increasing soil productivity by fixing nitrogen in the atmosphere and converting unavailable soil N into available forms to be absorbed by plants (Simarmata *et al.*, 2017).

The viability of rhizobacteria that is well maintained during storage is the key to the quality of biofertilizer, one of the crucial factors is the carrier material. The carrier material must be able to maintain a high viability and effectiveness of the inoculant (Putri *et al.*, 2010). The growth and viability of microorganisms during the storage period are determined by the type of carrier (Ferreira and Castro, 2005). The carrier material must also have a high ability to hold water and maintain pH during the storage period in order to make it easier for the bacteria inside to exchange gases, especially oxygen to survive (Abd El-Fattah *et al.*, 2013). Apart from its ability to maintain microbial viability, carriers are expected to be cheap, easy to obtain, easy to handle, high in organic matter and non-toxic (Prihastuti, 2012).

Carrier material is a temporary habitat medium for microbes before being applied to the soil, both in solid and liquid form (Setiawati *et al.*, 2017). Solid carriers can consist of a variety of materials, for example peat, compost, biochar, dolomite and guano. Peat and compost (humic materials) are commonly used as a mixture of biofertilizer carriers because they have high pore space, nutrient content and cation exchange capacity (Santi and Goenadi, 2010). The use of biochar as a carrier is proven to have a high water holding capacity which allows the carrier material to maintain moisture, thereby creating an environmental carrying capacity for bacterial cell proliferation (Saito and Marumoto, 2002). The results of Suryantini's research (20162017) also revealed that the use of peat, bokashi and their combination with dolomite and charcoal provides high viability of the P solubilizing bacteria population (Pseudomonas sp.) and can increase soybean crop yields. Guano comes from bat or bird droppings that contain Carbon (C) minerals, which are rich in Nitrogen (N), Phosphate (PO4) and urea from digestive waste that accumulate and settle on the cave floor. These can be used by rhizobacteria as food reserves during the biofertilizer storage period (Bird *et al.*, 2007). Nutrients in the form of Ashby's and Okon growth media are useful as additional food ingredients for halotolerant N-fixing rhizobacteria in biofertilizer. Ashby's and Okon media are nitrogen-free selective media widely used to isolate nitrogen fixing bacteria (Setiawati *et al.*, 2015).

The application of biofertilizers on saline soils must be in the right dosage. The research of Mieke Setiawati *et al.* (2020) suggested that the application of biofertilizer at a dosage of 25 kg ha^{-1} in combination with Azolla can increase the growth and yield of rice plants. The use of biofertilizers at a dose of 5 liters ha^{-1} was also reported to increase the number of productive tillers of rice plants (Baba *et al.*, 2021). However, no one has reported the effect of the dose of halotolerant N biofertilizer on saline soils.

Based on the above problems, this study aims to obtain the exact composition of carrier material that can maintain bacterial viability,

water content, and pH of nitrogen biofertilizer and to obtain the right dosage to increase the growth of rice plants to be used in saline ecosystems.

Materials and Method

Research materials and tools. Materials and tools used in this study were the inoculant consortium of halotolerant nitrogen fixing rhizobacteria (Pseudomonas stutzeri, Klebsiella pneumonia, Bacillus cereus, Delftia tsuruhatensis) which had been cultured on salinized Nutrient Agar media (6 dS m^{-1}) with a density of 10^7 CFU mL^{-1}, carrier materials (peat, compost, biochar, dolomite, guano), liquid nutritional materials (Ashby''s and Okon media), rice seed of variety Inpari 34 rice variety, urea, SP-36, KCl, bucket, test tubes, tube racks, bunsen, beaker glass, erlenmeyer, shake bottles, stirring rods, autoclave, laminar air flow, aluminum foil, petridishes, labels, cotton wool, cling wraps, heat resistant plastics, syringes, scissors, pH meters, ovens, analitic scales, documentation tools and stationery.

Experimental design. The research consisted of two stages. ; Tthe first stage was the viability test of the halotolerant N-fixing rhizobacteria on different carrier media. The second stage was the application dosage test of halotolerant N biofertilizer that can increase the growth of rice plants grown in saline ecosystems.

Viability test was conducted from February to September 2020 at the Microbiology Laboratory of CV Bintang Asri Arthauly Bandung. The experimental design used was a completely randomized design (CRD) consisting of 9 (nine) treatments and repeated three times.

A = Peat 50% + compost 50% + nutrition 0%
B = Peat 50% + 45% compost + 5% nutrition
C = Peat 50% + compost 40% + nutrition 10%
D = Peat 50% + 25% compost + 25% biochar + 0% nutrition
E = Peat 50% + 22.5% compost + 22.5% biochar + nutrition 5%
F = Peat 50% + 20% compost + 20% biochar + 10% nutrition
G = Peat 50% + 20% compost + 20% biochar + 5% dolomite + 5% guano + 0% nutrition
H = Peat 50% + 17,5% compost + 17,5% biochar + 5% dolomite + 5% guano + 5% nutrition
I = Peat 50% + 15% compost + 15% biochar + 5% dolomite + 5% guano + 10% nutrition

Application dosage of halotolerant N biofertilizer on saline soil test was conducted from September until November 2020 in pot experiment at greenhouse of Jayamukti Village, Banyusari District, Karawang Regency. The experimental design used was randomized block design (RBD) consisting of thirteen (13) treatments and repeated three times. Treatments were control (0 g ha^{-1}), d_1t_1 (500 g ha^{-1} + seed treatments), d_1t_2 (500 g ha^{-1} + nursery treatments), d_1t_3 (500 g ha^{-1} + seed and nursery treatments), d_2t_1 (1000 g ha^{-1}+ seed treatments), d_2t_2 (1000 g ha^{-1}+ nursery treatments), d_2t_3 (1000 g ha^{-1} + seed and nursery treatments), d_3t_1 (1500 g ha^{-1} + seed treatments), d_3t_2 (1500 g ha^{-1} + nursery treatments), d_3t_3 (1500 g ha^{-1} + seed and nursery treatments), d_4t_1 (2000 g ha^{-1} + seed treatments), d_4t_2 (2000 g ha^{-1} + nursery treatments), d_4t_3 (2000 g ha^{-1} + seed and nursery treatments). N biofertilizer used in this experiment was formula H (Peat 50% + 17,5% compost + 17,5% biochar + 5% dolomite + 5% guano + 5% nutrition) based on the best viability test results obtained.

Viability Test

Carrier preparation. Each carrier material was mashed to a size of 0.5 - 1.5 mm, then mixed according to each treatment. As much as 40 g of the carrier material was put into 50 g aluminum foil and sterilized using an autoclave with a temperature of 121 °C, pressure at 15 atm for 15 minutes. Then 8 mL of the isolate suspension is injected into the carrier or the equivalent of 20% of the volume of the carrier material in aluminum foil.

Population of Halotolerant N Fixing Rhizobacteria. Test was carried out during the storage period of 0, 2, 4, 12, 20, and 28 weeks. The viability test used Total Plate Count (TPC) method.

A total of 10 g of the carrier material sample was inputted into 90 ml of physiological solution (0.85% NaCl) in a small test tube, then pulverized until homogeneous. Then made a series of dilutions up to 10^{-7} by pipetting 1 ml of solution into 9 ml of aquadest up to 7 times. 0.1 ml of the suspension at the seventh dilution was put into a petridish containing saline NA media (6 dS m^{-1}), then incubated for 48-72 hours at room temperature (27-28 °C). The formed bacterial colonies were then counted using an electric bacterial colony counter.

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Water content. Determination of water content and pH was carried out at storage time periods of 0, 2, 4, 12, 20 and 28 weeks using the William (2000) method.

A sample of 10 g of carrier materials biofertilizer was put into a steel bowl with a known weight. Then dried in the oven for one night at 105 °C, cooled in a desiccator, weighed it using an analytical scale, and calculated the water content using formula:

$$\text{Water content (\%)} = \frac{W-W1}{W}\ x\ 100$$

Where,
W = original sample weights (g)
W1 = the sample weight after drying (g)
100 = conversion factor to %

pH. Weighed 10 g of carrier materials sample and put it in a shake bottle containing 50 mL of aquadest then shake it for 30 minutes. Soil suspension was measured with a pH meter that had been calibrated using a buffer solution of pH 7.0 and pH 4.0.

Application Dosage of Nitrogen Biofertilizer. The experiment was started by transferring saline soil (moderately saline) into a bucket with a capacity of 10 kg. Soil was taken from a depth of 0 cm - 25 cm and had been cleaned from plant debris. For application in seed treatment, halotolerant nitrogen biofertilizer were mixed with rice seeds according to the respective treatment doses, namely 0, 500, 1000, 1500, and 2000 g ha^{-1} equals to 0, 20, 40, 60, and 80 g kg^{-1} rice seeds. For nursery treatments, halotolerant nitrogen biofertilizers were spread on the nursery media. For the combination between seed and nursery, 20 g halotolerant nitrogen biofertilizer was mixed 1 kg of seeds and the remaining was spread in the nursery according to each treatment. Nursery activities were done in a pot tray with a length 30 cm x width 40 cm x height 10 cm by planting the seeds on the soil seedling medium and organic fertilizer with a ratio of 1: 1 v/v. Seeds were spread on the media, then stored in a place that is protected from direct sunlight. After 14 days after sowing (DAS), seeds were transplanted shallowly in the bucket and then the dead or damaged seedlings were sewn for one to two weeks after planting. Weeding was suggested when rice plants were 21 to 35 days old. Irrigation was carried out in an intermittent manner until the conditions were crumbled with a water level of 1 cm from the ground surface. Intermittent watering was done by controlling between dry and inundated conditions, consecutively. Inorganic fertilization used urea 300 kg ha^{-1}, SP-36 200 kg ha^{-1}, and KCl 150 kg ha^{-1} . Plants were maintained until 58 days or at the end of vegetative period.

Variables observed were plant height and biomass at the end of vegetative period at the age of 58 days after planting (DAP).

Data analysis. Viability of halotolerant N fixing rhizobacteria, water content of nitrogen biofertilizer, plant height and biomass were analyzed using one-way analysis of variance (ANOVA) and treatment means were compared by Duncan's multiple range test at $p \leq 0,05$. All the statistical tests were performed using the SPSS 16.0 software.

Result and Discussion

Viability of Halotolerant N Fixing Rhizobacteria. The viability of Halotolerant N-fixing Rhizobacteria in different carrier materials up to the storage period of 28 WAI showed a significant difference between treatments. The data on the average population numbers of halotolerant N-fixing rhizobacteria at different carrier material compositions are presented in Table 1.

Table 1. Effect of different carrier composition on halotolerant n-fixing rhizobacteria populations.

Carrier Composition	Storage time					
	0 WAI	2 WAI	4 WAI	12 WAI	20 WAI	28 WAI
	10^7	10^7	10^9	10^7	10^7	10^7
	----- CFU mL^{-1} -----					
A	8.91 ab	9.25 ab	12.29 d	9.96 d	9.72 ab	9.34 a
B	9.25 b	9.93 cde	11.17 ab	9.20 ab	9.81 abc	9.42 a
C	8.70 a	10.32 e	12.02 cd	9.64 bcd	9.69 a	9.58 ab
D	8.88 ab	9.17 a	12.04 cd	9.71 cd	9.83 abc	9.64 ab
E	9.12 ab	9.47 abc	11.32 ab	9.54 abcd	9.82 abc	9.52 a
F	8.89 ab	10.07 de	11.73 bcd	9.12 a	9.98 bc	9.41 a
G	8.90 ab	9.73 bcd	11.40 ab	9.72 cd	10.02 c	9.89 abc
H	9.76 c	10.35 e	11.58 bc	9.97 d	10.45 d	10.22 c
I	9.75 c	9.45 abc	10.86 a	9.34 abc	10.41 d	10.09 bc

Note: The average value of treatment in the same column followed by the same letter showed no

significant difference based on Duncan's multiple range test at the 5% significance level. WAI: Week After Inoculation.

The average Halotolerant N-Fixing Rhizobacteria (HNFR) population in all compositions showed that HNFR is still able to survive at a 7 month shelf life even though the population size has decreased from the previous storage period (Figure 1). Minister of Agriculture Regulation No. 70/Permentan/SR.140/10/2011 regarding organic fertilizers, biological fertilizers and soil amendments, states that the technical requirements for the number of colonies in biological fertilizers for bacteria with the type of carrier material in the form of a solid formula are $\geq 10^7$ CFU mL^{-1}. This supports that the biological fertilizers tested in this study meet the quality standard requirements for the number of colonies in the nitrogen biofertilizer. The high density of bacterial cells in biological fertilizer formulations is expected to dominate the root area when applied, so that it can compete with other microorganisms in the soil (Pudjiwati and Hamid, 2020).

Furthermore, at 28 WAI it can also be seen that the H carrier composition of the H carrier material (50% peat + 17,5% compost + 17,5% biochar + 5% dolomite + 5% guano + 5% nutrition) was able to maintain a significantly larger population than other treatments amounted up to 10.22 x 10^7 CFU mL^{-1}, eventhough not significantly different with treatment G and I. This is presumed to be due to the use of the proper composition of peat, compost, biochar, dolomite, guano and nutrition. The right type and composition of carrier material is essential for maintaining the number of inoculants and a longer life. The chemical and physical properties of different carrier material formulas can have an impact on the effectiveness of biological fertilizers in supporting plant productivity (Arora *et al.*, 2014).

Several properties of biochar suggest suitability as an inoculant carrier and seed-coating material. The physicochemical properties of biochar can sustain bacterial life, because: highly porous structure and surface area can be inhabited by bacteria and protect microorganisms from predation. Additinally, high water holding capacity of biochar can prevent bacterial desiccation.; Reduced carbon is a potential source of energy, finally biochar can provide some mineral nutrients. Therefore, it is believed that biochar can sustain the growth and survival of bacteria over time (Glodowska *et al.*, 2017). The use of dolomite can increase the pH of the fertilizer so that it creates the right acidity conditions for microbial growth and survival in the biofertilizer carrier (Suryantini, 2017). Guano contains a lot of magnetic minerals which are useful as microbial food ingredients (Sari *et al.*, 2014).

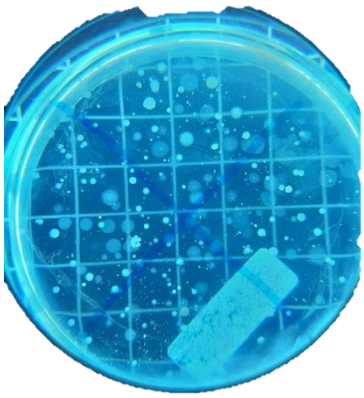

Figure 1. Population of Halotolerant N-fixing Rhizobacteria at 28 weeks after inoculation which in H Composition Carrier (50% peat + 17,5% compost + 17,5% biochar + 5% dolomite + 5% guano + 5% nutrition).

Water content. The water content of nitrogen biofertilizers with different carrier materials until the storage period of 28 WAI showed significant differences between treatments. Data on average water content are presented in Table 2.

Table 2. Effect of different carrier composition on water content of nitrogen biofertilizer.

Carrier Composition	Storage time					
	0 WAI	2 WAI	4 WAI	12 WAI	20 WAI	28 WAI
	----- % -----					
A	35.27 c	33.60 a	35.97 d	31.70 b	35.90 c	23.17 a
B	33.67 abc	35.43 bc	34.57 cd	34.17 bc	35.83 c	34,50 d
C	35.63 c	35.93 c	34.83 d	35.90 c	35.97 c	37.73 e
D	32.20 ab	33.40 a	30.90 ab	28.67 a	32.00 b	30.07 b
E	34.60 bc	34.27 ab	32.80 bc	32.47 b	32.20 b	34.23 d
F	34.30 abc	36.10 c	32.63 bc	34.27 bc	47.10 d	32.20 c
G	31.60 a	33.00 a	29.20 a	41.43 d	27.67 a	31.50 c
H	34,50 abc	36.63 c	35.07 d	34.43 bc	35.37 c	34,50 d

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I	34.43 abc	33.83 a	31.37 b	32.47 b	29.07 a	32.70 c

Note: The average value of treatment in the same column followed by the same letter showed no significant difference based on Duncan's multiple range test at the 5% significance level. WAI: Week After Inoculation

The average water content of the nitrogen biofertilizer in all compositions showed that it was able to maintain its water content at 7 months of shelf life even though it fluctuated in each storage period. Minister of Agriculture Regulation No. 70 / Permentan / SR.140 / 10/2011 concerning organic fertilizers, biological fertilizers and soil amendments, states that the technical requirements for the water content of biological fertilizers with the type of carrier in the form of solid formulas are 30-35%. This supports that the biological fertilizers tested in this study, except A carrier composition A, meet the water content quality standard requirements of the biofertilizer. In the A carrier composition A, which consisted of 50% peat + 50% compost at 28 WAI, the water content value was lower than the water content quality standard requirements for solid biological fertilizers based on the Regulation of the Minister of Agriculture no. 70/Permentan/SR.140/10/2011, this was suspected because the composition did not contain biochar and nutrients. The available water capacity of biochar carrier material is high so it can hold more water (Santi and Goenadi, 2010). The quality of biological fertilizers is largely determined by the material of origin, for example fertilizers derived from guano with low C-organic content, low C / N ratio, and high water content (Suriadikarta and Setyorini, 2006).

pH Level. The pH level of nitrogen biofertilizer with different carrier materials up to the storage period of 28 WAI was not statistically analyzed, but from the means data from each compositions showed a slightly difference between treatments. Data on the average pH level of nitrogen biofertilizer with different carrier materials compositions are presented in Table 3.

Table 3. The effect of different carrier composition on the ph level of nitrogen biofertilizer.

Carrier Composition	Storage time					
	0 WAI	2 WAI	4 WAI	12 WAI	20 WAI	28 WAI
A	7.7	7.6	7.8	8.0	7.6	7.7
B	7.9	7.5	8.2	8.0	8.0	7.6
C	8.2	7.8	7.7	8.4	7.9	7.6
D	7.9	7.9	7.9	8.1	8.0	7.7
E	8.0	7.7	7.6	8.2	8.0	7.8
F	8.0	7.7	7.8	8.1	8.5	7.8
G	7.9	7.7	7.8	8.0	8.0	8.0
H	8.1	7.8	8.1	8.1	8.1	7.9
I	8.0	7.6	7.8	8.1	8.0	7.7

Note: Data were not analyzed statistically; WAI: Week After Inoculation

The growth of bacterial colonies on a carrier depends on the type of isolate and the conditions of the carrier such as pH and nutrients contained in the carrier material (Rohmah *et al.*, 2016). The pH of the biofertilizer affects the viability and activity of the inoculum in it (Pindi and Satyanarayana, 2012). In this study, the pH of the carrier material during the study was in the pH range for viable nitrogen-fixing bacteria, indicated by a high colony count up to 28 WAI.

The average pH level of the G, H, and I treatments tend to be greater than the other treatments, this was because these three treatments contained dolomite while the other treatments did not. The content of Ca and Mg in dolomite can replace the position of acidity-causing ions in the uptake complex so that it can increase the pH (Setiawati *et al.*, 2020). Dolomite lime or which has the chemical formula $CaCO_3.MgCO_3$ contains Ca^{2+} and Mg^{2+} ions which can replace the position of Al^{3+} ions which cause soil acidity and then form bonds with HCO_3 so that the pH rises (Shaaban *et al.*, 2015).

Plant Height and Biomass. Plant height and biomass at several application dosage of N biofertilizer showed significant differences between treatments. Data on plant height and biomass are presented in Table 4.

Table 4. The effect of different carrier composition on the ph level of nitrogen biofertilizer.

Treatments	Plant Height (cm)	Biomass (g)
control	28.87 a	2.83 ab
d_1t_1	31.83 a	2.75 a
d_1t_2	30.63 a	2.75 a
d_1t_3	32.80 a	2.85 ab
d_2t_1	29.87 a	2.67 a
d_2t_2	30.97 a	2.67 a
d_2t_3	28.70 a	2.78 a
d_3t_1	34.13 b	3.05 b
d_3t_2	29.30 a	3.03 b
d_3t_3	34,50 b	3.14 b
d_4t_1	29.27 a	2.62 a
d_4t_2	32.47 a	2.62 a
d_4t_3	33.07 ab	2.72 a

Note: The average value of treatment in the same column followed by the same letter showed no significant difference based on Duncan's multiple range test at the 5% significance level.

Based on the data in Table 4. It can be seen that the application of nitrogen biofertilizer at a dosage of 1500 g ha^{-1}, both in seed treatment and the combination of seed and nursery treatment, significantly increased plant height compared to control, 500 and 1000 g ha^{-1} but did not differ significantly with dosage of 2000 g ha^{-1}. The average height of this plant was still much lower than the height of the Inpari 34 rice plant at the age of 56 DAP in non-saline conditions which can reach 93 cm (Prayoga *et al.*, 2018). This phenomenon was proved that soil salinity has an effect on reducing plant growth. Salt stress causes several disturbances in plants such as nutritional imbalance, decreased stomatal conductance, low photosynthetic activity, which causes a decrease in plant growth and yield (Ivanova *et al.*, 2015). Munns and Tester (2008) also added that salt stress in plants can cause morphological changes in plants such as a decrease in plant height, number of leaves, plant size, root length and fruit production, as well as changes in secondary metabolites, such as hormones and oxidative compounds.

The application of nitrogen biofertilizer at a dosage of 1500 g ha^{-1} also had a tendency to increase plant biomass although the results of statistical analysis were not significantly different from the control and 500 g ha^{-1} applied to seed and nursery treatment. The active ingredient of the nitrogen biofertilizer used in this study was halotolerant N-fixing rhizobacteria that function as N fixers to provide N for plants that were able to survive, grow and dominate the root area of rice plants to carry out their functions well on saline soils (Bano and Fatima, 2009). These halotolerant bacteria have the ability to balance osmotic pressure to avoid denaturation caused by salt in their environment by accumulating salts and osmolytes (organic molecules) in their cytoplasm (Oren and Rodríguez-Valera, 2001). So it was expected that there was a positive and significant relationship between N availability and plant biomass, where the availability of N through the N_2 fixation process can increase N levels in rice plant so that plant biomass increases (Aon *et al.*, 2015).

Conclusion and Suggestion

Results showed that the composition of the H carrier composition material H (50% peat + 17,5% compost + 17,5% biochar + 5% dolomite + 5% guano + 5% nutrition) was able to maintain the high viability of halotolerant N-fixing rhizobacteria compared to other treatments, namely 10.22 x 10^7 CFU mL^{-1}. The water content and pH level in the H carrier composition H also met the biofertilizer quality standards, namely 34,50% and 7.9 respectively. Rhizobacterial viability, water content, and pH levels can be maintained up to 28 Weeks After Inoculation or 7 months of storage. Nitrogen biofertilizer with thea composition of 50% peat + 17,5% compost + 17,5% biochar + 5% dolomite + 5% guano + 5% nutrition at a dosage of 1500 g ha^{-1} applied to seeds and nursery phase can increase the height and biomass of rice plants grown under saline conditions. Further research is needed on the application of nitrogen biofertilizers in saline soils that can increase the effectiveness of N fertilization.

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