

## Research Article

Reginawanti Hindersah\*, Agusthinus Marthin Kalay, Henry Kesaulya, Cucu Suherman

# The nutmeg seedlings growth under pot culture with biofertilizers inoculation

<https://doi.org/10.1515/opag-2021-0215>

received May 30, 2020; accepted October 6, 2020

**Abstract:** Nutmeg is important for national and community revenue mainly in Maluku Province where nutmeg seedlings are grown in low-fertility soil without fertilizer. A greenhouse experiment was performed to evaluate the response of nutmeg seedlings following the application of two different biofertilizer consortia. The experimental design was completely randomized block design, which tested the combination treatments of two rates and the application methods of biofertilizer consortium. The rates of “bacillus biofertilizer” was 0.15 and 0.3%, while the rates of “mixed biofertilizer” was 0.5 and 1.0%. Both biofertilizer were inoculated by foliar spray and soil application. The results verified that at 24 weeks after inoculation, biofertilizers increased the seedling growth traits which included plant height, shoot dry weight, leaf surface area, root number, and root dry weight over the control. Soil application by 1% of “mixed biofertilizer” consists of nitrogen-fixing bacteria and phosphate-solubilizing microbes resulted in better seedlings performance. However, the highest plant height was demonstrated by seedlings treated with 0.3% “bacillus biofertilizer” composed of phosphate solubilizing *Bacillus*. Biofertilizer inoculation also enhanced soil microbes and leaf surface area but did not change the root-to-shoot ratio of the seedlings. The results showed that biofertilizer inoculation improves the growth of nutmeg seedlings.

**Keywords:** nitrogen-fixing bacteria, phosphate-solubilizing microbes, phytohormones, seedling growth

## 1 Introduction

Indonesia is the world’s second largest exporter of nutmeg (*Myristica fragrans* Houtt). The native of nutmeg is Maluku Islands including Ambon where nutmeg is cultivated for generations with other crops in agroforestry system known as *Dusung* (Matinahoru 2014; Leatemia et al. 2017). In addition to nutmeg, communities grow perennial and annual food crops such as fruit, spices, horticultural, and medicinal plants in the *Dusung* (Rehatta and Raharjo 2014). Nutmeg productivity in Ambon Island is low, 0.39–0.77 t ha<sup>-1</sup> (Leatemia et al. 2017) even though the potency of nutmeg production in Ambon island is predicted up to 3 t ha<sup>-1</sup> (Basir et al. 2018).

The obstacles to increase nutmeg production include the unavailability of good-quality seedlings in the nursery. The local farmers believe that growing nutmeg naturally from seed is the best way to have best and long-term plant productivity compared to vegetative-propagated seedlings. In Maluku, nutmeg seedlings were produced mainly from mature seeds with soil as the only growth media without fertilizer. Mature fruits were obtained from mother trees with varied leaf, fruit, seed, and mace characteristics (Hetharie et al. 2015), so that the performance of the seedling was diverse. The constraint of tree seedling production in tropical soil is low nitrogen (N) and phosphorus (P) contents that have to be overcome by chemical fertilizer. In most islands of Maluku, the chemical fertilizer price is high and occasionally not available in the market.

Biofertilizer is an alternative technology to substitute chemical fertilizer and can be used in nutmeg seedling production. The active ingredients of biofertilizers are commonly N-fixing bacteria (NFB) and P-solubilizing microbes (PSMs) isolated from the soil. Both bacterial groups play an important role in nutrient cycling in soil. The nitrogenase of NFB catalyzes the fixation of N<sub>2</sub> to available NH<sub>3</sub>. Organic acid excretion is a main mechanism of PSMs to change the solubility of unavailable P to soluble phosphate. They assist nutrient acquisition and increase the availability of N and P for root uptake (Rubio et al. 2013; Sharma et al. 2017).

\* **Corresponding author: Reginawanti Hindersah**, Faculty of Agriculture, Department of Soil Science, Universitas Padjadjaran, Sumedang 45363, West Java, Indonesia, e-mail: reginawanti@unpad.ac.id

**Agusthinus Marthin Kalay, Henry Kesaulya:** Faculty of Agriculture, Pattimura University, Ambon 97233, Maluku, Indonesia

**Cucu Suherman:** Faculty of Agriculture, Department of Agronomy, Universitas Padjadjaran, Sumedang 45363, West Java, Indonesia

Biofertilizer usually contains phytohormones indole acetic acid (IAA), gibberellins (GAs), and cytokinins (CKs) excreted by certain beneficial microbes. Phytohormones production by NFB *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Bacillus* have been documented (Fibach-Paldi *et al.* 2012; Yu *et al.* 2012; Rubio *et al.* 2013; Li *et al.* 2017). Phosphate-solubilizer *Bacillus*, *Pseudomonas*, *Aspergillus*, and *Penicillium* were also reported to secrete the same phytohormones (Araújo *et al.* 2005; Mittal *et al.* 2008; Sharma *et al.* 2017). Certain soil microbes also produced siderophore and exopolysaccharide (EPS) to improve the uptake of essential metals (Emtiaz *et al.* 2004, Ahmad *et al.* 2008). Microbial EPS has been documented to enhance soil aggregation and improve nutrient uptake (Alami *et al.* 2000; Costa *et al.* 2018).

Biofertilizer application in tree and forest nursery have not only improved nutrient uptake and plant growth but also induced plant tolerance to abiotic stress (Asif *et al.* 2018). In addition to nutrients' supply by the soil and fertilizer, woody plants require phytohormones to induce their growth (Aloni 2007; Yuan *et al.* 2019). Actually, the composition of chemical fertilizer consist of only macro- and micronutrients, so that biofertilizer application is a way to supply phytohormones to the tree. Nowadays nutmeg is considered as the medicinal plants that have been studied broadly. Microbial inoculation is beneficial for the growth, nutrient uptake as well as active substance of medicinal plants (Solaiman and Anawar 2015). Moreover, biofertilizers are ecofriendly and cost-effective inputs for the farmers since they are renewable by using appropriate technology. Though the biofertilizer has a positive effect on plant growth, sole application of biofertilizer might not be effective to provide all nutrients needed by nutmeg seedling. Organic matter and reduced dose of chemical fertilizer application are still needed.

Research in biofertilizer inoculation for nutmeg seedlings is very limited. Inoculating nutmeg seedlings by beneficial microbes give plant roots the access to form the root-microbe partnership which will be effective to promote plant growth and development once the seedlings grow in the fields. Nair and Chandra (2001) stated that *Azospirillum* and *Azotobacter* inoculation is beneficial for increasing nutmeg seedling growth. However, information concerning the biofertilizer rates and application method of NFB and PSB co-inoculation for nutmeg are not yet available. Therefore, the objective of this pot experiment was to evaluate the response of nutmeg seedlings, mainly the growth of shoots and roots to different rates and the application methods of two kinds of liquid biofertilizer that contain NFB and PSM as well as phytohormones.

## 2 Material and method

### 2.1 Experimental site

The study was conducted in the greenhouse of Faculty of Agriculture, Pattimura University, Ambon city, Maluku Province, Indonesia, in July 2018–January 2019. The research location is in the tropics at an altitude of 10 m above sea level (asl) with average day temperature of 24–30°C and humidity of 75–80%. First-transplanted seedlings (10 weeks old) of nutmeg var Banda were prepared from mother seeds by the Nutmeg Nursery Community of Lilibooi Village, Leihitu District, Maluku Tengah Regency, Maluku. The Lilibooi Village is located 2 m asl with the climate similar to the Ambon city.

### 2.2 Biofertilizer

Two kinds of liquid biofertilizer consortia used in this trial were bacillus biofertilizer (BB) and mixed biofertilizer (MB). First biofertilizer contained three *Bacillus* species prepared by the Plant Physiology Laboratory, Faculty of Agriculture, Pattimura University. The second one was formulated by using NFB and PSM microbes belonging to Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. In the preparation of both biofertilizer formulations, all microbes (Table 1) of equal volume were mixed and the total bacillus and fungal densities were counted. The density of *Bacillus* spp. in BB was at least  $10^7$  colony-forming unit (CFU) per 1 mL, while bacterial and fungal populations in MB were at least  $10^7$  and  $10^5$  CFU mL<sup>-1</sup>, respectively. The activity of N fixation and P dissolution as well as phytohormones production of fertilizers are depicted in Table 1. Based on the *in vitro* test, all PSMs produced organic acid, *Bacillus* produced siderophore and *Azotobacter chroococcum* produced EPS in significant amounts.

### 2.3 Experimental design

The greenhouse experiment was carried out in completely randomized block design with nine treatments and three replications. The treatments were the combination of two rates and two application methods of each biofertilizers. The application rates of BB were 0.15 and 0.3% while that of MB was 0.5 and 1%. Both biofertilizers were inoculated to nutmeg seedlings by foliar spray and

**Table 1:** Nitrogen-fixing and phosphate-solubilizing capacity and phytohormone production of *Bacillus* and mixed biofertilizers

Microbes	Acetylene reduction (nmol g <sup>-1</sup> h <sup>-1</sup> )	Phosphate solubilizing (mg L <sup>-1</sup> )	Phytohormones (mg L <sup>-1</sup> )
<b>Bacillus biofertilizer<sup>a</sup></b>			
<i>Bacillus subtilis</i> SW16b	—	12.4	IAA 5.6; GAs 6.3
<i>Bacillus mojavensis</i> JEN3	—	13.2	IAA 0.4; GAs 5.7
<i>Bacillus subtilis</i> HPC21	—	12.3	IAA 0.3; GAs 4.2
<b>Mixed biofertilizer</b>			
<i>Azotobacter chroococcum</i>	74.1	—	IAA 1.08; CKs 0.5, GAs 0.3
<i>Azotobacter vinelandii</i>	142.1	—	IAA 1.3; CKs 4.6
<i>Azopsirillum</i> sp.	219.9	—	IAA 12.2
<i>Acinetobacter</i> sp.	96	—	IAA 10.6
<i>Pseudomonas cepacia</i>	—	2.69	IAA 7.8; CKs 5.1; GAs 10.6
<i>Penicillium</i> sp.	—	4.15	IAA 10.1; CKs 2.9; GAs 10.9

IAA: indole acetic acid, CKs: cytokinins, GAs: gibberellins.

<sup>a</sup> Source: Kesaulya et al. (2017).

soil application. The control treatment received no biofertilizer.

## 2.4 Experimental establishment

Inceptisols collected from Lilibooi area was silty clay loam and had pH of 4.7. The soil contained 1.27% organic carbon, 0.1% total N, 14.13 mg kg<sup>-1</sup> total P<sub>2</sub>O<sub>5</sub>, 22.19 mg kg<sup>-1</sup> total K<sub>2</sub>O, 4.14 mg kg<sup>-1</sup> soluble P, and cation exchange capacity of 10.62 cmol kg<sup>-1</sup>. Soil was taken from the top soil (20 cm depth) of *Dusung* agroforestry in Lilibooi Village and cleaned of the plant debris. The growth medium was prepared by mixing the soil evenly with chicken manure at a volume ratio of 3:1. The chicken manure had pH of 6.7, 28% water content, C/N ratio of 14.2, 1.1% N, 2.7% P, and 0.9% K. As much as 5 kg of growth media was put into a 20 × 30 cm (width × height), black polyethylene bag and incubated for 1 week prior to transplanting of the 10-week-old seedlings.

Each biofertilizers were diluted according to the treatment rates with ground water shortly before inoculation. For each concentration and application method, 20 mL of biofertilizer was inoculated every 3 weeks from 1 week to 20 weeks after transplanting. A total of 3 g of compound fertilizer (NPK 15-15-15) was applied to individual plants including control plants at 4, 12 and 20 weeks after transplanting. All seedlings were maintained in the greenhouse for 24 weeks. During the experiment, there was no diseases or pests attack and therefore no pesticides were applied. Seedlings were irrigated with groundwater 2–3 times a week depending on the weather.

## 2.5 Plant growth evaluation and statistical analysis

Plant height, shoot dry weights, leaf surface area (leaf area [LA]), secondary (lateral) root number, root weight, leaf number, and stem diameter were measured at 24 weeks after transplanting. Plant height was measured from the base of the stem to the shoot apex. Leaf surface area was measured by using “easy leaf area,” an automated digital image analysis (Easlon and Bloom 2014). Total root length was calculated by summing the length of each roots of individual plant. The dry weight of shoots and roots was weighed after heating the biomass at 60°C for 72 h. Secondary root number was counted based on roots extending laterally from the primary root; while the leaf number included all leaves that perfectly open. Stem diameter was measured at 10 cm from the stem base. Root-to-shoot ratio (R/S) was calculated based on the root and shoot dry weights of 24-week transplants.

For individual polybag, soil samples for bacterial counting were taken up from the soil 5 cm away from the roots. Soil samples were collected at the depth of 10 cm and evenly mixed. Soil samples were transferred into sealed plastic bag and stored at 4°C prior to microbiological analysis. Those soil samples have also been used for soil acidity analysis. Population of NFB and PSB were enumerated at 24 weeks after transplanting by serial dilution plate method (Suliasih and Widawati 2005). Nitrogen-free Ashby’s mannitol and Pikovskaya media were used to count NFB and PSB, respectively.

All data were subjected to analysis of variance (ANOVA) and then the least significant difference (LSD) test ( $P < 0.05$ ) was done. All statistical analyses have been done by using Minitab 18.

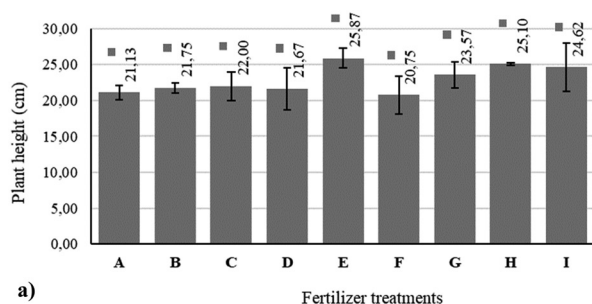
**Ethical approval:** The conducted research is not related to either human or animal use.

## 3 Results

### 3.1 Shoot growth traits

Biofertilizer treatments significantly enhanced plant height and dry weight at 24 weeks after transplanting. Seedlings inoculated with 0.3% BB by soil application and 1% MB by foliar as well as soil application demonstrated higher plant height over the control and other treatments (Figure 1a). Seedling that received 1% MB through soil application produced the highest dry weight (up to 9.90 g) but statistically did not significantly differ with 0.3% BB and 1% MB by foliar spray as well as 0.5% MB by soil application (Figure 1b).

Biofertilizer treatments have no effect on stem diameter and leaf number (Table 2). We found no great variation in stem diameter between treatments, and the average diameter was 0.54 cm. The leaf number was 14.7–20.0, which depended on the treatment. The control seedling had the lowest leaf number due to leaf fall. The seedlings that received 1% MB by foliar dressing showed higher leaf number but not significantly different from other treatments. Based on LSD test ( $p < 0.05$ ), biofertilizer-treated seedlings showed appreciable increase in LA during 24 weeks in the greenhouse (Table 3). Generally, LA of all inoculated seedlings was 25.4–43.8% higher than the control. Irrespective of the statistical analysis, the highest LA (57.8 cm<sup>2</sup>) was seen in the seedling treated with 1% MB by soil application.



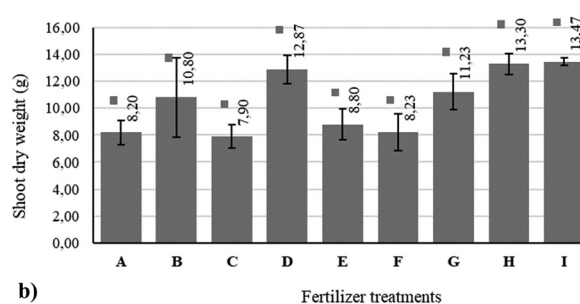
### 3.2 Root traits

Based on ANOVA, no significant difference was found among treatment for root length but did for root dry weight at 24 weeks after transplanting. The total root length of all treated seedlings was 21.42–24.88 cm, which was not significantly different with the control (Figure 2a). However, biofertilizer treatment had a potency to increase the total root length up to 9.1% in average compared to the control. Based on the LSD test ( $p < 0.05$ ), root dry weight in seedling with 1% MB through either foliar or soil dressing was significantly higher compared with the control and other treatments (Figure 2b). Both treatments gain dry root weight of 62 and 60%, respectively, over the control.

Fertilizer treatment was significant at  $p < 0.05$  for root number. Based on LSD test ( $p < 0.05$ ), seedlings treated with 1% MB by soil dressing had higher root number over the control and other treatments (Figure 3). Arrosing 1% MB around the stem produced 16.8 lateral roots which is 71.4% higher than the control.

### 3.3 Root-to-shoot ratio

Biofertilizer treatments did not change the R/S ratio based on LSD test ( $p < 0.05$ ), suggesting that both biofertilizer and their application methods had no effect on R/S (Table 3). The average R/S ratio of nutmeg seedlings at 24 weeks after transplanting was 0.38–0.45. We found that 0.3% BB by soil application had a potency to increase the R/S ratio of seedling compared to the other treatments.



**Figure 1:** Effect of biofertilizer on plant height (a) and shoot dry weight (b) of nutmeg seedling at 24 weeks after transplanting in the greenhouse. A: control, B: 0.15% BB foliar spray, C: 0.15% BB soil application, D: 0.3% BB foliar spray, E: 0.3% BB soil application, F: 0.5% MB foliar spray, G: 0.5% MB soil application, H: 1% MB foliar spray, I: 1% MB soil application. BB: bacillus biofertilizer, MB: mixed biofertilizer.

**Table 2:** Effect on biofertilizer on leaf number and leaf surface area of nutmeg seedlings at 24 weeks after transplanting in the greenhouse

Treatments	Stem diameter (cm)	Leaf number	Leaf surface area (cm <sup>2</sup> )
A: Control	0.53 ± 0.020	14.7 ± 1.28	40.2b ± 2.29
B: 0.15% BB, foliar spray	0.56 ± 0.006	18.8 ± 3.30	51.5a ± 7.05
C: 0.15% BB, soil application	0.57 ± 0.050	13.2 ± 1.89	50.4a ± 5.00
D: 0.3% BB, foliar spray	0.56 ± 0.041	18.2 ± 5.42	55.0a ± 3.33
E: 0.3% BB, soil application	0.53 ± 0.040	17.3 ± 1.15	50.7a ± 4.84
F: 0.5% MB, foliar spray	0.53 ± 0.032	18.7 ± 0.57	52.2a ± 6.32
G: 0.5% MB, soil application	0.53 ± 0.061	17.5 ± 2.29	55.2a ± 4.15
H: 1% MB, foliar spray	0.53 ± 0.036	20.0 ± 0.86	51.4a ± 4.51
I: 1% MB, soil application	0.55 ± 0.142	18.2 ± 2.25	57.8a ± 1.91

Means followed by the same letter in a column are not significantly different at  $p < 0.05$  according to the least significant different test. BB: bacillus biofertilizer, MB: mixed biofertilizer.

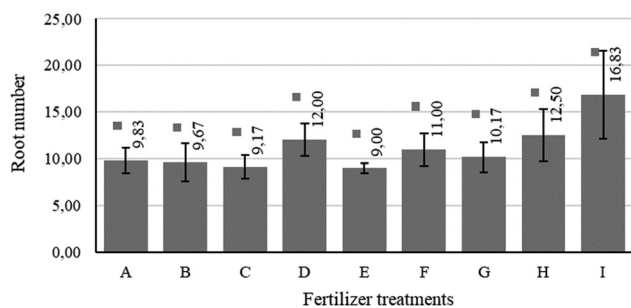
**Table 3:** Effect of biofertilizer on root-to-shoot ratio of nutmeg seedlings at 24 weeks after transplanting in the greenhouse

Treatments	Root-to-shoot ratio
A: Control	0.38 ± 0.06
B: 0.15% BB, foliar spray	0.39 ± 0.08
C: 0.15% BB, soil application	0.41 ± 0.03
D: 0.3% BB, foliar spray	0.31 ± 0.03
E: 0.3% BB, soil application	0.45 ± 0.09
F: 0.5% MB, foliar spray	0.40 ± 0.06
G: 0.5% MB, soil application	0.35 ± 0.03
H: 1% MB, foliar spray	0.38 ± 0.12
I: 1% MB, soil application	0.37 ± 0.10

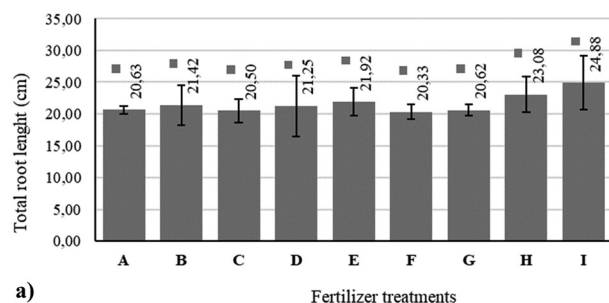
A: control, B: 0.15% BB foliar spray, C: 0.15% BB soil application, D: 0.3% BB foliar spray, E: 0.3% BB soil application, F: 0.5% MB foliar spray, G: 0.5% MB soil application, H: 1% MB foliar spray, I: 1% MB soil application. BB: bacillus biofertilizer, MB: mixed biofertilizer.

### 3.4 Bacterial population and soil acidity

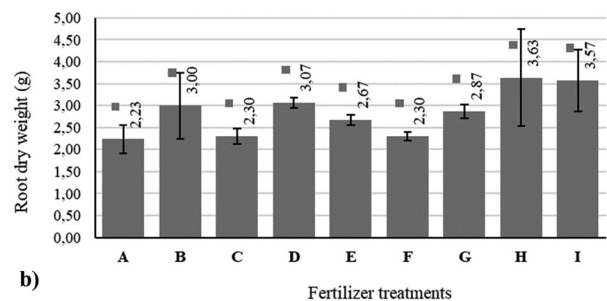
Biofertilizers significantly increased NFB and PSB count in soil around the seedling roots (Table 4). Before experiment,

**Figure 3:** Effect of biofertilizer on root number of nutmeg seedlings at 24 weeks after transplanting in the greenhouse. A: control, B: 0.15% BB foliar spray, C: 0.15% BB soil application, D: 0.3% BB foliar spray, E: 0.3% BB soil application, F: 0.5% MB foliar spray, G: 0.5% MB soil application, H: 1% MB foliar spray, I: 1% MB soil application. BB: bacillus biofertilizer, MB: mixed biofertilizer.

the untreated soil contained  $10^3$  CFU g<sup>-1</sup> of NFB and  $10^6$  CFU g<sup>-1</sup> of PSB. The increase in NFB was clearly demonstrated by MB treatment but the higher population of PSB was shown by BB treatments. In general, the higher increase in both bacterial population was



a)



b)

**Figure 2:** Effect of biofertilizer on total root length (a) and root dry weight (b) of nutmeg seedlings at 24 weeks after transplanting in the greenhouse. A: control, B: 0.15% BB foliar spray, C: 0.15% BB soil application, D: 0.3% BB foliar spray, E: 0.3% BB soil application, F: 0.5% MB foliar spray, G: 0.5% MB soil application, H: 1% MB foliar spray, I: 1% MB soil application. BB: bacillus biofertilizer, MB: mixed biofertilizer.



caused by higher concentration of biofertilizer by soil application.

The acidity of soil taken from Lilibooi Village before the experiment was 4.7, which is categorized as strongly acid soil (Table 4). At the end of experiment, the range of pH in soil was raised to 5.27–5.40.

## 4 Discussion

Biofertilizer increased the plant height, shoot dry weight, LA, root number, and root dry weight over the control. Stem diameter, leaf number, root length, and R/S ratio remained unchanged after inoculation. The highest plant height demonstrated by seedling received 0.3% BB by soil application, but the value was not significantly different with the said trait of 1% MB treatments. Seedling inoculation with 1% MB by soil application significantly produced highest shoot dry weight, LA, root number, and root dry weight. In addition, lower rate of MB also significantly increased the plant height, shoot dry weight, and LA over the control and BB. The results agree with the increased in plant height and shoot and root dry weight of walnut seedling after treating with NFB *Arthrobacter* and PSB *Pseudomonas chlororaphis* (Yu *et al.* 2012).

The consistency of MB to affect plant growth traits over BB could be related to the microbial composition in biofertilizer formulation. The soil in this trial contained low total nitrogen and available P. Nitrogen content in soil was as low as 0.1% and may induce nitrogenase activity of NFB since nitrogenase is inhibited by high nitrogen available such as  $\text{NH}_4\text{Cl}$  (Yin *et al.* 2015). This

repression is in accordance with the sensitivity of *nif* genes in NFB to fixed nitrogen (Yan *et al.* 2010; Poza-Carrión *et al.* 2014).

Low content of soluble  $\text{P}_2\text{O}_5$  ( $4.14 \text{ mg kg}^{-1}$ ) in Inceptisols used in this experiment induced the PSMs to produce phosphatase for organic P mineralization, and organic acid for solubilizing inorganic P which is unavailable in lower soil pH (Sharma *et al.* 2013; Kalayu 2019). All PSMs in both biofertilizer produce organic acid and based on *in vitro* assay were able to produce available P. This biological properties agree with the metabolism of another PSM; *F. Agrobacterium* spp., and *Bacillus circulans* (Babalola and Glick 2012), and *Aspergillus* and *Penicillium* fungi (Saxena 2013).

Since the soil was low in N and available P, nitrogen and P contents in nursery growth media might be increased for root uptake after inoculation. Both nutrients are essential for perennial plant growth seedling. Vegetative growth needs a lot of N for amino acid synthesis to build the cell and metabolism (de Oliveira Ferreira *et al.* 2016). Hence, amino acid (N) portion in leaves improved the efficiency of photosynthesis (Perchlik and Tegeder 2018). The role of P in vegetative growth is in biosynthesis of ATP for energy transfer as well as proteins and carbon. Under P deficiency circumstances, ATP production is limited and then  $\text{CO}_2$  fixation through photosynthesis is reduced (Carstensen *et al.* 2018).

The records concerning fertilization on nutmeg seedling included in Indonesia was very limited although the Indonesian Spice and Medicinal Research Institute recommended the application of 4 g NPK fertilizer (15:15:15) for individual nutmeg seedlings. The N-fixer

**Table 4:** Effect of biofertilizer treatments on nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and pH in soil at 24 weeks after transplanting in the greenhouse

Treatments	Microbial population		Soil acidity
	NFB ( $10^6 \text{ CFU g}^{-1}$ )	PSB ( $10^8 \text{ CFU g}^{-1}$ )	
A: Control	0.05d $\pm$ 0.24	0.73g $\pm$ 0.09	5.29 $\pm$ 0.11
B: 0.15% BB, foliar spray	0.19c $\pm$ 0.24	1.22f $\pm$ 0.05	5.41 $\pm$ 0.13
C: 0.15% BB, soil application	0.22c $\pm$ 0.09	3.36bc $\pm$ 0.05	5.38 $\pm$ 0.11
D: 0.3% BB, foliar spray	0.30c $\pm$ 0.33	2.57cd $\pm$ 0.02	5.38 $\pm$ 0.04
E: 0.3% BB, soil application	0.29c $\pm$ 0.24	5.83a $\pm$ 0.03	5.33 $\pm$ 0.14
F: 0.5% MB, foliar spray	1.33b $\pm$ 0.17	1.84ef $\pm$ 0.22	5.35 $\pm$ 0.16
G: 0.5% MB, soil application	2.55b $\pm$ 0.07	1.98de $\pm$ 0.03	5.27 $\pm$ 0.10
H: 1% MB, foliar spray	2.56b $\pm$ 0.04	2.81c $\pm$ 0.01	5.40 $\pm$ 0.04
I: 1% MB, soil application	4.65a $\pm$ 0.06	4.27ab $\pm$ 0.05	5.26 $\pm$ 0.18

Note: Means followed by the same letter in a column are not significantly different at  $p < 0.05$  according to least significant different test. BB: bacillus biofertilizer, MB: mixed biofertilizer. NFB: nitrogen-fixing bacteria, PSB: phosphate-solubilizing bacteria.

and P-solubilizer microbes can be beneficial to plant growth mainly in N- and P-deficient soil. In walnut seedling, the N-fixing *Arthrobacter* inoculated together with P-solubilizing *P. chlororaphis* resulted in maximum content of available N and P in soils (Yu et al. 2012). Moderately elevated N content in shoots and roots and P content in roots of mangrove seedlings following mixed inoculation of NFB and PSB have also been reported (Xiong et al. 2016).

Plant growth inoculated with high rates of biofertilizer showed more improvement relative to the lower rates. The improvement was caused by an increase in NFB and PSB population around the root seedlings. Before experiments, the soil contained  $10^3$  CFU  $g^{-1}$  of NFB and  $10^6$  CFU  $g^{-1}$  of PSB. Despite the presence of NFB and PSB in preplant soil, the effectivity and competence of indigenous beneficial microbes might be lower than that of biofertilizer. Increase in NFB and PSB population in growth media at the end of experiment (Table 4) could have an impact on the ability of both microbial groups to provide N and P for root uptake and then plant growth. Microbial inoculation along with seedling establishment provides a niche in the rhizosphere for microbial growth although competitive and synergic effect with indigenous microbes may have taken place (Trabelsi and Mhamdi 2013). The higher growth of nutmeg seedling with higher rate of biofertilizer application might be attributed to the synergic effect between native and introduced microbes. This positive interaction may increase N fixation, P solubilization, and phytohormone production. Synergetic effects between PSB and NFB have been demonstrated to enhance soil ammonium, inorganic N, and available P, which then resulted in performance improvement of the 60-day-old *Cyclocarya paliurus* seedling (Wang et al. 2019).

The soil acidity was very strongly acid (pH 4.7) before experiment and became less acid at the end of the experiment. The acid soil was less supportive to NFB growth which is optimum at neutral soil although some *Azotobacter* and *Azospirillum* isolates are able to tolerate the acid condition (Singh 2011; Verma et al. 2011). Low soil pH is more suitable for the growth of PSMs *Bacillus* and *Penicillium* (Koni et al. 2017). The better effect of MB on the growth of nutmeg seedlings may be due to the adaptability of NFB and *Penicillium* in acid soil (pH 5.29–5.40) of this experiment.

Seedling inoculation with high rate of MB by soil application enhanced plant growth (plant height, shoot dry weight, and root number) and microbial population compared to foliar spray. This is due to the increase in NFB and PSB population in soil after the trial (Table 4). Soil application of liquid biofertilizer allows microbes to

colonize the rhizosphere faster over foliar spray. Once the biofertilizer inoculated to the plant, the desired beneficial microbes proliferate and colonize the rhizosphere where they metabolize the root exudates and affect plant growth (Huang et al. 2014) and then interact with plant roots to enhance plant growth. Biofertilizer application by foliar spray allow beneficial microbes to colonize the phyllosphere. Compared to the microbes in the rhizosphere, phyllosphere microbes will face more abiotic stress such as nutrient and water limitations as well as solar radiation (Thapa et al. 2017; Truchado et al. 2017).

Irrespective of the biofertilizer dose and type, we found that LA of seedling treated with biofertilizer enhanced about 31.9% over control. Further, the biomass and the growth of nutmeg seedling that received both the biofertilizers in the field might be better. The results are in line with the findings of Rashid et al. (2018) who demonstrated that *Azospirillum* combined with PSMs and P-mobilizing microbes enhanced LA of physic nut (*Jatropha curcas* L.) seedlings.

The results showed that biofertilizer treatments had not change the R/S ratio. We found the average R/S ratio of nutmeg seedling at the end of trial was about 0.38 due to higher proportion of shoots over roots (Figures 1a and 2b). Plants with high proportion of shoots adsorb more light photon and synthesize more photosynthate and hence greater shoot biomass, but limited water and nutrient uptake by the seedling roots of 17 eudicot species (Mašková and Herben 2018). Nonetheless soil treatments in the field can lead to better tree growth since R/S ratio is not strongly relevant to the imbalance partitioning of the resource between shoots and roots (Rogers et al. 2019).

Researchers have concluded that inoculation of soil beneficial microbes in estate plantation plays a significant role in plant establishment and ensures the robustness of the seedlings (Nair and Chandra 2001; Taryo-Adiwiganda et al. 2006; Xiong et al. 2016). Moreover, biofertilizer application enables to decrease the rate of chemical fertilizer (Çakmakç et al. 2012) and increase the yield (Rohman et al. 2019). The results of the experiment verified that biofertilizers are the promising nutrient input for nutmeg seedling production.

## 5 Conclusion

Some shoot and root traits of nutmeg seedlings treated with biofertilizer types and their method of application showed better performance compared to the control. In

general, nutmeg seedling inoculation with 1% MB by soil application produced the highest shoot dry weight, LA, root number, and root dry weight. Dressing the soil with MB was preferred than BB to increase plant growth although both treatments had not change the R/S ratio at 24 weeks. Soil application of 0.3% BB also resulted in the highest plant height. The biofertilizers enhanced leaf surface area but the R/S ratio remained unchanged following inoculation of both biofertilizers. The R/S ratio was lower than 1, so that growth of nutmeg seedlings aerial part in the greenhouse was higher than roots. For better quality of local variety nutmeg seedling, we recommend to treat nutmeg seeds with microbial preparations before planting in the substrate.

**Acknowledgement:** We thank the Community of Nutmeg Nursery Center in Lilibooi Village, Leihitu District, Maluku Tengah Regency, and Regional Planning and Development Agency of Maluku Province.

**Funding information:** The research was funded by Maluku Corner Centre of Excellence of Universitas Padjadjaran year 2018 (contract number: 542/UN6.WR3/TU/2018).

**Authors' contributions:** Reginawanti Hindersah wrote the proposal and manuscript, controlled the quality of the mixed biofertilizer, and supervised the experiment; Agusthinus Marthin Kalay did the experiment, traits' measurement, and statistical analysis as well as wrote the materials and methods section; Henry Kesaulya controlled the quality of bacillus biofertilizer and did the soil and microbial analyses; and Cucu Suherman wrote the manuscript.

**Conflict of interest:** Authors declare no conflict of interest.

**Data availability statement:** The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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