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Application of inorganic and liquid organic bio-fertilizers affects the vegetative growth and rhizobacteria populations of eight banana cultivars

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Abstract. *Handayani T, Dewi TK, Martanti D, Poerba YS, Antonius S, Witjaksono. 2021. Application of inorganic and liquid organic bio-fertilizers affect the vegetative growth and rhizobacteria populations of eight banana cultivars. Biodiversitas 22: 1261-1271.* Banana (*Musa* spp.) is generally cultivated subsistent as backyard crop or intensively in plantation with application of large amounts of inorganic fertilizers to improve yields. An application of bio-fertilizer may reduce the use of inorganic fertilizer and therefore may ensure sustainable production including banana production. This research evaluated the application of liquid organic bio-fertilizer (LOB) and inorganic fertilizers on vegetative growth and soil microbial population of banana plants. The LOB and inorganic fertilizer were tested in composition of (i) 100% inorganic fertilizer (NPK), (ii) 50% NPK + 100 % LOB, (iii) 100% LOB; on eight banana cultivars, i.e., Mas Kirana (AA), Cavendish (AAA), Barangan (AAA), Raja Bulu (AAB), Raja Lido (AAB), Raja Sereh (AAB), Tanduk (AAB), and Kepok (ABB). The results showed that for most of the cultivar tested, the vegetative growth rates were not significantly different between treatment of 100% NPK and LOB in combination with 50% NPK fertilizer. The LOB application improved soil microbial population, including PGPR with a specific function such as IAA producing, phosphate solubilizing, and protease enzyme activity. The application LOB alone was insufficient to support banana's vegetative growth to the level incurred by even a moderate rate of inorganic fertilizer application.

Keywords: Banana, liquid organic bio-fertilizer, microbial population, PGPR, vegetative growth

Abbreviations: LOB: Liquid Organic Bio-fertilizer; PGPR: Plant Growth Promoting Rhizobacteria; WAP: Week After Planting

INTRODUCTION

Banana is one of the fruits that is widely grown and consumed by the Indonesian people. Bananas are generally grouped into cultivated, mostly seedless bananas and some seedy banana for spices and wild seeded bananas. Indonesia is known to have a wide variety of bananas, both cultivated and wild bananas (Hapsari 2014, Poerba et al. 2018; Ernawiati et al. 2018, Poerba et al. 2019); this is because Indonesia is the center of origin and genetic diversity of the wild *Musa acuminata Colla* species (Nasution and Yamada 2001; Perrier et al. 2011; Li et al. 2013) which is one of the ancestors of the cultivated bananas that exist. In Indonesia, bananas are commonly planted as a backyard crop, home garden, or mix with other crops in a smallholding system (≤ 1 ha). The varieties planted depend on local commercial varieties (Suyamto and Sutanto 2012; Danarto and Hapsari 2015). Bananas are also grown as border plants that mark land ownership, planted subsistent at the steep hills, riverbanks with very minimum tending. However, some large commercial plantations have been operational for some time, including a private company and a few state-own companies (Molina et al. 2019).

While as a backyard crop, bananas may not be intensively fertilized inorganically, in commercial cultivation, a large number of inorganic fertilizers, especially macronutrients like nitrogen, phosphate, and potassium, are intensively applied to the soil to improve yields. However, those common practices cause high production costs and hazardous to the environment, especially in soil fertility in the long run. Recently, eco-friendly biofertilizers have been applied to reduce inorganic fertilizers costs and avoid pollution (El Moniem et al. 2008).

Bio-fertilizers are composed of plant growth-promoting rhizobacteria (PGPR), a group of bacteria that is actively colonizing plant roots, increasing plant growth, and suppressing phytopathogens (Gupta et al. 2015; Santosa et al. 2018). Rhizobacteria promote the growth and yield of agriculturally important crops grown under different soil and climatic conditions (Mia et al. 2005). PGPR produce plant hormones, i.e., auxin, gibberellin, and cytokinin, promote phosphate and potassium solubilization, and perform nitrogen fixation, which constitutes important natures organic bio-fertilizers (Dewi et al. 2015). PGPR-based bio-fertilizers with their natures may play important roles in maintaining soil fertility (Posada et al. 2016; Gamez et al. 2019).

Many bio-fertilizers are available in the market, with single, double, or multiple microbes as the active agents.

Many institutions have also developed their bio-fertilizer formulations with their specific microbe isolates for their particular purposes. According to the Minister of Agriculture, all formulations are subject to quality testing and registering based on Indonesian National Standards (SNI) (PP No. 01/2019). We also have developed our bio-fertilizer 'Beyonic' and formulated it either in solid or liquid form. The liquid organic bio-fertilizers (LOB) 'Beyonic' LIPI contains selective PGPRs isolated and screened from various Indonesian ecosystems. Application LOB 'Beyonic' increased yields 25-30% on multiple crops in various ecosystems, which corresponded with increasing urease, phosphomonoesterase, and respiration activity of the soil, yet reducing greenhouse gas (GHG) emission (Antonius et al. 2012). However, this formulation has not been applied to a banana plantation.

Information on bio-fertilizer application for increasing banana production for diverse cultivars in the Indonesian agro-ecological system is still lacking. Some reports from other countries such as Malaysia demonstrated increased yield in the range of 35-51% with bio-fertilizer inoculation combined with reduced N application (Mia et al. 2010). Bio-fertilizer also significantly promoted plant growth, increased chitinase, and β-1,3-glucanase activities by 55%-65% and 17.3%-120.1% banana roots (Zhang et al. 2014). Gurav and Jadhav (2013) reported that using bio-fertilization from feather biomass for banana cultivation not only increased protein, amino acid, reducing sugar, chlorophyll, and proline content, but also enhanced the antioxidant potential of banana fruit, which was assessed using 2, 2-diphenyl-1-picrylhydrazyl, ferric reducing/ antioxidant power, and N, N-dimethyl-p-phenylenediamine. Application of organic amendment could improve biological properties and enhance the production plant (Gurav and Jadhav 2013).

This study aimed to evaluate the effect of liquid organic bio-fertilizer 'Beyonic' application on eight cultivated varieties of bananas on their vegetative growths and their root zone's soil microbial abundance especially IAA producing bacteria, protease producing bacteria, and phosphate solubilizing bacteria.

MATERIALS AND METHODS

The banana plants were grown at the experimental field in Cibinong Bogor (S 06°29; E 106°51). Soil classified as clay loam with soil pH 4.9-5.8, soil fertility levels is low with 1-2 %C; 0.1-0.2%N and C/N about 10-11, with the rainfed condition, during 2016-2017.

The experimental design used was a split-plot randomized complete block design, with banana cultivars as the main plot and fertilizer application (Table 1) as the subplot, and five banana plants as replications. Fertilizer application combination (Table 1) was tested on 8 banana cultivars; (i) Mas Kirana (AA), (ii) Cavendish (AAA), (iii) Barangan (AAA), (iv) Raja Lido (AAB), (v) Raja Bulu (AAB), (vi) Raja Sereh (AAB), (vii) Tanduk (AAB), and (viii) Kepok (ABB).

Table 1. Fertilizer treatment description and nutrient rates used in the field experiment

Treatments	Fertilizer rate per plant		
	NPK1	**KCl2**	**LOB 'Beyonic'**
F1 = 100 % NPK	250 g	160 g	-
F2 = LOB+ 50% NPK	125 g	80 g	1 L per plant (25 mL L^{-1})
F3 = 100 % LOB	-	-	1 L per plant (25 mL L^{-1})

Note: Inorganic fertilizer 1) NPK 16:16:16 = 16% N (Nitrogen), 16% P_2O_5 (Phosphate), 16% K_2O (Kalium); 2) KCl = fertilizers contents 60% K_2O (Kalium); The inorganic fertilizer applied at 0, 12 and 24 week after planting (WAP); LOB 'Beyonic' applied every 2 weeks until 12 WAP, LOB concentrate 25 mLLOB per liter water

Seedlings of bananas were derived from tissue culture propagation and had at least 4 to 6 leaves. Seedlings from each cultivar were planted in the planting holes, with a hole size of 50 cm x 50 cm x 50 cm (length x width x depth) and a spacing distance of 2.5 m x 2.5 m. Decomposed manure (15 kg per planting hole) and dolomite (1 ton ha^{-1}) were applied one week before planting. Weeding was carried out at bimonthly intervals. Inorganic fertilizer was applied at 0, 12, and 24 WAP, while LOB 'Beyonic' was applied every two weeks until 12 WAP. LOB was diluted in the proportion of 25 ml in 1 liter of water. The diluted LOB with a 1 liter per plant dose was applied by pouring it into the root zone.

LOB was prepared by inoculating PGPR formula as an inoculant in the mixing growth media consisting of a mixture of brown sugar, molasse, green bean sprout, fish meal, cornmeal, agar, eggs, rock phosphate, and coconut water of young fruits. All broth components that included raw materials were boiled except the eggs and the coconut water. The starter of LOB was inoculated into the prepared fermentation broth with egg and young coconut water and fermented for 21 days under continuous aeration and agitation at about 40 rpm. Inoculant formula consisted of 10 isolates of Burkholderia's family, including *Pseudomonas*, three species of *Bacillus*, *Pseudochrobactrum*, *Ochrobactrum*, *Brevundimonas*, *Brevibacillus*, and *Microbacterium* (Antonius et al. 2019 with ID Patent No. 000064813).

The data on plant vegetative growth, including the pseudostem height, the diameter of pseudostem, number of leaves, leaf length, and leaf width, were collected every four weeks (0, 4, 8, 12, and 16 weeks after planting, WAP). The pseudostem height was measured from the ground level to the base of emerging leaves (crown). The pseudostem diameter was measured at 5-10 cm above ground, while the leaf length and width were observed on the third from the newest leaf.

Soil samples were collected at 0 WAP shortly before the treatment of fertilizer and at 12 WAP. Soil and root were collected from the banana plant's rhizosphere at a depth of 10 cm to 15 cm. One hundred grams of soil were taken from each treatment in three replicates using the composite method. The soil and root samples obtained was mixed and collected into the polybag and stored in the cooler box. Ten grams of soil sample was stored in Erlenmeyer flask and homogenized with distilled water (90 mL) by shaker for 30 min and decimally diluted (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) in sterile distilled water. The last dilutions were taken with a micropipette and spread on different specific cultural media, namely skim milk agar medium, tryptic soya agar medium and Pikovskaya agar medium for showing PGPR activity indicator of protease, IAA producing and phosphate solubilization, respectively. The general total population of bacteria was observed on nutrient agar medium. Phosphate solubilizing and protease enzyme-producing bacteria activities were scored from clear zone formation on the colonies. Total Plate Count Method was used to calculate the total population of bacteria (Cappucino and Sherman 1987; Moat et al. 2002, SNI 2897:2008)

Collected data on plant growth parameters were analyzed using Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) comparison at a 5% level of significance using SAS System 9.1.

RESULTS AND DISCUSSION

Plant growth response

The ANOVA analysis (Table 2) showed that both fertilizer treatments and banana cultivars significantly affect plant vegetative growth responses. However, there was no interaction between the fertilizer treatments and the banana cultivars tested.

Fertilizer treatments demonstrated that the treatment of 100% LOB + 50% NPK was not significantly different from 100% NPK. Still, both were significantly higher than the treatment of LOB alone regarding all growth variables observed, including pseudostem height, the number of leaves, the diameter of pseudostem, leaf length, and leaf width (Table 3).

Generally, the average vegetative growth responses of 8 banana cultivars to fertilizer applications showed differences among cultivars. The diploid with genome Acuminata (AA), Mas Kirana, had significantly smaller plant morphology as indicated by pseudostem height and diameter with smaller leaf length and width than other cultivars tested. Meanwhile, cultivar Kepok, a triploid with the predominance genome of Balbisiana (ABB) showed the biggest plant morphology as indicated by pseudostem height and diameter and leaf size, even though its number of a leaf was among the lowest. Other cultivar tested, such as Raja (Raja Lido, Raja Bulu, and Raja Sereh), which are triploids with 2 A genome and 1 B, tend to cluster and significantly bigger than the triploid AAA such as Cavendish and Barangan. Tanduk, even though it is a triploid AAB, had small morphology and tended to be smaller in diameter than triploid AAA (Table 3).

As represented by the pseudostem height up to 16 WAP, the bananas growth curve seemed to follow regular sigmoid growth curve, but at the early stage which was characterized by slow growth up to 8 WAP followed by more rapid growth henceforth (Figure 1). The effect of fertilization treatments on eight different banana cultivars showed significantly different (Pr>f = <0.0001). Still, the interaction between the two factors was no significant (Pr>f = 0.1196) for the vegetative growth variable of pseudostem height at 12 WAP. Observation on the cultivar's growth regarding the pseudostem height (Figure 1) showed that they did not differ until 4 WAP but started to differ at 8 WAP. Referring to Figure 1, the difference in pseudostem height among three fertilizer treatments depends on the cultivars. However, generally, the fertilization treatment of inorganic fertilizer NPK resulted in the pseudostem's highest height, but not significantly different from the combination of LOB and 50%NPK. Pseudostem height resulted from both treatments were significantly greater than that of the treatment of LOB alone. Cultivars of Cavendish and Barangan demonstrated significantly different growth rates of pseudostem height with inorganic fertilizer application and reduced it (50% NPK + 100% LOB and LOB alone) could reduce the growth significantly (Figure 1). Therefore, compared to inorganic fertilizer application, the application of LOB alone generally incurred about 50% reduction on the pseudostem height for Cavendish, Barangan, Raja Lido, Raja Bulu, Mas Kirana, Raja Sereh, even though for Tanduk the growth reduction could reach over 60%, while for Kepok the growth reduction occurred only around 23%.

Table 2. Analysis of variance (ANOVA) summary for vegetative growth parameter of eight banana cultivars at different fertilizer treatments at 12 WAP

Source	Pseudo-stem height	Number of leave	Diameter of pseudostem	Leaf length	Leaf width
Fertilizer	<0.0001 **	<0.0001 **	<0.0001 **	<0.0001 **	<0.0001 **
Banana Cultivar	<0.0001 **	<0.0001 **	<0.0001 **	<0.0001 **	<0.0001 **
Replication	0.3878 ns	0.2718 ns	0.6261 ns	0.2546 ns	0.2869 ns
Fertilizer*Replication	0.7241 ns	0.0188 *	0.4735 ns	0.4581 ns	0.6302 ns
Fertilizer*Cultivar	0.1196 ns	0.0338 *	0.1931 ns	0.1806 ns	0.2460 ns
%CV	18.548	15.629	18.288	16.876	16.447

Note: Value at each column are pr>f, * significant at the 0.05 level, ** significant at the 0.01 level, ns not-significant. WAP: week after planting

Table 3. The responses of vegetative growth variables at different fertilizer application treatments on eight banana cultivars at 12 WAP

Treatments	Pseudo-stem height (cm)		Number of leave		Diameter of pseudostem (cm)		Leaf length (cm)		Leaf width (cm)	
Fertilizers application										
NPK	120.30 ± 27.49	a	11.76 ± 1.62	a	10.62 ± 2.18	a	112.16 ± 26.61	a	51.12 ± 8.23	a
LOB+50%NPK	115.76 ± 40.65	a	11.57 ± 2.01	a	10.14 ± 2.94	a	108.73 ± 33.19	a	48.61 ± 12.54	a
LOB	67.68 ± 28.79	b	8.00 ± 2.62	b	6.25 ± 1.93	b	70.45 ± 24.75	b	32.68 ± 11.01	b
Banana cultivars										
Mas kirana (AA)	69.69 ± 26.63	e	10.23 ± 3.06	c	6.08 ± 2.08	f	65.15 ± 24.65	d	32.73 ± 10.90	e
Cavendish (AAA)	74.62 ± 27.80	e	10.54 ± 1.90	bc	8.24 ± 3.05	ed	76.08 ± 27.32	d	40.46 ± 12.67	cd
Barangan (AAA)	89.53 ± 22.56	cd	11.73 ± 3.41	ab	8.41 ± 2.06	ed	96.40 ± 21.31	c	42.73 ± 9.69	c
Tanduk (AAB)	79.73 ± 40.80	de	9.00 ± 2.83	d	7.34 ± 3.31	e	70.27 ± 30.53	d	36.23 ± 15.62	ed
Raja lido (AAB)	97.85 ± 31.45	c	10.77 ± 3.14	bc	9.15 ± 2.44	cd	113.92 ± 25.30	b	44.04 ± 8.50	c
Raja bulu (AAB)	123.00 ± 34.47	b	12.21 ± 1.19	a	10.51 ± 2.90	b	120.14 ± 29.70	ab	52.04 ± 12.39	b
Raja sereh (AAB)	116.40 ± 34.77	b	10.53 ± 2.39	bc	10.02 ± 2.96	bc	100.27 ± 28.07	c	44.20 ± 11.23	c
Kepok (ABB)	153.64 ± 26.42	a	8.36 ± 1.28	d	11.90 ± 1.83	a	131.93 ± 18.18	a	59.43 ± 5.72	a

Note: Value means ± St Dev from 8 banana cultivars and different fertilizer applications. The same letter in the same column does not differ significantly at the 0.05 level by DMRT

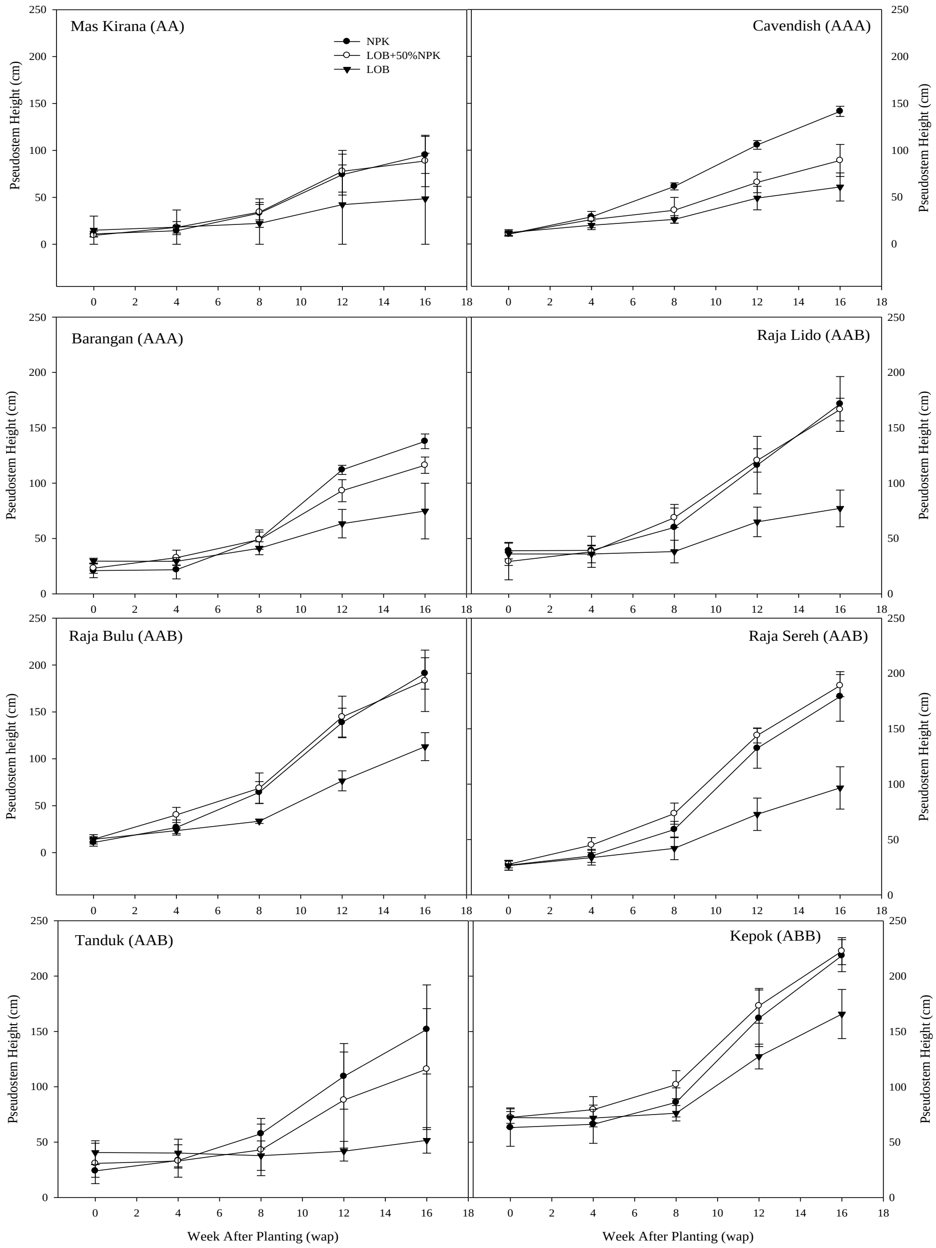

Figure 1. Growth curve of pseudostem height (cm) of 8 banana cultivars in 3 different fertilization treatments up through 16 WAP

Table 4. Vegetative growth parameters of eight cultivars at different fertilizer application treatments at 12 WAP

Cultivars	Fertilizer application	Diameter of pseudostem (cm)		Number of leaves		Leaf length (cm)		Leaf width (cm)	
				Mean ± Std error -					
Mas Kirana (AA)	NPK	6.20 ± 2.43	a	11.40± 0.71	a	61.60±28.55	a	35.10±12.99	a
	LOB+1/2NPK	6.88 ± 1.95	a	12.00± 1.52	a	75.60±25.94	a	34.50±11.31	a
	LOB	4.04 ± 1.13	b	6.80± 2.39	b	47.20±13.81	b	23.00± 6.09	b
Cavendish (AAA)	NPK	11.62 ± 0.53	a	11.60± 0.89	a	107.00± 7.81	a	53.80± 4.49	a
	LOB+1/2NPK	7.54 ± 1.12	b	10.67± 0.58	a	67.00±10.58	b	39.33± 6.03	b
	LOB	5.29 ± 1.16	c	9.40± 2.61	a	50.60± 7.33	c	27.80± 4.44	c
Barangan (AAA)	NPK	9.90 ± 0.42	a	13.60± 2.61	a	111.40± 6.27	a	49.40± 2.82	a
	LOB+1/2NPK	9.52 ± 0.85	a	14.00± 1.14	a	104.80±14.65	a	46.80± 5.97	a
	LOB	5.82 ± 1.16	b	7.60± 0.71	b	73.00±16.78	b	32.00± 8.05	b
Raja Lido (AAB)	NPK	10.65 ± 2.09	a	12.50± 1.29	a	129.80±18.38	a	49.50± 3.70	a
	LOB+1/2NPK	10.83± 0.58	a	12.75± 0.96	a	132.75± 5.32	a	50.50± 1.73	a
	LOB	6.60± 1.05	b	7.80± 3.11	b	86.80±12.03	b	34.50± 4.36	b
Raja Bulu (AAB)	NPK	11.42± 1.57	a	12.80± 0.84	a	132.80±13.74	a	56.40± 7.96	a
	LOB+1/2NPK	12.28± 2.85	a	12.60± 1.30	a	138.20±22.59	a	59.60± 9.66	a
	LOB	6.40± 1.89	b	10.80± 0.55	b	74.00±19.65	b	33.50± 9.51	b
Raja Sereh (AAB)	NPK	11.47± 1.78	a	11.40± 2.35	a	122.20±13.22	a	49.80± 2.77	a
	LOB+1/2NPK	12.23± 1.02	a	12.20± 0.45	a	113.20± 4.60	a	53.00± 2.00	a
	LOB	6.37± 1.08	b	8.00± 1.34	b	65.40±15.21	b	29.80± 5.89	b
Tanduk (AAB)	NPK	9.68± 2.81	a	10.60± 1.52	a	90.80±25.65	a	46.80±10.57	a
	LOB+1/2NPK	7.74± 3.61	ab	9.60± 3.11	ab	75.60±31.89	ab	39.00±16.39	ab
	LOB	4.59± 0.97	b	6.80± 2.51	b	44.40±13.72	b	22.90±10.13	b
Kepok (ABB)	NPK	11.96± 2.36	ab	8.60± 0.55	ab	131.20±33.5	ab	58.90± 9.95	ab
	LOB+1/2NPK	13.22± 0.72	a	8.60± 1.52	a	141.80± 8.73	a	62.80± 1.79	a
	LOB	9.80± 0.62	b	7.40± 1.67	b	111.60± 3.97	b	53.30± 3.15	b

Note: value in each column are means ± Std Error, the same letter in the same column and same cultivar do not differ significantly at the 0.05 level by DMRT

The effect of fertilization and cultivar on other vegetative growth response variable such as pseudostem diameter, number of leaves, and leaf length and width were essentially similar to that of pseudostem height (Table 4), i.e., no significant difference on those growth response variables between inorganic fertilizer treatment and the use of LOB but with 50% reduction of inorganic fertilizer, there were significant growth reductions in treatment of LOB alone for cultivar, except for Cavendish. Cavendish, being the most importantly traded cultivars, showed significant growth reduction with reduction of inorganic fertilizer as demonstrated in the pseudostem diameters and leaves' size, but not in the number of leaves. Growth reduction due to the replacement of inorganic fertilizer with LOB alone accounts for about 40-50% for most of the cultivar tested, the reduction of more than 50% occurred for Tanduk while reducing only 20-30% occurred on Kepok. Application of LOB 'Beyonic' could prevent significant plant growth reduction if combined with inorganic fertilizers at least 50% of the recommended dosage.

The population of plant growth-promoting rhizobacteria

The total population of bacteria was observed under a specific medium. The result showed that the soil samples contain bacteria with some activity, including phosphate solubilizing bacteria, IAA hormone-producing bacteria, and protease producing bacteria. The number of the total population of bacteria in the banana plant's soil was affected by each treatment. The different number of bacterial populations might be affected by nutrient content in the soil. The condition soil may have a variety of nutritional content that affects the number of microbial populations. Organic fertilizer (manure) was added in the planting hole before treatment affects the soil microbial population at 0 WAP, respectively.

The ANOVA of 0 WAP showed that the total population of bacteria on fertilizer treatment was significantly different; however, the total population of IAA producing bacteria, protease producing bacteria, and phosphate solubilizing bacteria was not significant. At 0 WAP, the interaction between fertilizer and cultivar was significant, with each cultivar not significantly different, even though the fertilizer treatment was not applied. At 0 WAP, the planting method that included decomposed manure before treatment and the banana planting materials that could already carry some bacteria in their root zone, which may be different with different cultivars altogether, might have affected the total population bacteria IAA producing bacteria and phosphate solubilizing bacteria. Due to these circumstances, the experiment was designed in a split-plot randomized complete block design with banana cultivars as the main plot and fertilizer application as the subplot to reduce error in soil microbial population differences.

Table 5. Analysis of variance (ANOVA) summary for microbial population parameter of eight banana cultivars at different fertilizer treatment at 0 and 12 WAP

Treatment	Total Bacteria (CFU mL^{-1})	IAA Producing Bacteria (CFU mL^{-1})	Protease Producing Bacteria (CFU mL^{-1})	Phosphate Solubilizing Bacteria (CFU mL^{-1})
0 WAP				
Fertilizer	0.0397 *	0.0861 ns	0.1628 ns	0.0836 ns
Banana Cult.	0.1308 ns	0.8401 ns	0.0111 *	0.5127 ns
Rep	0.0019 **	0.6612 ns	0.6663 ns	0.0603 ns
Fertilizer*Rep	0.5537 ns	0.1563 ns	0.6984 ns	0.6199 ns
Fertilizer*Cult.	<.0001 **	0.0005 **	0.0704 ns	0.0009 **
%CV	21.66882	31.87862	31.73933	33.11039
12 WAP				
Fertilizer	0.0488 *	<.0001 **	<.0001 **	<.0001 **
Banana Cult.	0.2263 ns	0.0111 *	0.0028 **	0.0016 **
Rep	0.0027 **	0.0120 *	0.0128 *	0.0013 **
Fertilizer*Rep	0.9767 ns	0.2451 ns	0.4080 ns	0.3811 ns
Fertilizer*Cult.	0.3717 ns	0.6976 ns	0.4341 ns	0.0753 ns
%CV	59.88767	61.92463	53.43413	38.49258

Note: value at each column are pr>f, * significant at the 0.05 level, ** significant at the 0.01 level, ns non-significant

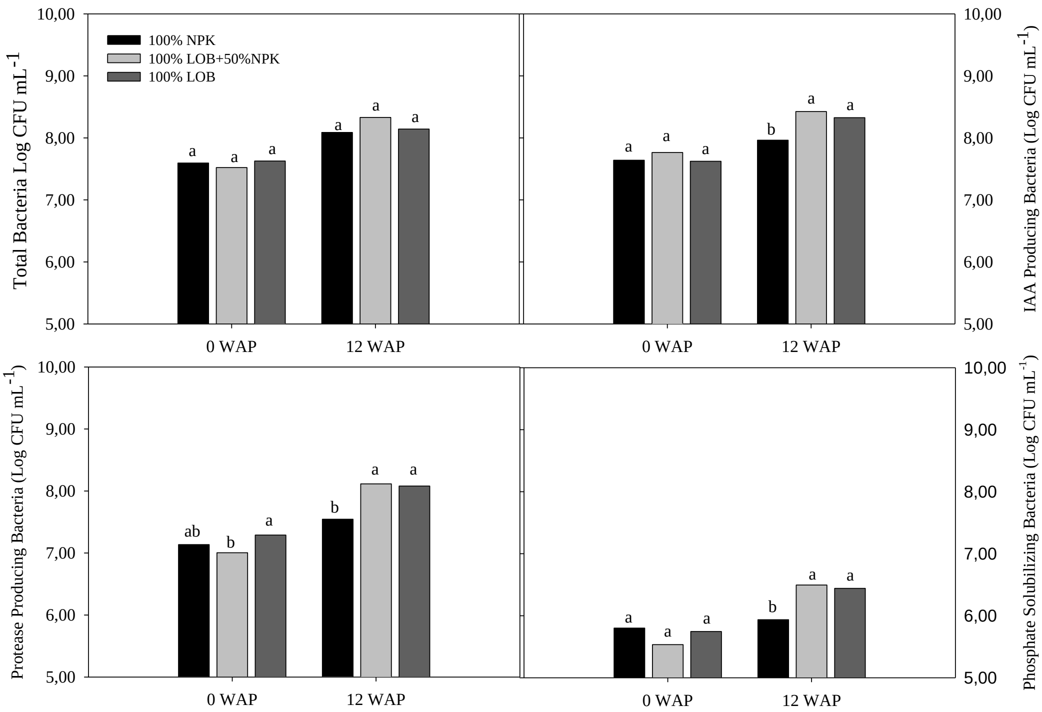

Figure 2. The bacterial population on each fertilizer application treatment at 0 and 12 WAP. Note: The same letter in the same week of each graph do not differ significantly at the 0.05 level by DMRT; the value of each histogram is means from 8 cultivars of banana at different fertilizer application

The ANOVA of 12 WAP compared to that of 0 WAP (Table 5) showed that there were generally increases in the level of significance of fertilizer application and cultivar on the total bacteria population, IAA producing bacteria, protease producing bacteria and phosphate solubilizing bacteria. However, at 12 WAP, the interaction between fertilizer and cultivar that were significant at 0 WAP became less pronounced regarding the PGPR variable, indicating that the effect of fertilizer application tends to have a similar effect for each cultivar, even though each cultivar were significantly different also.

A more detailed analysis of the PGPR population indicated a virtually homogenous population at 0 WAP when there was no fertilizer application to the plants. After 12 weeks of banana plant growth, there was a significant increase in those populations, at a rate of 2.4-17.4% depending on the kind of PGPR (Figure 2). Generally, PGPR was the lowest with 100%NPK, while a significantly higher PGPR population was found in the rhizosphere of plants treated with 50%NPK+LOB, which was not significantly different from treatment LOB only (Figure 2). It is worth noting that inorganic fertilizer treatment only allowed the PGPR population to increase only 2.4-6.5%, while with the application of 50% NPK+LOB, the increase of PGPR population was 8.5-17.4%, while with LOB only, the increase was only 6.8-12.1% (Figure 2).

Although the banana cultivar tested significantly affecting the PGPR population at 0 WAP, their population was around the scale of 5 x 10^7 CFU mL^{-1} (7.7 Log CFU mL^{-1}). However, after plant growth of 12 weeks, the PGPR population increased variably from 2.0% to over 23.0% regarding total bacteria population and PGPRs but depending on their specific function and the banana cultivar (Figure 3). Therefore, the application of fertilizer treatment increased bacterial population in each cultivar with different magnitude. For example, Mas Kirana (AA), Raja Bulu (AAB), Cavendish (AAA), Barangan (AAA) showed higher total bacterial count than that of Raja Lido (AAB), Raja Sereh (AAB), Tanduk (AAB), Kepok (ABB). Total population of IAA producing bacteria, protease producing bacteria in Barangan cultivar showed the highest number. Total population of phosphate solubilizing bacteria in Raja Lido cultivar was the highest.

Treatment of different fertilizers gave significant effect on total population of bacteria (Table 6). For the whole cultivars' tested application of LOB + 50% NPK increased total population of bacteria. Total population of IAA producing bacteria of Barangan cultivar was highest (9.32x10^8 CFU mL^{-1}) on 12 WAP. On the other hand, total population bacteria in Raja Sereh cultivar significantly increased in all activities with LOB + 50% NPK treatment.

The increase of bacterial population as indicated by total bacteria after 12 weeks of planting were mostly attributed to the application LOB or LOB+50% NPK in almost all banana cultivars tested, except in 'Cavendish' and 'Tanduk'. For Cavendish, the increase of total bacteria occurred with NPK fertilizer, while for Tanduk there was no increase in total bacteria population. Total bacterial population in coinciding with increase of all functionally specific PGPR tested included IAA producing bacteria, protease producing bacteria and phosphate solubilizing bacteria for banana cultivars of Barangan, Raja Lido, Raja Bulu, and Kepok. While for Mas Kirana and Cavendish there were no significant increase in IAA producing bacteria. Interestingly, high population of bacteria due to NPK fertilizer did not correlate with high population of functional PGPR, in fact, that the increase of protease-producing bacteria occurred at treatment of LOB+50%NPK. It is also interesting to note that for Tanduk, even though total bacteria did not increase significantly after 12 weeks of plant growth, notable increase happened in population of IAA and protease-producing bacteria as well as phosphate solubilizing bacteria at the LOB only. Total population of phosphate solubilizing bacteria in Raja Lido cultivar showed the highest increase compared to other cultivars at the application of LOB only. Application LOB only or combined with reduced NPK generally increased population of rhizobacteria. However, even though total bacteria population did not seem to increase with LOB application, increase in population of one or more functional PGPR occurred in all cultivar tested. Therefore, application of LOB with or without NPK evidently increased population of functional PGPR in banana rhizosphere.

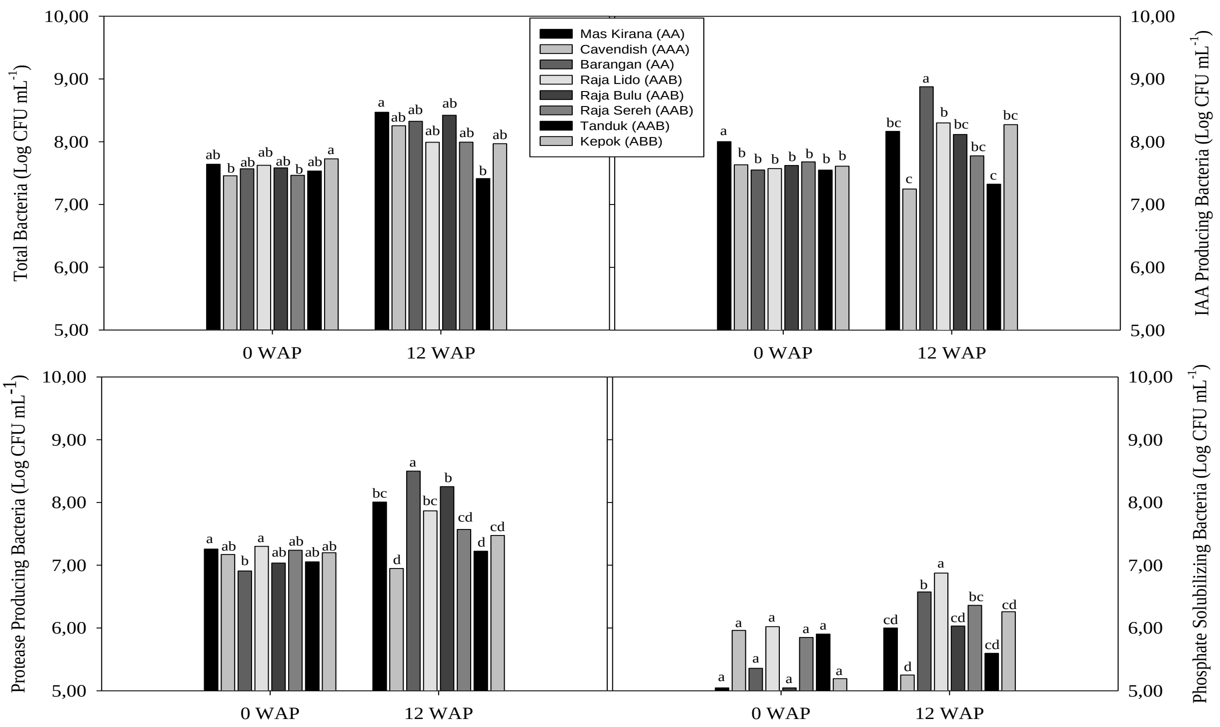

Figure 3. The bacterial population on each banana cultivars at 0 and 12 WAP. Note: The same letter in the same week on each graph do not differ significantly at the 0.05 level by DMRT; the value of each histogram cultivars is means from three different fertilizer application.

Table 6. The microbial population on 0 WAP to 12 WAP of eight cultivars banana at different fertilizer treatment

Treatments		Total bacteria		IAA producing bacteria		Protease producing bacteria		Phosphate solubilizing bacteria	
Cult.	Fertilizer	0 WAP	12 WAP	0 WAP	12 WAP	0 WAP	12 WAP	0 WAP	12 WAP
					(CFU mL^{-1}) $x10^6$				
Mas Kirana	NPK	16.167	29.500	29.667	5.833	29.667	14.000	nd	0.567
(AA)	LOB+1/2 NPK	67.333	**633.333**	225.333	**278.833**	9.500	**189.500**	nd	0.817
	LOB	48.000	220.667	46.500	154.833	15.000	100.833	0.333	**1.617**
Cavendish	NPK	39.333	**311.667**	61.167	**19.167**	17.167	2.500	1.217	nd
(AAA)	LOB+1/2 NPK	21.500	130.000	30.833	17.500	6.500	10.500	0.167	**0.300**
	LOB	25.333	98.167	37.000	16.333	18.000	**13.667**	1.367	0.233
Barangan	NPK	38.000	177.333	43.667	471.667	5.833	111.833	0.000	1.750
(AAA)	LOB+1/2 NPK	18.667	**319.667**	22.000	**931.833**	4.000	**431.667**	0.183	4.267
	LOB	54.500	137.000	40.833	861.000	14.500	401.833	0.500	**5.250**
Raja Lido	NPK	49.500	66.667	50.667	77.333	13.667	41.000	3.050	2.267
(AAB)	LOB+1/2 NPK	52.500	52.500	27.333	247.333	14.167	42.667	0.100	8.800
	LOB	24.333	**175.000**	34.500	**276.000**	32.167	**137.333**	nd	**11.400**
Raja Bulu	NPK	88.667	285.500	44.500	52.667	15.667	46.333	0.283	0.100
(AAB)	LOB+1/2 NPK	10.500	**313.500**	57.000	104.500	5.500	**283.833**	nd	**2.783**
	LOB	15.667	193.833	24.333	**235.000**	11.333	206.000	0.050	0.333
Raja Sereh	NPK	13.667	46.667	23.000	45.500	6.167	34.500	nd	1.900
(AAB)	LOB+1/2 NPK	22.667	**192.833**	32.000	**69.667**	10.500	**53.667**	1.817	**4.817**
	LOB	51.000	55.000	88.667	64.333	35.333	23.500	0.300	0.167
Tanduk	NPK	27.500	16.167	61.000	14.000	13.333	8.833	0.500	0.167
(AAB)	LOB+1/2 NPK	17.500	17.667	18.333	14.000	9.000	7.333	nd	0.167
	LOB	57.167	**43.667**	27.000	**35.500**	11.667	**34.000**	1.900	**0.850**
Kepok (ABB)	NPK	41.667	42.667	35.000	47.833	7.750	21.667	nd	0.167
	LOB+1/2 NPK	55.000	49.000	51.333	**465.000**	21.667	24.167	0.467	**3.050**
	LOB	63.333	**187.500**	36.333	50.333	18.000	**43.667**	nd	2.233

Note: value are means from 3 replication of observed sample; nd-not detected; bold number in 12 WAP are higher value in each fertilizer and cultivar banana treatment

Discussion

The vegetative growth of bananas in the plantation is generally supported heavily with inorganic fertilizer and organic fertilizer to guarantee a desirable yield. The recommended inorganic fertilizer doses per plant per cycle are about 90 g N, 28 g P, and 182 g K_2O doses per plant (Smithson et al. 2001) to 600 g Urea, 400 g TSP, 1550 g KCl (Suhartanto et al. 2012). Nomura et al. (2017) reported that to achieve maximum yield of banana cultivars like 'Nanicão IAC 2001' and 'Grand Naine', the farmers need application about 525 kg N ha^{-1} $year^{-1}$ and 855 kg K_2O ha^{-1} $year^{-1}$. Our inorganic fertilizer application dose we applied in this research are around 64 kg N ha^{-1}, 64 kg P ha^{-1} and 218 kg K_2O ha^{-1} is considered a moderate-rate fertilizer for bananas.

Our results showed that it was possible to reduce the use of inorganic fertilizer to 50% if compensated with the application of LOB without significantly reduced the growth rate as measured by pseudostem high and diameter, leaf number, and leaf size except for the most commercially important cultivar of Cavendish. The application of biofertilizer in combination with 50% inorganic fertilizer in bananas has been demonstrated to show the best growth especially in some cultivars like Raja and Kepok; that could be due to the development of the root. Perdani et al. (2020) reported that the application of bio-organic fertilizer with 50% inorganic fertilizer increased fibrous root formation and the rate of mycorrhizal colonization in the roots of upland rice around 34%. Ye et al. (2020) suggest using liquid organic bio-organic fertilizers combined with the correct dosage of chemical fertilizers for maximum yield benefits regarding yield, quality and fertilizer savings since it increases microflora abundance and improves soil fertility and in turn increases soil microbial activity. The benefit of biofertilizer application to reduce inorganic fertilizer doses without decreasing vegetative growth and yield have been demonstrated for many crops such as watermelon (Antonius and Agustiyani 2011), sorghum (Agustiyani et al. 2015), and tomato (Ye et al. 2020).

Application of LOB and 50% inorganic fertilizer in sorghum resulted in comparable yield to the 100% inorganic fertilizer application, however with Brix value higher than that of 100% inorganic fertilizer application (Agustiyani et al. 2015). The synergistic effect of PGPR and inorganic fertilizer on plant growth may be dialectic as it is explained as follows. The addition of N and P through mineral fertilizer (inorganic fertilizer) has influenced the biological process both directly and indirectly, including soil microbe (Damodaran et al. 2016). Bioorganic fertilizer when applied with NPK fertilizer increased cfu of bacteria. The increase in the number of bacteria in the root zone will increase the availability of nutrients that plants can absorb (Damodaran et al. 2014). Different fertilizer applications change the soil physical and chemical properties, which affects the soil bacterial and structure in rhizospheres soil (Rubiao et al. 2020). Mahdi et al. (2010) indicate that bio-fertilization is to accelerate the microbial processes necessary for nutrient availability that can be easily assimilated by plants and increase the number of useful microorganisms in the soil.

For Cavendish, interestingly, both treatments of inorganic fertilizer and combination of 50% inorganic fertilizer and LOB did not result in significantly different in regard to the number of leaves which is a significant determinant for fruit yield, even though the leaf size decreased significantly. A reduction in the leaf size of Cavendish due to limited availability of inorganic fertilizer could be one of the indicates of potassium deficiency that would further reduce the bunch yield (Taulya 2013). Mustafa and Kumar (2012) reported that the inorganic fertilizer application in Dwarf Cavendish showed increased growth and yield when plants supplied with (g plant^{-1}) 72-200 N; 90-96 P; and 150-480 K. The nutrient of N, P, and K rates given in this experiment may have been limited compared to the recommended doses for bananas, especially for Cavendish (Mustafa and Kumar 2012). Banana is a potassium loving crop, and has a very high demand for this nutrient. In India, the applied dose of K varies from 800 to 1600 kg ha^{-1} (Kumar et al. 2008).

In this work, different cultivar showed different growth rates that may require different fertilizers doses. Differences between cultivars generally are difficult to assess from the literature since many cultivars have not been grown in the same climate with a differential nutrient supply. However, by comparing the nutrient concentrations in lamina-3 of 30 banana cultivars, Lahav (1995) reported that cultivars with a *balbisiana* constitution had lower concentrations of most elements than other species of *acuminata* based cultivars. Furthermore, this effect was carried over to cultivars with the *balbisiana* genome. Thus, each set of B genome contributed to lowering the concentrations of nutrients, i.e., cultivars with the AAA genome had higher levels than those with the AAB, and the latter higher than the ABB. The report of nutrient in banana by Lahav (1995) is in line with our result that 'Kepok' (ABB) that have two *balbisiana* genome were more responsive to a lower fertilizer dose (treatment 100% LOB+50% NPK or 100% LOB alone) than that of cultivars with more A genome.

Application of PGPR to enrich compost compared to regular compost has been demonstrated to increase yield of broccoli, carrot, and corn in an organic farming setup (Antonius et al. 2015). On the contrary, our result indicated that the application of LOB alone did not support satisfactory growth of banana plants; in fact, the vegetative growth of eight cultivars tested underwent severe reduction up to 50%. The nutrient availability due to microbe nutrient solubilizing activity may be sufficient to support plant growth with little biomass but may not be sufficient for large biomass plant growth such as bananas. However, it is also interesting to note that the growth reduction on Kepok due to LOB application alone was merely 20-30%. Thus, this result indicates the direct benefits of application LOB alone, which is easy and inexpensive to produce for smallholder farmers planting Kepok for cash crop or subsistence use.

LOB ‘Beyonic’ we used in this work contains plant growth-promoting rhizobacteria that have functions for nitrogen-fixing, potassium and phosphate solubilization, proteolytic activity, and growth hormone (IAA) secretion. Consequently, LOB inoculant is not a single strain but a mixed strain to bring the synergistic effect of nutrient mobilization, to enhance efficacy, stability, and uniformity when applied to different fields. LOB ‘Beyonic’ contains phosphate solubilizing bacteria that solubilize the fixed phosphate and make it bioavailable to plants. Phosphate solubilizing bacteria can transform insoluble phosphates into soluble forms by the excretion of organic acids in the rhizosphere. These organic acids decrease the soil pH and cause the dissolution of phosphate complexes and make them available to plants (Gupta, 2004). Protease producing bacteria might be related to biocontrol activity based on the ability to degrade the protein. PGPRs as biological agents have evidently to be alternatives to chemical agents for providing resistance against various pathogens' attacks (Backman and Sikora 2008). Itelima et al. (2018) reported *Bacillus subtillis* N11 and mature compost were found to control *Fusarium* infestation on the banana root. Microbes that can produce IAA can increase root growth and enhance root surface; eventually, plants can absorb more soil nutrients (Bolero et al. 2007).

During plant growth, application of LOB with or without inorganic fertilizer in the banana cultivars tested showed that the bacteria's total population generally increased significantly, and there was a synergistic effect of LOB and inorganic fertilizer in increasing the population of total bacteria population. LOB application as compost enrichment increases in the total population of bacteria and increases urease and phosphatase activity in the soil samples (Antonius et al. 2015). LOB application to ultisol soil alone or combined with compost and biochar increased the total microbe population significantly after ten weeks and coincided with the increasing soil enzyme activity of phosphomonoesterase and soil P availability (Antonius et al. 2018).

The increase in bacteria population with a specific function, such as protease activity, phosphate solubilizing activity and IAA producing activity, were attributed to the application of LOB. The increase of those bacteria populations with specific function due to LOB application was dependent upon the cultivars. For example, the total population of phosphate solubilizing bacteria in Raja Lido cultivar was the highest in LOB application only. Barangan cultivar has the highest total population of IAA producing bacteria compared to other cultivars. Barangan cultivar also has the highest population protease producing bacteria in the application of LOB and LOB +50% NPK. The total population of protease-producing bacteria of Mas Kirana, Raja Bulu, Raja Sereh, Tanduk and Kepok increased in LOB application. The PGPR population difference may eventually affect biomass and yield. The different response in biomass and yield of genotypes to PGPR application has been demonstrated in many crops such as chickpea (Imran et al. 2015), barley (Shirinzadeh et al. 2013), and wheat (Rothballer et al. 2003).

To conclude, we have demonstrated that LOB composed of a consortium of bacteria with many different plant growths promoting activities, such as IAA producing, phosphate and potassium solubilizing activities, protease activity; that have been proven to be effective to many plants could be applied with reduced inorganic fertilizer up to 50% without significant reduction on the plant growth variables in many different banana varieties, except for cultivar Cavendish. That treatment combination of inorganic and biofertilizer also increased the population of PGPR such as IAA producing, phosphate and potassium solubilizing, protease producing bacteria in the banana plant rhizospheres. Fine-tuning the microbial composition or the percentage of inorganic fertilizer reduction should be worthwhile for Cavendish since it is the most widely grown and traded banana cultivar in the world. Application of LOB alone in the event where the inorganic fertilizer is lacking, may be beneficial for Kepok as it was demonstrated that its vegetative growth reduction even though significant, was in a small percentage.

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