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# The Effect of Several Rhizobacteria Consortia Formulation and Bacterial Density to The Growth and Yield of Five Maize Varieties in Indonesia

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**Abstract.** Maize is one of the important crop so production improvement is must be done to sufficient the needs. Plant growth promoting rhizobacteria has become a feasible, efficient and sustainable approach to increase maize productivity. This study investigated the effect of several rhizobacteria consortia formulations and bacterial density on the growth and yield of five maize varieties in Indonesia. The experiment was carried out using a nested-split plot randomized complete block design (RCBD). The treatments consisted of two rhizobacteria consortia formulation (granule and liquid) as nested, four rhizobacterial density (0, 10<sup>7</sup>, 10<sup>8</sup>, and 10<sup>9</sup> cfu/ml) as main plot, and five maize varieties (Bisi 18, Bisi 2, Pertiwi 3, Bisi 228, and Bisi 220) as subplot. The growth and yield data were analyzed through ANOVA and by means using DMRT  $\alpha$  5% to determine the best treatment. Application of rhizobacteria consortia formulation on five maize varieties did significantly affect the growth and yield of maize although bacterial density did not showed the same results. Rhizobacteria consortia with granule formulation appeared significantly higher yield performance than liquid. Pertiwi 3 tended to produce higher average yield than other varieties for both rhizobacteria consortia formulation although the varieties did not significantly contribute on ear weight variables.

**Keywords:** Maize, PGPR, productivity

## 1 Introduction

Maize (*Zea mays* L.) is an important crop for staple food, feed, and raw materials for manufacture [1]. During 2017-2018, there was an imbalance of total production of maize compared to the demand of about 0.6 million tonnes however it fulfilled through import [2]. There are many maize varieties grown in Indonesia, including five varieties used in this study. These varieties have high potential yield of about 12-15 t/ha but the actual yield only around 4.5 t/ha in dryland [3, 4]. This is could be caused by environmental factors especially nutrient availability and biotic or abiotic stress. An effort should be taken to increase the maize yield so the maize national demand could be fulfilled.

Plant growth-promoting bacteria (PGPB) in the form of rhizobacteria consortium, particularly in maize, are capable to improve plant growth and yield when applied

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as biofertilizer through few mechanisms including producing phytohormone and alleviating biotic or abiotic stress [5–7]. To the best of our knowledge, the study on rhizobacteria consortia formulation with several bacterial densities on various maize varieties is still rare. In this research, the effect of several rhizobacteria consortia formulations and bacterial density on the growth and yield of five maize varieties in Indonesia was investigated. The rhizobacteria consortia formulation consisted of granule and liquid formulation with bacterial density ranged from  $10^7$  to  $10^9$  CFU/ml. This paper could provide information for further research on the formulation and application of rhizobacteria as biofertilizer for improving maize productivity in Indonesia.

## 2 Materials and methods

### 2.1 Preparation of Rhizobacteria Consortia Formulation

Rhizobacteria isolates were obtained from rhizosphere of vigorous and healthy maize after isolation and purification procedure. The rhizobacteria consortia formulation were composed of seven rhizobacteria isolates which is consisted of 4 identified (3 isolates identified as *Enterobacter asburiae* and 1 isolate classified as *Enterobacter cancerogenus*) and 3 unidentified rhizobacteria isolates (L1S1, L2S4, and L5S4). All isolates was prepared as described by Kurniawan [8] and Putra [9].

The rhizobacteria consortia was formulated into two form i.e granule and liquid. The granule formulation was composed of rice husk charcoal, peat, kaolin, and dolomite with rasio 7:1:2:5 (w/w) and produced using granulator. The liquid formulation was comprised of 5% (v/v) molasses, 1 g/L of each commercialized fertlitzer Grow More hijau (N-total 32%, P<sub>2</sub>O<sub>5</sub> 10%, and K<sub>2</sub>O 10%) and Grow More merah (N-total 10%, P<sub>2</sub>O<sub>5</sub> 55%, and K<sub>2</sub>O 10%), and 0.1 M NaCl.

### 2.2 Experimental design

Field study was conduted in Singosari, Malang, East Java. The experiment was carried out using a nested-split plot randomized complete block design (RCBD). The treatments consisted of two rhizobacteria consortia formulation (granule and liquid) as nested, four rhizobacterial density ( $0$ ,  $10^7$ ,  $10^8$ , and  $10^9$  cfu/ml) as main plot, and five maize varieties (Bisi 18, Bisi 2, Pertiwi 3, Bisi 228, and Bisi 220) as subplot. The treatments combination were replicated three times and consisted of 25 samples for each treatments combination.

Application of rhizobacteria consortia formulation was done once in the beginning of seed planting with a dose of 100 g/planting hole for granule formulation or 100 ml/planting hole for liquid formulation. Furthermore, inorganic fertilizer was applied two times i.e in the form of urea and NPKS with a dose of 1.87 g dan 5.25 g per plant at 10 days after sowing and urea with a dose fo 5 g/plant at 28 days after sowing.

### 2.3 Observational variables

The variables observed included growth and yield variables. the growth variables consisted of plant height (from collar diameter to growing point), number of leaves (fully opened and formed leaf blade), dan stem diameter (measured at  $\pm 5$  cm above root collar) while the yield variables included ear weight (with and without husk; g), ear length (cm), ear diameter (mm), tip filling (%), number of kernel per row (row), and 100 seeds weight (g).

### 2.3 Statistical and data analysis

The statistical and data analysis was completed using Minitab v.19. The analysis of variance (ANOVA) was performed to find out the effect of the treatments. Then, duncan multiple range test (DMRT)  $\alpha$  5% was used to determine the best treatment.

### 3 Results and discussion

Rhizobacteria can be found in the rhizosphere, a thin layer of soil that covers the root surface and has a positive effect on plant growth. There are several genera of rhizobacteria reported as plant growth promoting rhizobacteria (PGPR), namely *Pseudomonas*, *Enterobacter*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Bacillus* and *Serratia* [10]. Abiala, Odebo, Hsu, & Blackwood [11] exert that bacteria isolated from the rhizosphere of maize growing in southwestern Nigeria were classified to the following genera i.e *Myroides*, *Enterobacter*, *Bacillus*, *Lysinibacillus*, *Citrobacter*, *Stenotrophomonas*, and *Pseudomonadaceae*.

**Table 1.** The growth variables of five maize varieties under application of various rhizobacteria consortia formulations and bacterial density at the end of vegetative growth stage.

Treatment		Plant height (cm)	Number of leaves	Stem diameter (mm)
<i>Formulation</i>				
Granule		220.94 a*	14.07 a	29.41 b
Liquid		175.59 b	14.54 a	31.44 a
<i>Formulation</i>	<i>Bacterial density(cfu/ml)</i>			
	0 ( <i>control</i> )	222.35 a	14.03 a	29.10 a
Granule	10 <sup>7</sup>	225.31 a	14.45 a	29.39 a
	10 <sup>8</sup>	218.82 a	13.52 a	30.18 a
	10 <sup>9</sup>	217.26 a	14.26 a	28.96 a
Liquid	0 ( <i>control</i> )	168.08 a	14.04 a	31.58 a
	10 <sup>7</sup>	191.33 a	15.31 a	31.20 a
	10 <sup>8</sup>	167.29 a	14.36 a	30.37 a
	10 <sup>9</sup>	175.67 a	14.45 a	32.60 a
<i>Formulation</i>	<i>Variety</i>			
Granule	Bisi 18	227.71 a	14.51 ab	28.67 ef
	Bisi 2	227.98 a	14.52 ab	32.47 b
	Pertiwi 3	196.79 b	13.77 c	28.62 ef
	Bisi 220	229.32 a	13.70 c	28.15 f
	Bisi 228	222.88 a	13.83 c	29.13 def
Liquid	Bisi 18	175.90 d	14.58 ab	31.31 bc
	Bisi 2	186.92 c	15.08 a	35.09 a
	Pertiwi 3	161.30 e	13.93 bc	30.52 cde
	Bisi 220	176.40 d	14.47 ab	30.67 bcd
	Bisi 228	177.45 cd	14.63 a	29.60 cdef

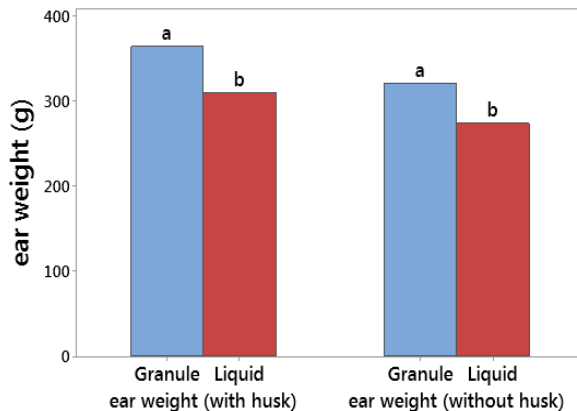
\* The mean value which was followed by the same letter in the same column showed that the difference was not significant based on the DMRT with  $\alpha$  5%

Identified rhizobacteria used in this study classified as *Enterobacter* sp. which are included in the rhizobacteria group and originate from the Enterobacteriaceae family to produce protein enzymes that have proteolytic activity. Aeron et al. [12] revealed that the bacteria *Enterobacter* sp. produce commercially important enzymes such as amylase, protease, gelatinase, lipase, deoxyribonucleate, phosphatase and urease. In addition, bacteria belonging to the enterobacteriaceae group that have proteolytic activity have the ability to produce protease enzymes which are secreted into their environment. This protease enzyme

then works to hydrolyze protein compounds into oligopeptides, short chain peptides and amino acids. Furthermore, the rhizobacteria isolates used in this study potentially produce extracellular compound which is categorized as osmoprotectan, fitohormon, and organopesticide according to GCMS analysis (not reported in this paper).

The growth variables of five maize varieties under application of various rhizobacteria consortia formulations and bacterial density at the end of vegetative growth stage is shown in Table 1. The rhizobacteria consortia formulation had a significant effect on the growth variables of maize (plant height and stem diameter), while the bacterial density had no significant effect. This results indicated that rhizobacteria consortia formulation has more effect on the growth of maize compared with the bacterial density of its formulation.

The effect of rhizobacteria consortia formulations on five maize varieties was seen on the growth variables of maize. Bisi 2 shows a higher average plant height, more leaves and wider stem diameter than other varieties for both rhizobacteria consortia formulation (granule and liquid). Besides, Pertiwi 3 tended to exhibit lower plant height, leaf number and stem diameter compared to other varieties. The difference in the growth of maize is due to the specific characteristics of each variety and its response to the application of rhizobacteria formulation.



**Fig. 1.** The maize ear weight under application of several rhizobacteria consortia formulations.

The yield variables of five maize varieties under application of various rhizobacteria consortia formulations and bacterial density showed in Table 2. Rhizobacteria consortia formulation and maize variety did significantly affect yield variables of maize while bacterial density did not contribute significant effects on the yield components. Rhizobacteria formulation did significantly affect ear weight (with and without husk), ear length, and 100 seeds weight with granule appeared significantly higher than liquid (Table 2; Fig. 1). The effect of rhizobacteria consortia formulations on five maize varieties was seen on ear diameter, tip filling, number of kernel per row, and 100 seeds weight (Table 2). Pertiwi 3 tended to produce higher average yield variables than other varieties for both rhizobacteria consortia formulation (granule and liquid) although the varieties did not significantly contribute on ear weight (with and without husk) variables (Fig. 2).

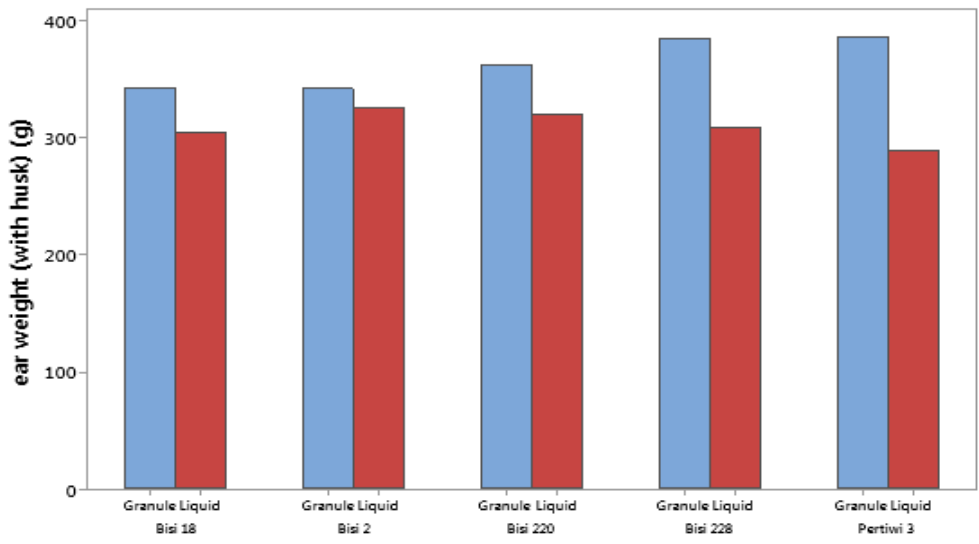


Fig. 2. The ear weight of five maize varieties under application of several rhizobacteria consortia formulations.

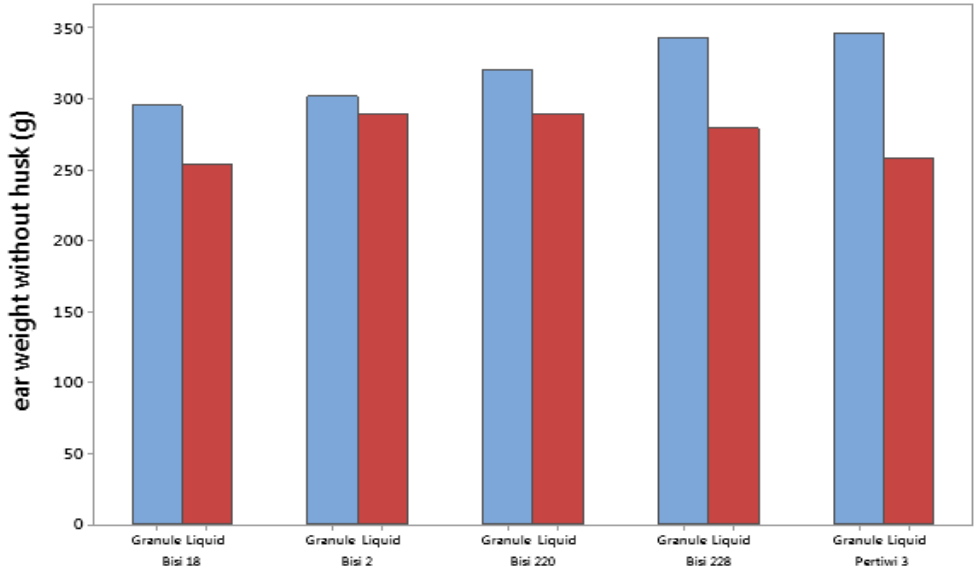


Fig. 2. The ear weight (without husk) of five maize varieties under application of several rhizobacteria consortia formulations.

Microbial consortia did increased yield variables of crop which is promoted by the abilities of PGPR as phytostimulation, biofertilization (N), and biocontrol (several root pathogens) [6, 13]. Nezarat & Gholami [14] reported that application of PGPB did significantly affect yield variables e.g 100 seed weight and number of kernel per ear. In term of yield, Pertiwi-3 tend to produce higher value in yield variables than other varieties (Table 2) which is in accordance with the studies reported before [15–17]. This study also emphasized that application of rhizobacteria with several bacterial density ranged from  $10^7$  to  $10^9$  CFU/ml produced the same results.

**Table 2.** The yield variables of five maize varieties under application of various rhizobacteria consortia formulations and bacterial density.

Treatment	Ear length (cm)	Ear diameter (mm)	Tip filling (%)	number of kernel per row (row)	100 seeds weight (g)	
<i>Formulation</i>						
Granule	29.07 a*	50.72 a	92.92 a	15.42 a	40.00 b	
Liquid	27.53 b	49.24 a	90.48 a	15.77 a	45.97 a	
<i>Formulation Bacterial density (cfu/ml)</i>						
Granule	0 (control)	29.63 a	50.73 a	93.12 a	15.24 a	39.79 a
	10 <sup>7</sup>	29.02 a	50.95 a	92.71 a	15.67 a	39.85 a
	10 <sup>8</sup>	28.78 a	50.64 a	93.48 a	15.28 a	40.32 a
	10 <sup>9</sup>	28.83 a	50.57 a	92.36 a	15.49 a	40.04 a
Liquid	0 (control)	26.12 a	49.58 a	91.14 a	15.45 a	45.74 a
	10 <sup>7</sup>	28.17 a	49.42 a	91.59 a	15.87 a	44.83 a
	10 <sup>8</sup>	27.24 a	48.91 a	88.43 a	15.97 a	45.69 a
	10 <sup>9</sup>	28.57 a	49.06 a	90.75 a	15.79 a	47.60 a
<i>Formulation Variety</i>						
Granule	Bisi 18	28.36 a	51.30 c	96.42 a	15.03 b	41.37 de
	Bisi 2	27.04 a	43.27 g	91.15 bc	12.07 c	33.80 f
	Pertiwi 3	28.43 a	57.70 a	93.97 ab	16.20 ab	44.28 bcd
	Bisi 220	29.70 a	52.39 bc	89.35 c	16.88 a	41.63 de
	Bisi 228	31.80 a	48.95 de	93.70 ab	16.92 a	38.92 e
Liquid	Bisi 18	28.45 a	49.25 de	90.82 bc	15.93 ab	47.50 a
	Bisi 2	27.03 a	46.20 f	89.37 c	16.40 a	46.38 ab
	Pertiwi 3	25.78 a	53.70 b	91.15 bc	16.10 ab	45.82 abc
	Bisi 220	27.02 a	49.46 d	89.22 c	15.57 ab	46.90 ab
	Bisi 228	29.35 a	47.60 ef	91.83 bc	14.85 a	43.23 cd

\* The mean value which was followed by the same letter in the same column showed that the difference was not significant based on the DMRT with  $\alpha$  5%

## 4 Conclusion

Application of rhizobacteria consortia formulation on five maize varieties did significantly affect the growth and yield of maize although bacterial density did not showed the same results. Rhizobacteria consortia with granule formulation appeared significantly higher yield performance than liquid formulation. Pertiwi 3 tended to produce higher average yield variables than other varieties for both rhizobacteria consortia formulation (granule and liquid) although the varieties did not significantly contribute on ear weight (with and without husk) variables.

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