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

Ali Ikhwan[1*], *Aniek* Iriany[1], *Erny* Ishartati[1], *Shazma* Anwar[2], and *Faridlotul* Hasanah[3]

[1]Department of Agrotechnology, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Jl. Raya Tlogomas No 246, Malang 65144, East Java Indonesia
[2]Department of Agronomy, University of Agriculture Peshawar, 25130 Peshawar, Khyber Pakhtunkhwa, Pakistan
[3]Alumni of Soil Science Study Program, IPB University, Jl. Raya Dramaga, Bogor 16680, West Java, Indonesia

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 cultivars 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, 10^7, 10^8, and 10^9 cfu mL–^{1}) as main plot, and five maize cultivars (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 α 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: Biofertilizer, enviromentally friendly, plant growth promoting rhizhobacteria, productivity improvement, *Zea mays* L.

* Corresponding author: aliikhwan64@gmail.com

1 Introduction

Maize (*Zea mays* L.) is an important crop for staple food, feed, and raw materials for manufacture [1]. During 2017 to 2018, there was an imbalance of total production of maize compared to the demand of about 0.6×10^6 t however it fulfilled through import [2]. There are many maize varieties grown in Indonesia, including five cultivars used in this study. These cultivars have high potential yield of about 12 t ha^{-1} to 15 t ha^{-1} but the actual yield only around 4.5 t ha^{-1} in dryland [3, 4]. This 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 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 cultivars in Indonesia was investigated. The rhizobacteria consortia formulation consisted of granule and liquid formulation with bacterial density ranged from 107 CFU mL^1 to 109 CFU mL^{-1}. 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 rhizobateria consortia formulation

Rhizobacteria isolates were obtained from rhizosphere of vigorous and healthy maize after isolation and purification procedure. The rhizobacteria corsortia formulation were composed of seven rhizobacteria isolates which is consisted of four identified (three isolates identified as *Enterobacter asburiae* (Brenner *et al*. 1988 emend. Hoffmann *et al.* 2005) and one isolate classfified as *Enterobacter cancerogenus* [(Urosevic 1966) Dickey and Zumoff 1988] and three 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^{-1}) and produced using granulator. The liquid formulation was comprised of 5 % (v v^{-1}) molasses, 1 g L^{-1} of each commerciallized fertilizer Grow More “hijau” (N-total 32 %, P_2O_5 10 %, and K_2O 10 %) and Grow More “merah” (N-total 10 %, P_2O_5 55 %, and K_2O 10 %), and 0.1 M NaCl.

2.2 Experimental design

Field study was conduted in Singosari, Malang, East Java (Coordinat: 7.915, 112.641; 7°54’54” S 112°38’27.6” E). Soil characteristics are pH_{H2O} 7.4, pH_{KCl} 6.2, C-org 2.56 %, N-total 0.31 %, P_2O_5-$_{available}$ 105 mg kg^{-1}, K_{exch} 0.44 cmol(+) kg^{-1}, Ca_{exch} 16.82 cmol(+) kg^{-1}, Mg_{exch} 2.54 cmol(+) kg^{-1}, and Na_{exch} 0.63 cmol(+) kg^{-1}.

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^{-1}] 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)$^{-1}$ for granule formulation or 100 mL (planting hole)$^{-1}$ 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 d after sowing and urea with a dose for 5 g plant^{-1} at 28 d 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 ± 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).

2.4 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) α 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, Azospirilum, Azotobacter, Burkholderia, Bacillus and Serratia [10]. Abiala *et al* [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 cultivars 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)
Formulation				
Granule		220.94±23.92 a*	14.07±0.96 a	29.41±2.62 b
Liquid		175.59±22.50 b	14.54±1.13 a	31.44±3.43 a
Formulation	*Bacterial density (cfu mL^{-1})*			
Granule	0 (*control*)	222.35±23.22 a	14.03±0.92 a	29.10±2.53 a
	10^7	225.31±19.98 a	14.45±0.86 a	29.39±2.89 a
	10^8	218.82±27.87 a	13.52±1.07 a	30.18±2.76 a
	10^9	217.26±25.56 a	14.26±0.83 a	28.96±2.36 a
Liquid	0 (*control*)	168.08±19.16 a	14.04±1.25 a	31.58±2.98 a
	10^7	191.33±20.71 a	15.31±0.73 a	31.20±3.70 a
	10^8	167.29±28.37 a	14.36±1.27 a	30.37±3.79 a
	10^9	175.67±11.50 a	14.45±0.88 a	32.60±3.17 a
Formulation	*Cultivars*			
Granule	Bisi 18	227.71±19.11 a	14.51±0.89 ab	28.67±2.14 ef
	Bisi 2	227.98±28.66 a	14.52±0.70 ab	32.47±2.44 b

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Table 1. Continued.

Treatment		Plant height (cm)	Number of leaves	Stem diameter (mm)
Granule	Pertiwi 3	196.79±23.63 b	13.77±1.18 c	28.62±2.28 ef
	Bisi 220	229.32±18.25 a	13.70±1.05 c	28.15±1.95 f
	Bisi 228	222.88±12.96 a	13.83±0.71 c	29.13±2.04 def
Liquid	Bisi 18	175.90±21.59 d	14.58±0.97 ab	31.31±2.78 bc
	Bisi 2	186.92±23.41 c	15.08±0.75 a	35.09±3.65 a
	Pertiwi 3	161.30±19.24 e	13.93±1.38 bc	30.52±2.63 cde
	Bisi 220	176.40±22.78 d	14.47±1.12 ab	30.67±3.04 bcd
	Bisi 228	177.45±21.06 cd	14.63±1.21 a	29.60±2.53 cdef

The mean value was followed with standard deviation.
*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 α 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, deoxyribonuleate, 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 oligopetides, 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 cultivars 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 cultivars was seen on the growth variables of maize. Bisi 2 shows a higher average plant height, more leaves and wider stem diameter than other cultivars 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 cultivars. 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.

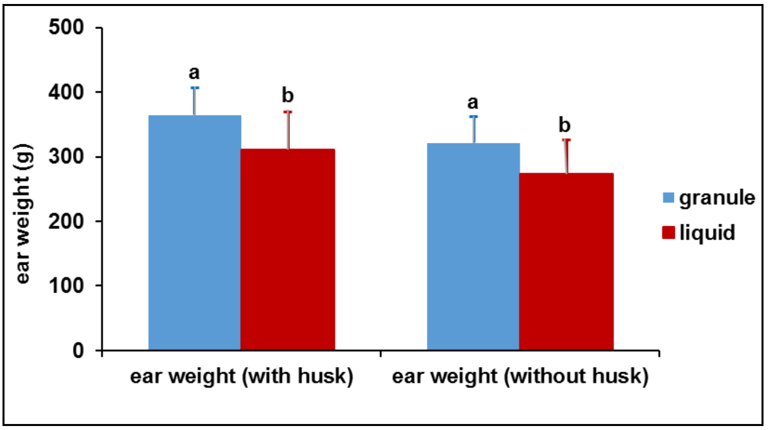

Fig. 1. The maize ear weight under application of several rhizobacteria consortia formulations.

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; Figure 1). The effect of rhizobacteria consortia formulations on five maize cultivars 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 (Figure 2).

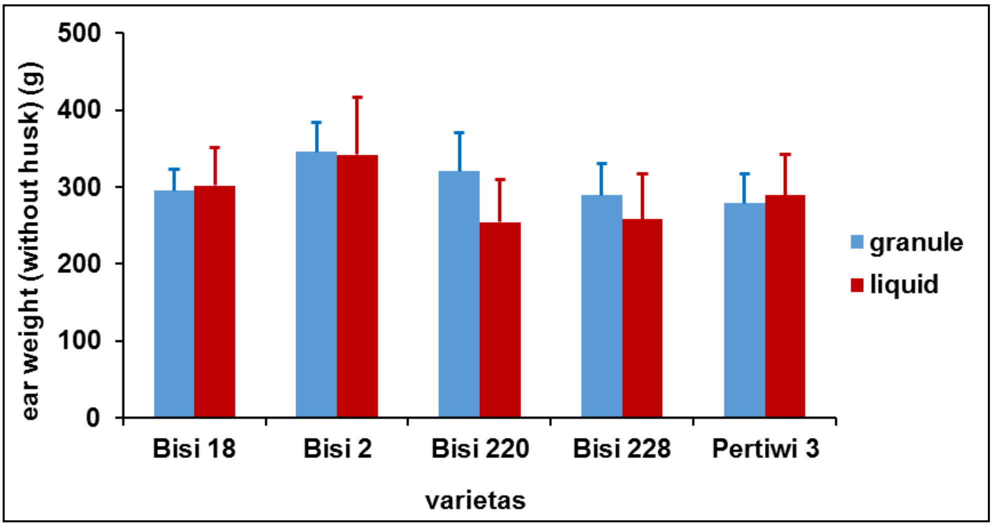

Fig. 2. The ear weight of five maize varieties under application of several rhizobacteria consortia formulations.

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 signifiantly 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^{-1} produced the same results.

Table 2. The yield variables of five maize cultivars 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)
Formulation						
Granule		29.07±2.13 a*	50.72±5.04	92.92±4.27	15.42±2.04	40.00±3.77 b
Liquid		27.53±2.46 b	49.24±3.51	90.48±6.21	15.77±1.78	45.97±4.58 a
Formulation	*Bacterial density (cfu mL^{-1})*					
Granule	0 (*control*)	29.63±1.95	50.73±5.12	93.12±3.12	15.24±2.08	39.79±4.17
	10^7	29.02±2.07	50.95±5.17	92.71±3.95	15.67±2.33	39.85±3.63
	10^8	28.78±2.21	50.64±5.85	93.48±3.12	15.28±1.88	40.32±3.94
	10^9	28.83±2.36	50.57±4.45	92.36±6.40	15.49±2.02	40.04±3.70
Liquid	0 (*control*)	26.12±2.50	49.58±3.10	91.14±4.21	15.45±1.90	45.74±5.19
	10^7	28.17±2.75	49.42±3.94	91.59±5.11	15.87±1.50	44.83±4.98
	10^8	27.24±2.05	48.91±3.54	88.43±9.31	15.97±1.99	45.69±3.68
	10^9	28.57±1.93	49.06±3.74	90.75±5.03	15.79±1.81	47.60±4.31
Formulation	*Cultivars*					
Granule	Bisi 18	28.36±1.79	51.30±1.28 c	96.42±1.67 a	15.03±0.91 b	41.37±0.87 de
	Bisi 2	27.04±1.20	43.27±2.30 g	91.15±3.72 bc	12.07±0.51 c	33.80±1.73 f
	Pertiwi 3	28.43±1.68	57.70±2.18 a	93.97±3.23 ab	16.20±0.91 ab	44.28±1.33 bcd
	Bisi 220	29.70±0.99	52.39±1.64 bc	89.35±2.57 c	16.88±0.96 a	41.63±1.45 de
	Bisi 228	31.80±1.33	48.95±1.22 de	93.70±5.61 ab	16.92±1.25 a	38.92±0.78 e
Liquid	Bisi 18	28.45±2.30	49.25±3.04 de	90.82±7.07 bc	15.93±1.71 ab	47.50±4.36 a
	Bisi 2	27.03±2.64	46.20±1.77 f	89.37±5.06 c	16.40±1.51 a	46.38±4.58 ab
	Pertiwi 3	25.78±2.28	53.70±3.02 b	91.15±4.86 bc	16.10±2.17 ab	45.82±5.11 abc
	Bisi 220	27.02±2.04	49.46±1.75 d	89.22±5.41 c	15.57±1.70 ab	46.90±4.51 ab
	Bisi 228	29.35±1.57	47.60±2.61 ef	91.83±8.50 bc	14.85±1.60 a	43.23±3.73 cd

The mean value was followed with standard deviation.
* 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 α 5 %

This research was carried out on the land with sufficient fertility, especially P_2O_5-$_{available}$, which was categorized as very high [18–20]. Future research should be carried out on soil with low fertility. Previous studies stated that PGPR could increase crop productivity on low-fertility land [21–23].

4 Conclusion

Application of rhizobacteria consortia formulation on five maize cultivars 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 cultivars did not significantly contribute on ear weight (with and without husk) variables.

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