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The Role of Rhizobacterial Inoculum and Formulated Soil Amendment in Improving Soil Chemical-Biological Properties, Chlorophyll Content and Agronomic Efficiency of Maize under Marginal Soils

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Abstract

Intensively use of chemical fertilizers accelerates organic matter and soil health degradation. Consequently, the soil capacity in providing the essential nutrient is decreasing significantly. The research was conducted to investigate the contribution of selected rhizobacteria inoculant as biofertilizers and formulated soil amendment (FSA) for enhancing the soil organic carbon, abundance of nitrogen fixer bacteria (NFB), phosphate solubilizing bacteria (PSB), chlorophyll content and yield of maize in marginal soils. The experiment was arranged as Randomized Complete Blocks Design (RCBD) consisting of two factors and provide with three replications. The first factor were biofertilizers (consortia of PSB and N-fixing bacteria combined with formulated soil amendment (compost 50%, biochar 20%, humid acid 1%, guano 9%) and the second factor were the rate of N,P fertilizers100%, 80%, 60%, 40% recommended doses (138; 110.4; 82.8; 55.2 kg ha^{-1} N and 36; 28.8; 21.6; 14.4 kg ha^{-1} P). The results revealed that application of biofertilizers and FSA increased soil C-org, population of PSB and N-fixing bacteria, chlorophyll leaves and yield of maize were increased significantly. The highest population of PSB and N-fixing bacteria, chlorophyll leaves and yield of maize were obtained by application of 1.200 g ha^{-1} of biofertilizers and 2 t ha^{-1} of organic FSA. The application of biofertilizers and FSA with 60-100% NP fertilizer could increase maize yields. Additionally, fertilizers efficiency was increased by 40 %. This finding recommends the use of 1200 g of biofertilizers inoculant and 2 t of FSA ~~to~~ improved the soil properties and increased the maize productivity on marginal soils.

Keywords: N-fixer, organic carbon, P-solubilizer, fertilizers efficiency

1. Introduction

Concern for environmental pollution due to the use of various chemicals especially inorganic fertilizers and synthetic pesticides as well as health and environmental reasons makes sustainable agriculture one of the alternatives of modern agriculture. One effective step that can be developed in sustainable agriculture is the use of microbes that are useful in facilitating or increasing the availability of soil nutrients or known as biofertilizers (Macik *et al*., 2020). Microbes which are commonly used as an active ingredient in biological fertilizers are nitrogen-fixing bacteria, phosphate solubilizing microbes, mycorrhizae, and phytohormone-producing bacteria (Wahane *et al.*, 2020).

Beneficial microbe that has a role to facilitate available phosphate is phosphate-solubilizing microbes (PSM). These microbes are able to improve plant growth and increase soil P availability (Jayakumara *et al.,* 2019; Wu *et al.,* 2019) and produce phosphatase enzyme (Chen and Liu, 2019) and produce phytohormone (Fitriatin *et al.*, 2020).

Some bacteria can convert atmospheric nitrogen into ammonia in symbiosis with plants or without symbiosis. Gentili and Jumpponen (2006) reported that *Azotobacter* sp. and *Azospirillum* sp. are non-symbiotic and free-living bacteria that play a role in N fixation. Steenhoudt and Vanderleyden (2000) stated that *Azospirillum* is the best genus from Plant Growth-Promoting Rhizobacteria (PGPR) genera group because its interactions with several plant roots are able to fix nitrogen and produce plant growth hormones.

Biofertilizers play an important role to sustainably increase land productivity and influence plant growth and yield positively (Itelima *et al*. 2018). Fitriatin *et al*. (2019) reported that the application of biofertilizers that contain consortium of phosphate-solubilizing microbes and nitrogen-fixing bacteria increase soil nitrogen and P availability. The application of biofertilizers and organic ameliorants is a step to reduce soil damage due to excessive use of inorganic fertilizers. The use of organic fertilizers is expected to supplement the use of chemical

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fertilizers. More research is needed to know more about the impact of biofertilizers which contain phosphate-solubilizing microbes and nitrogen-fixing bacteria with formulated soil amendment in increasing soil carbon, population of PSM, chlorophyll content and yield of maize.

2. Materials and Method

The field experiment was conducted at Horticultural Seed Development Centre and Various Plants Pasir Banteng, Sumedang, West Java on Inceptisols. A composite sample was taken from the field from depth 0-30 cm for physical and chemical analysis of the soil. The physic-chemical properties of the soil are as follows: texture dusty clay; pH 5.95; C_{org} 2.3%; N_{total} 0.21%; C/N 10; P_2O_5 HCl 25% 60.75 mg $100g^{-1}$; P_2O_5 (Bray) 11.36 ppm; and CEC 23.61 $cmol.kg^{-1}$.

The experiment was laid out in Randomized Complete Blocks Design (RCBD) consisting of two factors and three replications (The plot size was 3m × 1.5m with inter row spacing of 75 cm and intra row spacing of 25 cm). The first factors were biofertilizers and formulated soil amendment (FSA) (control; biofertilizers; FSA ; and biofertilizers + FSA). Biofertilizers with a peat base material carrier contain N-fixer bacteria (*Azotobacter chroococcum*, and *Azospirillum* sp.) and phosphate solubilizing bacteria (*Bulkholderia vietnamiensi* and *Enterobacter ludwigii)*. These bacteria were selected in isolation from several ecosystems including rice rhizosphere, peanut rhizosphere and sweet potato rhizosphere. *Azotobacter* and *Azospirillum* were cultured on JNFb media while *Enterobacter* and *Bulkholderia* were cultured on Pikovskaya media. Formulated soil amendment contains mixture of compost 50%, biochar 20%, humid acid 1%, and guano 9%. The second factor was the rate of NP fertilizers 100%, 80%, 60%, 40% recommended doses (138; 110.4; 82.8; 55.2 kg ha^{-1} N and 36; 28.8; 21.6; 14.4 kg ha^{-1} P) with Urea (46% N) and single super phosphate (36% P) which is applied around the planting hole. Maize seeds used BISI 2 with a germination rate of more than 85%.

2.1 Data collection

Soil Organic Carbon (SOC)

Was determined by oxidimetric titration Walkley and Black method (Nelson and Sommers 1982).

The number of bacterial population

Nitrogen fixing bacteria and phosphate solubilizing bacteria population were determined by serial dilution plate count technique (Zhang *et al*. 2012).

Content of chlorophyll

Chlorophyll content of leaf was measured by using Soil Plant Analysis Development (SPAD) meter (SPAD 502, Minolta, Japan). Five plants were randomly selected from each plot to measure chlorophyll content.

Grain yield

To calculate grain yield by using given formula:

$$Gr\,(Kg\,ha-1) = \frac{yield\ plot\ at\ 14\,\%\ moisture\ (Kg)}{plot\ size\ in\ m2}\ x\ 10000\ m2 \quad (1)$$

Agronomic efficiency

Agronomic efficiency was calculated by equation below (Zemichael *et al*. 2017):

$$AE\ (kg\ kg-1) = \frac{Gf - Gu}{Na} \quad (2)$$

If: Gf: the grain yield in the fertilized plot (Kg).

Gu: the grain yield in the un fertilized plot (Kg).

Na: the quantity of nutrient applied (Kg).

Data were processed using DSAASTAT (Dipartimento in the Scienze Agrarie ed Ambientali Statistic) and Duncan's multiple range test with a 95% confidence level to determine differences in mean values between treatments.

3. Results

3.1 Soil Organic Carbon

The soil organic C content of the soil was measured at the end of the vegetative phase. The experimental results show that there was no interaction between biofertilizers and FSA with NP fertilizers (Table 1).

Table 1. Soil organic carbon as affected by biofertilizers, FSA and N, P fertilizers

Treatments	Soil Organic C (%)
Biofertilizers and formulated soil amendment (FSA)	
- Control	3.80 a
- Biofertilizers	3.83 a
- FSA	4.12 b
- Biofertilizers + FSA	4.31 b
N, P Fertilizers	
100%	4.01 a
- 80%	4.02 a
- 60%	4.19 a
- 40%	3.84 a

Note: Numbers followed by the same letter are not significantly different according to Duncan's test at 5% significance level

Biofertilizers did not significantly increase soil organic carbon. Different results are shown by the treatment of FSA which was able to increase soil organic carbon significantly. The combination treatment of biofertilizers and FSA increased soil organic carbon up to 13.4% compared to control.

3.2 Chlorophyll content

The statistical analysis showed the interaction between biofertilizers and FSA with NP fertilizer on chlorophyll content of maize leaves (Table 2). In general, the application of biofertilizers and FSA at various doses of NP fertilizers increased the chlorophyll content. The combination of biofertilizers and FSA at various doses of NP fertilizers higher chlorophyll content compared to the treatment of only biofertilizers or FSA. Chlorophyll content tends to be higher in the treatment of high NP fertilizer doses. The treatment of 100% NP fertilizers and the combination of biofertilizers and FSA gives the highest C soil content (39.47).

Table 2. Chlorophyll content of maize leaves (SPAD)

Treatments	N, P Fertilizers			
	100%	80%	60%	40%
control	32.83 a (d)	31.87 a (c)	30.73 b (b)	28.90 a (a)
Biofertilizers	38.90 c (c)	31.80 a (b)	29.47 a (a)	29.77 ab (a)
FSA	35.07 b (c)	31.73 a (b)	30.30 ab (a)	29.50 ab (a)
Biofertilizers + FSA	39.47 c (d)	34.37 b (c)	32.20 c (b)	30.20 b (a)

Note: Numbers followed by the same letter are not significantly different according to Duncan's test at 5% significance level. Letters in parentheses are read horizontally. Letters without brackets are read vertically

3.3 Soil microbe population

Beneficial soil microbe populations measured were PSB and N-fixer bacteria at the end of vegetative period. The PSB population ranges between 2.7-57.55 x 10^7 colony forming unit (CFU) g^{-1} (Table 3).

The results of statistical analysis show a significant interaction between biofertilizers and FSA with NP fertilizer on PSB population. Biofertilizers and FSA increased population of PSB at various doses of NP fertilizers. Application of 80-100% NP fertilizers and the combination of biofertilizers and FSA provide a better influence on the PSB population (55.9-57.55 x 10^7 CFU g^{-1}).

Table 3. Population of phosphate solubilizing bacteria (10^7 CFU g^{-1})

Treatments	N,P Fertilizer s			
	100%	80%	60%	40%
Control	20. 67 a (a)	22.00 a (a)	21.67 a (a)	20.67 a (a)
Biofertilizers	52.25 b (c)	51.10 c (bc)	48.50 c (a)	48.81 c (ab)
FSA	27.35 c (b)	26.33 b (b)	24.73 b (ab)	23.63 b (a)
Biofertilizers + FSA	57.55 d (c)	55.90 d (c)	50.43 c (b)	47.18 c (a)

The population of N-fixing bacteria was affected by the application of biofertilizers and FSA at several doses of N and P fertilizers. The statistical analysis shows a significant interaction between biofertilizers and FSA with NP fertilizer on N-fixing bacteria population.

Application of 80-100% NP fertilizers and the combination of biofertilizers and FSA provided a better influence on the population of (N-fixing bacteria (47.37 x 10^7 CFU g^{-1}). Fertilizing low-dose NP tended to give a lower population number of N fixing bacteria compared to other treatments (Table 4).

Table 4. Population of N-fixing bacteria (10^6 CFU g^{-1})

Treatments	N,P Fertilizer s			
	100%	80%	60%	40%
control	12.45 a (a)	12.17 a (a)	10.83 a (a)	11.933 a (a)
Biofertilizers	25.18 b (a)	26.37 b (a)	22.933 c (a)	23.77 b (a)
FSA	16.92 a (a)	16.9 a (a)	15.78 b (a)	19.78 b (a)
Biofertilizers + FSA	47.37 c (d)	33.63 c (c)	28.17 c (b)	22.38 b (a)

3.4 Agronomic efficiency

Agronomic efficiency (AE) is calculated in units of yield increase per treatments of NP fertilizer applied. It more closely reflects the direct production impact of an applied inorganic fertilizer. The result showed that there was a significant effect of biofertilizers and FSA application and also application of inorganic fertilizers on yield of maize.

Biofertilizers application with 40% NP fertilizers increased grain yield of maize up to 5.28 Kg Kg $^{-1.}$ Furthermore, increasing the dosage of NP fertilizer to 80%, the application of biofertilizers decreased the yield to 0.86 Kg Kg $^{-1}$ (Table 5). Application of FSA with 40% NP fertilizers increased grain yield up to 6.22 Kg Kg^{-1}. However, the combination of biofertilizers and FSA applications with 100%, 80% and 60% NP fertilizer increased grain yield of maize by 5.40, 4.20, 2.16 Kg Kg $^{-1}$, respectively.

Table 5. Agronomic efficiency of NP fertilizers application on maize

Treatment	Grain yield of maize (Kg ha^{-1})	Agronomic efficiency (Kg Kg $^{-1}$)
Control (100% NP fertilizer)	2844	-
80% NP fertilizer	2660	1.32
60% NP fertilizer	2456	3.71
40% NP fertilizer	2237	8.72
Biofertilizers + 100% NP fertilizer	3447	3.46
Biofertilizers + 80% NP fertilizer	2965	0.86
Biofertilizers + 60% NP fertilizer	2672	1.64
Biofertilizers + 40% NP fertilizer	2476	5.28
FSA + 100% NP fertilizer	3044	1.14
FSA + 80% NP fertilizer	2860	0.11
FSA + 60% NP fertilizer	2545	2.86
FSA + 40% NP fertilizer	2411	6.22
Biofertilizers and FSA + 100% NP fertilizer	3785	5.40
Biofertilizers and FSA + 80% NP fertilizer	3430	4.20
Biofertilizers and FSA + 60% NP fertilizer	3070	2.16
Biofertilizers and FSA + 40% NP fertilizer	2715	1.85

4. Discussion

In general, the treatment of biofertilizers and FSA can significantly increase soil organic carbon, chlorophyll content, and PSB population. This shows that the biofertilizers will have an optimal effect if there is an energy source for growth obtained from FSA. This proves that biofertilizers is more effective to promote plant growth when combined with FSA. Application of biofertilizers combined with FSA increases the soil organic carbon. The soil amendment used consists of materials which are rich in organic carbon such as compost with raw materials of bagasse, biochar, dolomite, hemic acid and guano which can increase the organic carbon content *in* the soil.

The application of biofertilizers can also help increase organic carbon by the decomposition process carried out by bacteria. Therefore, soil amendment and biofertilizers can increase the organic carbon content higher than other treatments. Compost and biochar are the source of carbon and energy for microbes in biological fertilizer. In this atudy, the compost has a C/N ratio of 23. This is supported by the results of research by Husna *et al*. (2019) showing the highest viability of phosphate solubilizing microorganisms in biochar carriers.

Increasing soil organic carbon greatly affects soil health (Tully and McAskill, 2020). The organic carbon can affect soil structure, aeration, and water holding capacity (Colombi *et al.,* 2019). These soil properties can support plants to produce in the soil. Provision of soil amendment and biofertilizers can increase organic carbon in the soil. Provision of soil amendment and P solubilizing microbes and N fixers can increase organic carbon by up to 75% in the soil after three years of application (Debska *et al*., 2016). The application of soil amendment and biofertilizers can increase organic carbon and affect other chemical properties that can support soil health.

The advantages of biofertilizers include reducing the use of chemical fertilizers and environmental pollution, increasing the availability of nutrients and enhancing plant growth, and improve the physical, chemical and biological properties of the soil (Wahane *et al*., 2020). Yasmin *et al.* (2020) reported that beneficial microbes application increased yield and available nutrient (N,P,K) and reduced the nitrogen fertilizer rate. Furthermore, Albdaiwi *et al.* (2019) stated that some microbes as Plant Growth-Promoting Rhizobacteria (PGPR) increase nutrient availability, yield of crops and substitute to chemical fertilizers. The formulated soil amendment is a source of energy for soil macro and microbes. Addition of soil amendment will increase microbiological activities and populations, especially those related to the decomposition and mineralization of organic matter (Stevenson, 1986).

Application of biofertilizers resulted in considerable increase in the chlorophyll content of maize plant leaf tissue. In this study, biofertilizers which contain phosphate solubilizing bacteria and N-fixing bacteria improved the growth of maize and increased chlorophyll content of maize plant leaf tissue. Khan (2018) observed chlorophyll content increases with biofertilizers application. The application of biofertilizers has been reported to improve the soil health and escalate the efficiency of applied fertilizers (Simarmata *et al.*. 2017).

Chlorophyll content is one of the parameters for plants to grow well. Chlorophyll is a green pigment in plants playing a role in the photosynthesis process of plants (Li *et al.*, 2018). Chlorophyll is formed from several nutrients, so if the plant does not get the optimum nutrient, the chlorophyll content in the plant is not optimal. Table 2 shows that the application of biofertilizers and ameliorants combined with 100% of the recommended dosage of inorganic fertilizers can increase the chlorophyll content higher than other treatments. This could be due to the 100% dose of inorganic fertilizer affecting the chlorophyll content of leaves. The availability of nutrients in the soil, especially the essential nutrients N, P and K can increase chlorophyll content in plants (Eisvand *et al.,* 2018).

The higher the supply of nitrogen into plants, the higher the chlorophyll content of plants in the leaves so that the photosynthesis process is faster (Bassi *et al*., 2018). The N element received by plants is obtained from the application of inorganic fertilizers which are not reduced from the recommended dosage and the use of N-fixing bacteria.

Table 4 shows that biofertilizers and FSA combined with inorganic fertilizers 100% of the recommended dosage resulting in the highest of PSB population compared to other treatments. The biofertilizers in this study contained PSB (*Bulkhoderia vietnamiensi* and *Enterobacter ludwigii*). The increase of PSB population was accompanied by soil amendment applied to the soil which can be a source of energy for bacteria to reproduce. The provision of ameliorants also increased PSB population because it helped bacteria grow and get a source of energy.

The increase in the population of N-fixing bacteria was caused by the application of biofertilizers and soil amendment. The biofertilizers used in this study contained N-fixing microorganisms, namely *Azotobacter chroococcum* and *Azospirillum* sp. However, increasing the N,P fertilizer will inhibited the N-fixing bacteria. Increasing population of N-fixing bacteria improves soil health and increases plant productivity (Tahat *et al*., 2020).

Based on the measurement of agronomic efficiency, the application of biofertilizers and FSA with 60-100% NP fertilizer could increase maize yields. 100 % of the N, P fertilizer will inhibit the N-fixation bacteria. In addition, the combination of biofertilizers and FSA application increased fertilizers efficiency by 40%. This is in line with research by Cisse *et al*. (2019) that application of 20 kg ha^{-1} biofertilizer reduced at least 50% of the NPK fertilization.

5. Conclusion

Application of biofertilizers (N-fixer bacteria *Azotobacter chroococcum*, and *Azospirillum* sp. and phosphate solubilizing bacteria *Bulkholderia vietnamiensi* and *Enterobacter ludwigii*) and formulated soil amendment (mixture of compost 50%, biochar 20%, humid acid 1%, and guano 9%) can significantly increase soil organic carbon, chlorophyll content, PSB population and yield of maize. The highest population of PSB and N-fixing bacteria, chlorophyll leaves and yield of maize were obtained by application of 1.200 g ha^{-1} of biofertilizers and 2 t ha^{-1} of formulated soil amendment. Additionally, fertilizers efficiency was increased by 40 %.

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