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Some soil biological and chemical properties as affected by biofertilizers and organic ameliorants application on paddy rice

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Abstract

Biofertilizers are compounds that contain microorganisms capable of increasing the nutrient availability to plants and increasing plant growth rate. The purpose of this research is to study the effects of biofertilizers and organic ameliorants on some of soil bio-chemical properties. The pot experiment was conducted at the field of the Faculty of Agriculture, Universitas Padjadjaran Indonesia. The experiment was conducted in a randomized block complete design format consisting of twelve treatments and three replications. The experiment consisted of control, solid biofertilizer (50 kg ha^{-1}), liquid biofertilizer (5 L ha^{-1}), a combination of solid biofertilizers (50 kg ha^{-1}) with organic ameliorants (10t ha^{-1}) (composted straw, biochar and cow manure), a combination of liquid biofertilizer (5 L ha^{-1}) and organic ameliorants (10 t ha^{-1}), and each of ameliorants (10 t ha^{-1}) independently. The results of experiment revealed that the application of solid biofertilizers and organic ameliorants significantly improved some soil biological properties (population of phosphate solubilizing microbes, N-fixing bacteria and phosphatase activity) and increased some soil chemical properties such as total N, available P, organic C and cation exchange capacity.

Keywords: Ameliorant, biofertilizers, phosphatase activity, soil, microorganism.

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Introduction

Biofertilizers are compounds containing living cells of different types of microorganism which has an ability to mobilize important elements from non-available to available form through biological process. The main purposes of using biofertilizers to improve the soil quality, to substitute inorganic fertilizers, to increase productivity and to promote an eco-friendly a sustainable agriculture (Bargaz et al., 2018).

The main group of bacterial and fungal biofertilizers, such as symbiotic nitrogen-fixing biofertilizer (roots nodulation, stem nodulation and Azolla), non-symbiotic nitrogen bio-fertilizers, phosphate solubilizing and mobilizing organisms (including mycorrhizal fungi), potassium solubilizing bacteria, plant growth promoting rhizobacteria, endophytic bacteria, decomposers (mineralize of organic materials and resulting the available form of nutrients), siderophore and the mass production of biofertilizers and to evaluate the quality of biofertlizers. Islam et al. (2013) suggested that inoculation with N-fixing bacteria significantly increased chlorophyll content, and the uptake of different macro- and micro-nutrient contents enhancing

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also in red pepper shoots. Salamone et al. (2012) states that inoculation of *Azospirillum brasilense* and *Pseudomonas fluorescens* improved paddy rice production.

In the soil, numerous biological and chemical processes are carried out, some of which include; mineralization of organic matter and fixation of mineral matter atoms into organic compounds. The processes take place within ecosystems (groups of organism interacting with their abiotic environment). Phosphate solubilizing microorganisms (PSMs) can solubilize unavailable phosphate and make them available for plant (Kalayu, 2019). The PSMs produce organic acid, phosphatase and indole acetic acid (Fitriatin et al., 2020). They have an important role in increasing the availability of insoluble P to plants. Organisms which are capable of increasing the P availability in soil belongs to diversified groups including bacteria, actinomycetes and several groups of fungi (Sharma et al., 2013).

Some result reported that biofertilizer improve nutrient available and plant growth on stress condition. Fitriatin et al (2018) reported that biofertilizers (PSMs and nitrogen-fixing bacteria) at different salinity levels (2, 4 and 6 mmhos cm^{-1}) affected the P-availibilty in the soil, phosphatase activity and dry weight of paddy rice. Soni et al. (2018) suggested that the isolated halotolerant PSMs may to be used as source to supply phosphorous to the growing plant in saline conditions.

The application methods of biofertilizers depend on the type of biofertilizers in use and the active ingredient of their inoculants. The objective of the research was to study the effect of biofertilizers (both solid and liquid biofertilizer containing of PSMs and nitrogen-fixing bacteria) on some chemical and biological properties of Inceptisols.

Material and Methods

Soil

The soil used in this study was taken from paddy soil (a depth of 0-20 cm). The soil were containing 46% clay, 41% silt, 13% sand. Soil texture can be classified as silty clay. The pH (in H_2O) was slightly acidic (6.47), soil C_{org} was low (1.30%), total N (N_{total}) was low (0.13%), the soil C/N ratio 10, soil $P_{available}$ was high (18,92 mg kg^{-1}) and cation exchange capacity (CEC) was 17.26 cmol kg^{-1}. The soil were classified Inceptisols in Soil Survey Staff (1999).

Biofertilizer

Biofertilizer contained of PSMs (*Pseudomonas mallei, Pseudomonas cepaceae, Penicillium* sp., *Aspergillus* sp.) and nitrogen-fixing bacteria (*Azotobacter* sp. and *Azosprillum* sp.) obtained from the collection of Soil Biology and Biotechnology Laboratory, Faculty of Agriculture, Universitas Padjadjaran, Indonesia. Preparation of biofertilizer begins with augmentation of each isolate. Adding 10 mL 0,85% physiological NaCl to the test tube of pure culture isolate then transferred to a tube filled with 500 mL each selective media. PSMs used Pikovskaya medium (5 g $Ca_3(PO_4)_2$, 10 g glucose, 0.5 g $(NH_4)SO_4$, 0.2 NaCl, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.2 g KCl, 0.5 g yeast extract, 0.002 g $MnSO_4 \cdot H_2O$, 0.002 g $FeSO_4 \cdot 7H_2O$, 15 g agar, in 1 L distilled water, pH 7) (Nautiyal et al. 1999) and nitrogen-fixing bacteria used Nitrogen-free Ashby medium (5 g glucose, 5 g mannitol, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.1 g $MgSO_4 \cdot 7H_2O$, 5mg $Na_2MoO_4 \cdot 2H_2O$, 0.9 g K_2HPO_4, 0.1 g KH_2PO_4, 0.01 g $FeSO_4 \cdot 7H_2O$, 5 g $CaCO_3$, 15g agar in 1 L distilled water, pH 7.3) and Okon medium (5 g Malic acid, 4 g KOH, 0.5 g K_2HPO_4, 0.05 g $FeSO_4.7H_2O$, 0.01 g $MnSO_4.7H_2O$, 0.1 g $MgSO_4.7H_2O$, 0.02 g NaCl, 0.01 g $CaCl_2$, 0.002 g Na_2MoO_4, 2.00 ml Bromothymol blue (0.5% in 95% methanol), Agar 1.8 g (semi-solid)/18 g(solid), NH_4Cl 1 g, Water 1 litre (Narayan et al., 2018), then incubated for 72 hours by shaking it using shaker. Solid biofertilizer used a mix of peat and compost with a ratio of 1:1 as carrier, for liquid biofertilizers use molasses (2%) + NH_4Cl 0.01 %.

Organic ameliorant

Organic ameliorant used in this research were straw compost (Organic C: 29.63%, N 1.43%. P: 1.34%, K: 2.11%, C/N: 21, and pH: 8.64), biochar (Organic C: 42.42%, N: 0.49%, P: 0.02%, K_2O: 0.16%. C/N: 87, and pH: 8,18), and cow manure (Organic C: 43.60%, N: 1.13%. P: 0.60%, K_2O: 0.59%, C/N: 29, and pH: 7.80). The three ameliorants were selected based on selection in previous studies and had different nutrients content. Doses of organic ameliorants were 10 t ha^{-1}.

Experimental design

The experiment procedure include testing and optimizing the formula for consortia of PSMs (*Pseudomonas mallei, Pseudomonas cepaceae, Penicillium* sp. and *Aspergillus* sp.) and nitrogen-fixing bacteria (*Azotobacter* sp. and *Azosprillum* sp.) with organic ameliorant with the composition of straw compost, biochar, and cow manure. Experimental soil was used for planting the rice seedlings during the pot experiment. Two rice seedlings were obtained at 14 days after seedlings (DAS) in a plastic container 40 x 30 cm with a height of 35 cm set to be transferred to pots for further experiment.

The experiment was carried out with the randomized block design (RBD) consisted of twelve treatments with three replications in greenhouse of Agricultural Faculty, Universitas Padjadjaran Indonesia.. The experiment consisted of control, solid biofertilizer (50 kg ha^{-1}), liquid biofertilizer (5 L ha^{-1}), a combination of solid biofertilizers (50 kg ha^{-1}) with organic ameliorants (10 t ha^{-1}) (composted straw, biochar and cow manure), a combination of liquid biofertilizer (5 L ha^{-1}) and organic ameliorants (10 t ha^{-1}), and each of ameliorants (10t ha^{-1}) independently. The provision of water was carried out to maintain the condition of the field capacity. The variables observed were some soil chemical properties (N_{total}, available P ($P_{available}$), CEC, organic C ($C_{org.}$)) and some soil biological properties (acid phosphatase activity and population of PSMs and nitrogen fixing-bacteria) at the end of the vegetative period. Plants are harvested at the end of the generative period at the age of 95 days.

Soil biological properties

Soil biological properties analysed in this study were total population of PSMs and nitrogen-fixing bacteria. The serial dilution plates were used to determine population of PSMs on Pikovskaya agar. One g of soil sample was taken and serially diluted using sterile distilled water upto 10^{-6} dilutions. One ml of diluted sample from 10^{-4} to 10^{-6} dilutions were taken to plate with Pikovskaya agar. The plates were incubated at 28°C for 48-72 hours until halozones appeared around the colonies. The same method to determine population of nitrogen fixing bacteria. *Azotobacter* were calculated on Nitrogen-free Ashby medium while *Azospirillum* on Okon medium (Somasegaran and Hoben, 1994). The acid phosphatase activity in soils (EC 3.1.3.2) determined according to Eivazi and Tabatabai (1977).

Soil chemical properties

Soil chemical properties analysed in this study were N_{total} content in soil using Kjeldahl method $P_{available}$ using Bray I method, $C_{org.}$ using Walkley and Black method and CEC was determined using the 1N ammonium acetate at pH 7 (van Reeuwijk, 2002).

Data analysis

Data were collected to analysis of variance (ANOVA) and treatment means were compared using Tukey HSD Advance test at p = 0.05 probability.

Results and Discussion

Soil Biological Properties

The result of experiment revealed that biofertilizer and organic ameliorant increase soil biological properties significantly. Population of soil beneficial microorganisms gave different respons by biofertilizer and organic ameliorant. Type of biofertilizer influenced to soil biological properties. The treatment of liquid biofertilizer with biochar significantly affected on population of phosphate solubilizing bacteria up to 11.70 x 10^9 CFU g^{-1} (Table 1). This indicated that the application of biochar stimulate the growth of microorganisms. Ajema (2018) reported that biochar incorporation into soil improved beneficial soil microorganisms. According to Głuszek et al. (2017) that biochar influenced soil physical and chemical properties as well as beneficial soil microorganisms.

Table 1. Population of microorganisms and phosphatase activity in soil at the end of vegetative period

Treatments	Population of soil microorganisms (CFU g^{-1})								Acid phosphatase activity	
	PSB* ($x10^9$)		PSF ** ($x10^4$)		*Azotobacter* ($x10^5$)		*Azospirillum* ($x10^7$)		(µg pNP g^{-1} h^{-1})	
Control	1.30	a	4.33	a	2.10	ab	10.38	a	32.8	a
Solid biofertilizer (S-bio)	9.00	c	9.67	ab	4.23	c	13.57	ab	56.3	c
Liquid biofertilizer (L-bio)	10.33	d	6.33	ab	1.93	ab	14.17	ab	50.3	bc
S-bio + straw compost	9.28	cd	6.00	ab	1.97	ab	16.77	ab	55.3	c
S-bio-fertilizer + biochar	6.10	bc	5.33	ab	2.03	ab	21.17	bc	50.5	bc
S-bio + cow manure	9.08	cd	7.00	ab	2.73	b	15.93	ab	53.7	bc
L-bio + straw compost	5.90	bc	6.33	ab	2.23	ab	15.33	ab	50.6	bc
L-bio + biochar	11.70	d	10.67	b	2.77	b	30.67	c	59.6	c
L-bio + cow manure	3.62	ab	4.67	ab	2.07	ab	13.83	ab	56.9	c
Straw compost	2.52	a	2.67	ab	1.73	a	8.79	a	48.4	bc
Biochar	1.18	a	6.00	ab	2.13	ab	13.60	ab	42.7	ab
Cow manure	2.32	a	6.33	ab	2.03	ab	21.18	bc	51.9	bc

Remarks: The average score followed by the same letter is not significantly different according to the Tukey HSD Advanced Test at the 5% level. *PSB : phosphate solubilizing bacteria; **PSF : phosphate solubilizing fungi

The result showed that biofertilizers application better influence than ameliorant organic on population of soil microorganisms. A previous study reported that application biofertilizer containing *Bacillus aryabatthai* increased population of phosphate solubilizing bacteria (Zulaehah et al., 2019). Bhardwaj et al (2014) stated that biofertilizers play an important role in creating a better soil environment through increase nutrient availability. The other research showed that biofertilizer (*Azotobacter* sp. *Azospirillum* sp and *Rhizobium* sp.) combined with organic fertilizer (palm oil mill effluent) increased soil bacterial population (Suliasih and Widawati, 2017).

Biofertilizers and organic ameliorant influenced enzyme activity. The result showed that biofertilizers improve soil phosphatase activity. Soil phosphatase activity was significantly higher 59.6 µg pNP g^{-1} h^{-1} recorded with liquid biofertilizers + biochar. This indicated that combination of biofertilizers and organic ameliorant stimulated soil microorganisms to produce phosphatase enzyme. According to Laxman et al. (2017) concortia of microorganisms application to soil and foliar application of macro nutrients showed remarkable influence on activity of dehydrogenase, phosphatases and urease enzymes. Rahmansyah and Antononius (2015) reported that biofertilizers and compost application improved soil phosphatase and urease activites. Furthermore, the continuous application of compost increased soil organic matter content, population of soil micoorganisms and soil enzyme activity (Chang et al., 2007).

Soil Chemical Properties

Based on the statistical analysis in Table 2, the combination of biofertilizers and ameliorants significantly affected the N_{total} and $P_{available}$ content in soil. The application of biofertilizers and organic ameliorants improve the N_{total} and $P_{available}$ significanlty. The effect of biofertilizer and organic ameliorants on N_{total} increased in the range 38.5% to 138.5%. The highest of N_{total} was shown by combination of solid biofertilizer with straw compost which increasing up to 138.5%. Our study suggests that biofertilizers without organic ameliorant have lower N_{total} content than the combined treatments of biofertilizers and organic ameliorants.

The applications of organic ameliorant significantly improved N_{total}. However, differences in the types of organic ameliorants in this case straw compost, biochar and cow manure gave effect on N_{total} non-significant each other. The other studies showed that application of ameliorant (biochar, lime and organic fertilizer) increased abundances of *Nitrosomonas* and *Nitrospira* which causes an increasing in soil N_{total} (Zhang et al., 2017).

The effect of biofertilizers and organic ameliorant on $P_{available}$ increased significantly in the range 14.7% to 79.8 % (Table 2). The highest of $P_{available}$ was shown by combination of solid biofertilizer with straw compost which increasing up to 79.8%. In general, application of organic ameliorant without biofertilizer have lower $P_{available}$ than the combined treatments of biofertilizers and organic ameliorants. Acoording to Ye et al. (2020) biofertilizer combined with organic fertilizers increased abundance of soil microorganisms and enhanced the $P_{available}$ and K contents in soil.

Table 2. N_{total} and $P_{available}$ in soil at the end of vegetative period

Treatments	N_{Total} (%)		$P_{available}$ (mgkg^{-1})	
Control	0.13	a	16.36	a
Solid biofertilizer (S-bio)	0.20	bc	23.16	e
Liquid biofertilizer (L-bio)	0.18	b	19.20	bc
S-bio + straw compost	0.31	d	23.47	e
S-bio-fertilizer + biochar	0.20	bc	20.17	cd
S-bio + cow manure	0.22	bc	21.20	de
L-bio + straw compost	0.21	bc	19.90	cd
L-bio + biochar	0.24	c	21.88	de
L-bio + cow manure	0.21	bc	18.77	b
Straw compost	0.18	b	18.70	ab
Biochar	0.19	b	18.16	ab
Cow manure	0.18	b	17.77	ab

Remarks: The average score followed by the same letter is not significantly different according to the Tukey HSD Advanced Test at the 5% level

Increasing of N_{total} in soil in this research as affected by biofertilizers and organic ameliorants. This is due to the presence of N-fixing bacteria in the form of *Azotobacter* and *Azospirillum* in biofertilizers. According to Rodrigues et al. (2008) the use of organic matter with the addition of *Azotobacter* improves the fixation of N compared to without the addition of *Azotobacter*. The value of the increasing organic matter with *Azotobacte*r is 11.4 kg ha^{-1}. Piccinin et al. (2013) states nitrogen fixing bacteria is associated with the plant and provides a portion of N needed by the plants. This process is through direct secretion to the plant. The addition of *Azospirillum* inoculation increases the growth of roots and upper parts of plants, and also increases crop productivity. This addition also reduces the use of N fertilizer by 15-20 kg N ha^{-1} (Rodrigues et al., 2008).

Table 3 showed the combination of biofertilizer and organic ameliorant has a significant effect on $C_{org.}$ in soil (The treatment of solid biofertilizers with ameliorant increased significantly on $C_{org.}$. Combination of solid biofertilizers with straw compost gave better effect to increase $C_{org.}$. This can be caused by C/N ratio (C/N ratio is 21) of straw compost was smallest than biochar (C/N ratio is 87) and cow manure (C/N ratio is 29), futhermore it decomposed faster and then increased $C_{org.}$ In this study, biofertilizers combined with organic ameliorant increased C_{org} up to 15.27%. The other study showed that biofertilizers (containing lactic acid bacteria, *Pseudomonas* spp., *Penicillium* and *Actinomycetes* spp.) increased the soil organic matter stability (Dębska et al., 2016). According to Mijwel (2018) combined of organic with biofertilizer were the best treatment for increasing of soil content of organic substance, $C_{org.}$ and organic phosphorus.

Table 3. $C_{org.}$ and CEC at the end of vegetative period

Treatments	C_{org} (%)		CEC (cmol kg^{-1})	
Control	1,31	a	20,43	a
Solid biofertilizer (S-bio)	1,42	bc	25,12	b
Liquid biofertilizer (L-bio)	1,37	ab	24,12	ab
S-bio + straw compost	1,51	d	25,97	b
S-bio-fertilizer + biochar	1,50	d	24,01	b
S-bio + cow manure	1,50	d	25,61	b
L-bio + straw compost	1,46	cd	23,51	ab
L-bio + biochar	1,44	cd	23,64	ab
L-bio + cow manure	1,45	cd	22,10	ab
Straw compost	1,45	cd	23,14	ab
Biochar	1,46	cd	22,70	ab
Cow manure	1,46	cd	24,41	ab

Remarks: The average score followed by the same letter is not significantly different according to the Tukey HSD Advanced Test at the 5% level.

Our study showed that application of biofertilizers combined with organic ameliorant significantly increased CEC. It may be due to that biofertilizer and organic ameliorant increased nutrient availability which further affects increasing in CEC. Arabi et al. (2018) reported that the interaction of biochar and biofertilizer significantly increased CEC. The other study showed that organic fertilizer composted with liquid biofilm biofertilizer (3-21 ton ha^{-1}) improved soil N_{total}, $P_{available}$, exchangeable-K and CEC of Lithosols and spinach yields (Sudadi et al., 2018).

Conclusion

Our study showed that combined of biofertilizer and ameliorant organic increased some soil biological properties (population of PSMs, N-fixing bacteria and acid phosphatase activity) and some soil chemical properties (N_{total}, $P_{available}$, $C_{org.}$ and CEC). The solid biofertilizers with straw compost application gave better to increase N_{total} and $P_{available}$, while the liquid biofertilizers with biochar gave better to improve population of soil microorganisms and phosphatase activity. Further studies are needed to develop organic-based biofertilizers to improve soil quality, crop production and reduce chemical fertilizers.

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