P Status In Andisol And P Content In Arabica Coffee Seedling Leaves Due To The Application Of Phosphate Providing Microorganisms And Organic Matters In Bener Meriah District

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Abstract: Bener Meriah district is one of the arabica coffee producing regions in Indonesia. Most of arabica coffee in Bener Meriah district grown on Andisol. Generally, the availability of P in Andisol is very low. Phosphate providing microorganisms and organic matters can be used to increase Andisol P availability. The objective of this study was to examine the effect of the application of phosphate providing microorganisms and organic matters on P status in Andisol and P content in arabica coffee seedlings leaves in Bener Meriah district. The experiment used a randomized block design that consisted of two factors. Factor I was the application of phosphate providing microorganisms consisting of without microorganisms, Glomus sp., Kurthia sp., Corynebacterium sp., and Listeria sp. Factor II was the application of organic matters consisting of T. diversifolia and coffee bean skins. The results of the study showed that Glomus sp., Kurthia sp., Corynebacterium sp., and Listeria sp. decreased soil P-retention by 2.38, 5.12, 7.48, 9.17% respectively, increased soil P-available by 24.85, 36.03, 52.79, 77.33% respectively, and increased P-content in the arabica coffee seedling leaves by 22.22, 33.33, 37.03,72.27% respectively, compared to without the application of microorganisms. The application of coffee bean skins resulted in lower soil P-retention, higher soil P-available and P-content in arabica coffee seedling leaves than T. diversifolia. The application of Listeria sp.-coffee bean skins resulted in the lowest soil P-retention, the highest soil P-available and P-content in arabica coffee seedlings leaves.

Index Terms: Andisol, arabica coffee seedling, organic matter, P content, phosphate providing microorganisms, P status.

1 Introduction

Bener Meriah district, Aceh Province, is one of the arabica coffee producing regions in Indonesia. Most arabica coffee in this district grown on Andisol. Andisol is a type of soil that developed from volcanic materials such as volcanic ash, pumice, cinder, lava, etc., and/or volcanoclastic materials, whose colloidal fractions are dominated by short range order mineral (allophane, imogolite, ferrihydrite) minerals or Al-humus complex [22]. Generally, Andisol has high P-retention (> 85%) and very low availability of P due to the large number of free Al-OH groups on the surface of the allophane [9]. Al-free is derived from Al-OH in the clay mineral structure of allophane, imogolite and Al in humus complex [25]. Free Al-OH group can dissociate or undergo protonation so that it can be both acidic and alkaline. In the form of acid ($Al\text{-}OH_2^+$) on the surface of clay minerals, it has a high activity to bind anions, particularly F^- and PO_4^{-3} [20]. High P-retention and low availability of P in Andisol cause the soil unable to supply. sufficient P for coffee plants. Phosphate providing microorganisms are microorganisms capable of providing P in a form that is available for plants, including phosphate solubilizing microorganisms and mycorrhiza. These microorganisms can be used to increase the availability of P in Andisol. Several studies on Andisol showed that the used of phosphate solubilizing microorganisms increased soil P-available [10], and increased solubility of P in the soil, growth and yield of corn [2]. While the other study showed that the used of arbuscular mycorrhiza in Andisol also increased soil P-available and P-retention of mustard [28]. Furthermore, increasing the availability of P in Andisol can also be done by using organic matters. In Bener Meriah district, there are several sources of organic matters that can be used, i.e. Tithonia diversifolia plants and coffee bean skins. Several studies have shown that the application of organic matters T. diversifolia can increase phosphate availability and phosphate reserves in the soil [12]. In Bener Meriah district, large amount of coffee bean skins can be found in coffee processing plants and can be the source of environmental pollution if not managed properly. The objective of this study was to examine the effect of the application of phosphate providing microorganisms and organic matters on P status in Andisol and P content in arabica coffee seedlings leaves in Bener Meriah district.

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2 Materials and Methods

2.1 Materials

The materials used in this study were: Andisol with content of P-available 3.41 ppm and P-total 221.2 ppm, P-retention 87%, weight of soil content 0.98 g cm^{-3}; phosphate providing microorganisms i.e. Glomus sp., Kurthia sp., Corynebacterium sp., and Listeria sp. isolated from the rhizosphere of arabica coffee roots; organic matters i.e. fresh T. diversifolia and coffee bean skins; arabica coffee seedlings of 6-month-old (Abesinia-3 varieties); Urea, SP 36, and KCl fertilizers.

2.2 Experimental design

The experiment was conducted in polybags in the screen house, using a randomized block design with six replications. The experiment consisted of two factors. Factor I is the application of phosphate providing microorganisms consisting of without microorganisms, Glomus sp., Kurthia sp., Corynebacterium sp., and Listeria sp. Factor II is the application of organic matters consisting of T. diversifolia and coffee bean skins. Each polybag was filled with 10 kg of Andisol as planting medium. Inoculation of phosphate providing microorganisms was done at the time of planting and inoculated in the planting hole. The application of Glomus sp.

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(arbuscular mycorrhiza) was done by adding 10 g of corn root containing spores to the roots of arabica coffee seedling. The application of Kurthia sp., Corynebacterium sp., and Listeria sp (phosphate solubilizing microorganisms) was carried out by adding 10 ml of nutrient broth containing each of these microorganisms to the roots of arabica coffee seedlings. The organic matters were applied two times. The first application was done two weeks before planting and the second application was done six months after planting. The doses of organic matters tested for each application was 20 tons ha^{-1} or 102 g $polybag^{-1}$. Basic fertilizer was applied two times. The first one was at the time of planting and the second one was six months after planting. Basic fertilizer doses used for one application were urea 20 g $polybag^{-1}$, SP36 25 g $polybag^{-1}$, and KCl 15 g $polybag^{-1}$ [23]. Watering was done every day in the morning to maintain at 75% field capacity. The plants were kept for nine months (up to 15 months).

2.3 Soil, plants, microorganisms and organic matters analysis

The parameters observed were: P status in Andisol (soil P-retention using Blakemore method and soil P-available using Bray II method); P-content in arabica coffee seedling leaves using H_2SO_4 extraction method) measured at the end of the experiment. The organic matters (T. diversifolia and coffee bean skins) were analyzed for C-organic using Walkley and Black method; N using H_2SO_4 extraction method; P using HCl extraction method. The organic acids produced by phosphate providing microorganisms using H_2O extraction method and organic matters using acetonitrile extraction method were analyzed using High Performance Liquid Chromatography.

2.4 Statistical analysis

The data obtained were analyzed using analysis of variance at 5% level with Statistical Product and Service Solution (SPSS) software version 22. The significant difference between treatment proceed with Duncan Multiple Range Test at 5% level.

3 Results and Discussions

3.1 Andisol P-retention due to the application of phosphate providing microorganisms and organic matters

The results of the variance analysis showed that the application of phosphate providing microorganisms and organic matters had a significant effect on P-retention. Table 1 shows that the application of phosphate providing microorganisms decreased Andisol P-retention. The application of Glomus sp., Kurthia sp., Corynebacterium sp., and Listeria sp., decreased P-retention in Andisol by 2.38, 5.12, 7.48 and 9.17% respectively, compare to without the application of microorganisms The four phosphate providing microorganisms used in the experiment had different ability in decreasing soil P-retention. This difference is thought to be due to the different type of phosphate providing microorganisms. This difference in the type of phosphate providing microorganisms caused the difference in the amount and type of organic acids produced by the phosphate providing microorganisms (Table 2).

Table 1. *Average Andisol P-retention due to the application of phosphate providing microorganisms and organic matters*

Phosphate providing microorganisms	Organic matters: T. diversifolia	Organic matters: Coffee bean skins	Average
	...........................%...........................		
Without microorganism	86.67	85.90	86.29 e
Glomus sp	85.00	83.47	84.24 d
Kurthia sp	82.33	81.40	81.87 c
Corynebacterium sp	80.33	79.33	79.83 b
Listeria sp	78.83	77.90	78.37 a
Average	82.63 b	81.60 a	

Note: *The numbers followed by the same letter on the same column and row, show no significant difference in the test at 5% level.*

Table 2. *Organic acids produced by phosphate providing microorganisms and organic matters*

Phosphate providing microorganisms and Organic matters	Organic acids: Suc-cinic	Propi-onic	Buty-ric	For-mic	Oxalic	Ci-tric	Ace-tic
	..............................ppm..............................						
Glomus sp	-	-	5.6	3.8	7.1	-	-
Kurthia sp	5.1	-	5.5	45.4	9.8	-	-
Corynebacterium sp	3.2	3.6	4.6	10.4	8.1	-	-
Listeria sp	3.6	4.4	4.8	13.4	7.7	4.6	-
T. diversifolia	-	-	-	-	-	-	1568
Coffee bean skins	-	-	-	-	-	-	1625

The highest P-retention decrease was found in the application of Listeria sp. because Listeria sp. produce succinic acids, propionic acids, butyric acids, formic acids, oxalic acids and citric acids. Two of these organic acids i.e. oxalic acids and citric acids are very strong and effective in decreasing P-retention [18], [3]. The difference in the type of organic acids caused different affinity in chelating P adsorption agents. Oxalic acids and citric acids had higher affinity than other organic acids in chelating P adsorption agents [3]. In addition, different type of microorganisms also caused attractive differences in microorganism cells against soil minerals. The cells of the microorganisms were attracted to the molecular nano because they had a negative/positive surface charge [14]. The relative surface charge of the cells bound with the clay mineral charge of the soil, thereby decreasing phosphate retention of soil minerals [15]. Table 1 also shows that the application of coffee bean skins resulted in lower Andisol P-retention than T. diversifolia due to the decomposition of coffee bean skins that produced more acetic acid than T. diversifolia. The coffee bean skins produced 1625 ppm acetic acid, whereas T. diversifolia produced 1568 ppm acetic acid. The acetic acid produced during the decomposition process was able to decrease Andisol P-retention. The other study showed that acetic acid, citric acid and malic acid were able to decrease Andisol P-retention [21]. Lower P-retention due to the application of coffee bean skins was because coffee bean skins had C/N ratio and C/P ratio higher than T. diversifolia. Coffee bean skins had C/N ratio of 22.61, whereas T. diversifolia had C/N ratio of 15.74. Coffee bean skins had C/P ratio of 144, whereas T. diversifolia had C/P ratio of 131.17. Higher C/N ratio and C/P ratio of coffee bean skins showed that the coffee bean skins took longer time in the decomposition process. The longer the decomposition process, the longer the acetic acid releasing process and the more acetic acid generated, subsequently the more Andisol P-retention decreasing. The decomposition of organic matter releases organic acids that function as chelating agents, and will bind to Al and Fe, resulting in phosphate release. The decomposition of organic matters will produce humic acid and fulvic acid which serves as the protection of sesquioxide by blocking the exchange site so that the binding of P will not occur [5].

3.2 Andisol P-available due to the application of phosphate providing microorganisms and organic matters

The results of variance analysis showed that the application of phosphate providing microorganisms and organic matters had significant effect on Andisol P-available. Table 3 shows that the application of phosphate providing microorganisms increased Andisol P-available. The application of Glomus sp., Kurthia sp., Corynebacterium sp., and Listeria sp increased Andisol P-available by 24.85, 36.03, 52.79, and 77.33% respectively.

Table 3. *Average P-available in andisol due to the application of phosphate providing microorganisms and organic matters*

Phosphate providing microorganisms	Organic matters		Average
	T. diversifolia	Coffee bean skins	
	………………….....ppm………………..…		
Without Microorganism	7.83	9.70	8.77 a
Glomus sp	10.57	11.33	10.95 a
Kurthia sp	11.73	12.13	11.93 b
Corynebacterium sp	13.00	13.70	13.40 c
Listeria sp	14.83	16.27	15.55 d
Average	11.61 a	12.63 b	

Note: *The numbers followed by the same letter on the same column and row, show no significant difference in the test at 5% level.*

Glomus sp., Kurthia sp., Corynebacterium sp., and Listeria sp. have different ability in increasing Andisol P-available. This difference in increasing P-available was due to the different type of phosphate providing microorganisms. This difference in the type of phosphate providing microorganisms caused different adaptation ability in the soil, the type of organic acids and the amount of organic acids produced. The ability of microorganisms in increasing soil P-available varied greatly depending on the type of microorganisms, adaptation ability, and the ability to produce organic acids and enzymes [13]. The different type of organic acids produced by microorganisms resulted in different quality and quantity in releasing phosphate [7]. The highest Andisol P-available was found in the application of Listeria sp. This was because Listeria sp. produced succinic acids, propionic acids, butyric acids, formic acids, oxalic acids and citric acids (Table 2). Several authors argued that the solubilization mechanism of P by microorganisms was related to the production of organic acids [17], [8], [19], [11], [4]. These organic acids served as catalysts, chelates, and absorbing agents complex [16]. Two of the organic acids produced by Listeria sp. i.e. oxalic acids and citrate acids are very strong in solubilizing P. The citric acid and oxalic are stronger than other organic acids in increasing the mobility of aluminum and iron, as well as solubilizing P through the retention competition and also the formation of Al-ligand and Fe-ligand complex compound [18], [3]. The effect of the application of organic matters showed a significant difference on Andisol P-available. The application of coffee bean skins resulted in higher Andisol P-available than T. diversifolia. The increasing P-available in Andisol soil due to the application of coffee bean skins was also because during the decomposition, coffee bean skins produced more acetic acid than T. diversifolia. Coffee bean skins produced 1625 ppm acetic acid, whereas T. diversifolia organic matters produced 1568 ppm acetic acid. The other study showed that the application of acetic acid, citric acid and malic acid increased the unstable phosphate fraction in Andisol [27]. Moreover, higher P-available due to the application of coffee bean skins was because coffee bean skins had higher C/P ratio than T. diversifolia. Coffee bean skins had C/P ratio of 144, whereas T. diversifolia had C/P ratio of 131.17. The higher C/P ratio of coffee bean skins indicated that coffee bean skins required longer decomposition process than T. diversifolia. The longer the decomposition process, the longer the acetic acid releasing process and the more acetic acid produced which served as catalyst, chelating agent, and complexation of P absorbing agent so that more Andisol P-available. The decomposition of coffee bean skins that took longer time, slowly and continuously will produce humus, carbon dioxide and energy. Humus can increase P-available by: the formation of phosphorus humus complex which is more available for plants, the exchange of phosphate anion from the adsorption site by humid anion and sulfate, the formation of chelating compound with Al^{2+}, Fe^{2+}, Ca^{2+}, and Mg^{2+} ions, thus neutralizing its reactivity on P. In addition, the carbon dioxide produced in the soil solution will form carbonic acid. This acid is able to increase P solubility [5]. The C/P ratio of coffee bean skins indicated that coffee bean skins required longer decomposition process than T. diversifolia to mineralize P compounds. During the decomposition process, mineralization will occur resulting in the releasing P-mineral (PO_4^{3-}) and increasing P-available in the soil. Decomposition of organic matters will also release various organic compounds containing phosphate such as phospholipids, phosphoric sugars, nucleic acids, nucleoproteins that will eventually increase P-organic in the soil [5].

3.3 The interaction effect between the application of phosphate providing microorganisms and organic matters on P-content of arabica coffee seedling leaves.

The results of variance analysis showed that the interaction between the application of phosphate providing microorganisms and organic matters had significant effect on P-content of arabica coffee seedling leaves. Table 4 shows that the interaction of the application of phosphate providing microorganisms and organic matters increase P-content in arabica coffee seedling leaves. The four phosphate providing microorganisms used in the experiment increased P-content of arabica coffee seedling leaves both on the application of T. diversifolia and coffee bean skins. These four phosphate providing microorganisms had different ability in increasing P-content in arabica coffee seedling leaves. The application of coffee bean skins resulted with higher P-content than the application of T. diversifolia only on the treatment without the application of phosphate providing microorganisms. The

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highest P-content in arabica coffee seedling leaves was found in the application of Listeria sp-coffee bean skins that were significantly different from all treatments, but was not significantly different from Listeria sp-T. diversifolia application.

Table 4. *Average P-content of arabica coffee seedling leaves due to the interaction between phosphate providing microorganisms and organic matters*

Phosphate providing microorganisms	Organic matters		Average
	T. diversifolia	Coffee bean skins	
	............................%..................................		
Without Microorganisms	0.25 a	0.29 b	0.27
Glomus sp.	0.32 c	0.34 d	0.33
Kurthia sp.	0.35 de	0.36 ef	0.36
Corynebacterium sp.	0.37 fg	0.37 fg	0.37
Listeria sp.	0.38 gh	0.39 h	0.39
Average	0.33	0.35	

Note: *The numbers followed by the same letter on the same column and row, show no significant difference in the test at 5% level.*

The different increase of P-content in arabica coffee seedling leaves was due to the different type of phosphate providing microorganisms. This difference in the type of phosphate providing microorganisms resulted in the difference in the types and amounts of organic acids produced (Table 2) thus causing difference in the ability to increase P-available in the soil (Table 3). This difference in P-available in the soil caused difference in the ability of the soil to provide and meet the needs of P-nutrient for arabica coffee which subsequently caused the difference in P-nutrient uptake by arabica coffee which will eventually cause different P-content in arabica coffee seedling leaves. Glomus sp., Kurthia sp., Corynebacterium sp., and Listeria sp. had the ability to solubilize P-unavailable to the form of P-available for plants. These four phosphate providing microorganisms produced organic acids (Table 2) that play an important role in increasing soil P-available. In addition, the increased Andisol P-available was also due to the mineralization of P-organic into phosphate as a result of phosphatase and phytase activities secreted by phosphate providing microorganisms [24], [6], [1], [26]. The application of coffee bean skins resulted in higher P-content in arabica coffee seedling leaves than the application of T. diversifolia only on the treatment without the application of phosphate providing microorganisms and Glomus sp. The higher P-content in arabica coffee leaves in the application of coffee bean skins because coffee bean skins produced more acetic acid than T. diversifolia resulting in higher P-available in the soil (Table 3). High P-available in the soil caused high P nutrient uptake so that P-content in arabica coffee seedling leaves was also high. The highest P-content in arabica coffee seedling leaves was obtained in the Listeria sp.-coffee bean skins treatment. This was because each application of Listeria sp.-coffee bean skins resulted in the highest soil P-available (Table 3).

4 Conclusion

1. The application of Glomus sp., Kurthia sp., Corynebacterium sp., and Listeria sp. decreased Andisol P-retention by 2.38, 5.12, 7.48, 9.17% respectively, increased Andisol P-available by 24.85, 36.03, 52.79,77.33% respectively, and increased P-content in the arabica coffee seedling leaves by 22.22, 33.33, 37.03, 72,27% respectively, compared to without the application of microorganisms.
2. The application of coffee beans skins resulted in lower Andisol P-retention, higher Andisol P-available and P-content in Arabica coffee seedling leaves than T. diversifolia.
3. The application of Listeria sp-coffee bean skins resulted in the lowest Andisol P-retention, the highest Andisol P-available and P-content in arabica coffee seedlings leaves.

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