Available online at:
http://journal.unila.ac.id/index.php/tropicalsoil
DOI: 10.5400/jts.2011.16.3.225

# Estimation of Available Phosphorus in Soil Using the Population of Arbuscular Mycorrhizal Fungi Spores

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Received 17 November 2010 / accepted 16 May 2011

## ABSTRACT

Soil microbes, such as arbuscular mycorrhizal fungi (AMF) have the ability to dissolve unavailable phosphorus (P) and they can be used as an indicator of the P availability in soil. The study was conducted on upland soil in East Java. The soil was sampled twice, before and after planting at the harvesting time. The population of AMF spores and soil P availability were observed. The AMF spores were isolated using wet sieving method, decanting, and followed by sucrose density gradient centrifugation. The available P was observed using the Olsen extraction. The numbers of AMF spore was corelated with available P, moreover the numbers of AMF spore was compared to the availabality of P. The results showed that the total number of AMF spores at six sites were ranged from a little to midle, and the available P ranged from low to high level. All soil site samples had a linear corelation between numbers of AMF spore and available P in soil. The greater the number of AMF spore, the higher the available P in soil. It was likely that the availability of P in soil can be predicted by the population of AMF spores in soil. Therefore, the number of AMF spore can be need as a biological method to predict the available P in soil and to make a recommendation the use of P fertilizer.

***Keywords***: Available P, biological method, correlation, number of AMF spores

## INTRODUCTION

The important role of arbuscular mycorrhizal (AM) fungi in the capture of nutrients from the soil of all ecosystems is wellknown. However, it is often neglected in the soil and crop management about the impact of AM fungi on fertilizer application quantitatively, especially phosphorus (P). Phosphorus is the second essential nutrient after nitrogen (N) and it is required for plant growth and found in soils in organic and complex inorganic forms. Due to its low solubility and mobility, plants cannot readily utilise P in an organic or complex inorganic form (Schachtman *et al*. 1998). Thus, AM fungi enhances nutrient uptake through the spread of extra radical hyphae into the surrounding soil and hydrolysing any unavailable sources of P (Ezawa *et al*. 2004). The function of AM fungi are to enhance the uptake of phosphorus from the soil, which is then translocated to the host plant through hyphal networks in the soil (Owuzu-Bennoah and Wild 1980).

The association of AM fungi with plant roots contribute the plant to take P nutrient requirements, so that mycorrhizal plants will be fulfilled its phosphorus element for plant growth until plant harvested (O'Keefe and Sylvia 1991). The positive impact of AM fungi on the soil is maintaining the soil fertility levels after crop harvest, especially in the availability of P, which is indicated by the population or the number of AMF spores which are greater after harvesring than before planting.

Effendy *et al.* (2006) studied that there are a positive relationship between the number of AMF spores with available P in Pujon, Malang and Bumiaji, Batu. Their research indicated that the greater numbers of spores content in soil were followed by the improving availability of P. The benefits of AMF spore population are its efficiency for P fertilization, that soil which has a greater number of AMF spores was not need a large quantities of P.

From these phenomenon, a study to predict the available P by the numbers of AMF spores in the soil were conducted in order to examine whether this method can be used as an alternative methods for determination of phosphorus in soil other than chemical analysis.

## MATERIALS AND METHODS

### Soil Sampling

The sampling soil were taken on dry land from six centre of horticultural plantation in East Java. Detail of the locations are shown in Figure 1 which were (1) Jember, in sub district Balung and Wuluhan, (2) Lumajang, in sub district Senduro and Pasrujambe, (3) Probolinggo, in sub district Lumbang and Sukapura, (4) Pasuruan, in sub district Tosari and Tutur, (5) Malang, in sub district Ngantang and Pujon, and (6) Magetan, in sub district Plaosan. The horticulture plants were dominated by Caisin (*Brassica chinensis*) and carrot (*Daucus carota*).

Soil samples from every district was sampled at top soil from five points for every location and then composited. Soil samples were taken twice, first was just before planting of first season, and second was after harvest or before planting at second season. Each soil sample was dried to be air dry soil, sieved with Ø = 1 mm siever and prepared for analysis of soil chemical and pysical properties, available P as well as for AMF spores isolation and identification.

### Soil and AMF Analysis

Soil analysis and observation of AMF spore population were conducted in soil fertility laboratory and biology laboratory in Faculty of Agriculture, University of Pembangunan National (UPN) “Veteran” East Java from May to August 2008.

The soil properties which are related to plant growth medium are (1) soil physics: soil texture bulk density (BD), and particle density (PD) (2) soils chemistry: pH, exchangeable bases, Cation Exchange Capacity (CEC), total P, available P, retention P, and (3) soils biology: organic C, total N, and ratio C/N (Sulaeman *et al*. 2005).

Data of AMF spores were correlated to the available P from each soil sample at 1st and 2nd sampling time, so that it can be known the basic relationship between AMF and available P which were expreseed by the value of correlation coefficient (r). From the two series, the relationship may have differences due to different in sampling time. The relationship between available P and number of AMF spores was also created by correlation equation.

Available phosphorus was analyzed using the Olsen method if the pH = 5.6 or more, and using the Bray methods if the soil pH is less than 5.6 (Bray 1948; Setijono 1996).

Spore density was expressed as total number of spores occurring in 100 g of soil. The determination of the number of AMF spores and its species was carried out by isolation and identification to genus level using wet sieving methods (Gerdemann and Nicolson 1963), decanting and followed by sucrose density gradient centrifugation and the spores were observed by using a stereozoom microscope (Daniels and Skipper 1982).

### Data Analysis

Data on AMF spores number and available P obtained from each site were analized by a regression test and a correlation analysis.

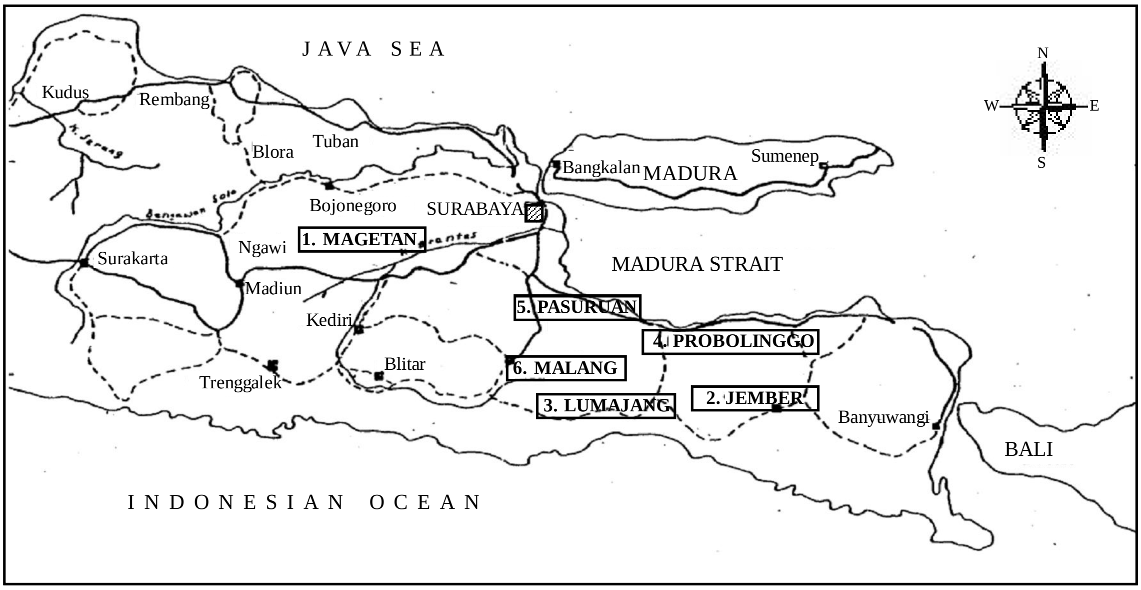


Figure 1. Map of six districts locations for sampling in East Java Province, Indonesia.

Table 1. Soil chemical and pysical properties of six districts in East Java.

| Soil properties | Location of soil sample in East Java | | | | | |
|---|---|---|---|---|---|---|
| | Malang | Magetan | Pasuruan | Probolinggo | Lumajang | Jember |
| $pH-H_2O$ | 5.9 | 5.6 | 6.0 | 6.1 | 6.2 | 6.2 |
| pH-KCl | 5.1 | 4.8 | 5.5 | 5.4 | 5.6 | 5.7 |
| pH-NaF | 10.60 | 10.82 | 10.78 | 10.70 | 10.66 | 10.80 |
| Organic-C (%) | 4.53 | 3.45 | 3.85 | 3.80 | 3.20 | 4.10 |
| Total-N (%) | 0.34 | 0.30 | 0.39 | 0.33 | 0.25 | 0.38 |
| C/N ratio | 13 | 11 | 9 | 11 | 12 | 10 |
| Total-P (mg $kg^{-1}$) | 1,212 | 1,175 | 1,874 | 1,415 | 1,311 | 2,459 |
| Available-P (mg $kg^{-1}$) | 30.13 | 23.55 | 72.67 | 39.05 | 25.91 | 37.52 |
| Retention-P (mg $kg^{-1}$) | 95.60 | 95.45 | 105.53 | 95.60 | 95.45 | 95.53 |
| CEC ($NH_4OAc$) (cmol $kg^{-1}$) | 32.24 | 35.17 | 41.74 | 32.24 | 35.17 | 41.74 |
| Ca (cmol $kg^{-1}$) | 7.18 | 6.59 | 9.30 | 7.18 | 6.59 | 9.30 |
| Mg (cmol $kg^{-1}$) | 1.27 | 0.85 | 0.63 | 1.27 | 0.85 | 0.63 |
| K (cmol $kg^{-1}$) | 0.86 | 0.94 | 0.38 | 0.86 | 0.94 | 0.38 |
| Na (cmol $kg^{-1}$) | 1.12 | 1.39 | 1.18 | 1.12 | 1.39 | 1.18 |
| Base Saturated (cmol $kg^{-1}$) | 32 | 28 | 29 | 32 | 28 | 29 |
| Texture | | | | | | |
| Sand (%) | 47 | 34 | 51 | 42 | 30 | 45 |
| Silt (%) | 40 | 46 | 41 | 44 | 40 | 37 |
| Clay (%) | 13 | 20 | 8 | 14 | 30 | 18 |
| Texture Class | Sandy loam | Clay | Sandy loam | Sandy loam | Clay | Sandy loam |
| BD (g $ml^{-1}$) | 0.92 | 0.98 | 0.96 | 0.92 | 0.98 | 0.96 |
| PD (g $ml^{-1}$) | 2.36 | 2.42 | 2.26 | 2.36 | 2.42 | 2.26 |
| Pores (%) | 53.0 | 52.0 | 52.0 | 53.0 | 52.0 | 52.0 |

## RESULTS AND DISCUSSION

### Characteristics of Soil Samples

The chemical and physical properties of soil samples from six district locations are presented in Table 1. The highest total P was at Jember District and the lowest at Magetan District. On the other hand, the highest available P were obtained from Pasuruan District and the lowest from Magetan District. Soil texture from six location were belonged to clay and sandy loam (Table 1).

### Numbers of AMF spores and Available-P in Soil

The number of AMF spores and available P in soil for each location are presented in Table 2. The dominan genus was *Glomus* sp. and the others genus were *Gigaspora*, *Acaulospora*, and *Scutellospora*.

### The Relationships between the Numbers of AMF Spore with Available-P

The relationships between the numbers of AMF spore and available-P (Table 2) are presented by regression equation at every district (Figure 2).

### Jember District

The relationships between the AMF spore numbers and available P in soil at Jember District are presented at Figure 2a and 2b.

At the 1$^{st}$ sampling time, the AMF spore numbers have a linear corelation with available P. The equation y = 0.105x - 6947 ($R^2$ = 0.49) with correlation coefficient (r = 0.70). For 2$^{nd}$ sampling time, the equation y = 0.056x + 8729 ($R^2$ = 0.49) with correlation coefficient (r = 0.70).

### Lumajang District

The relationships between the number of AMF spore and available of P in soils are presented in Figure 3a and 3b.

At the 1$^{st}$ sampling time the number of AMF spores have a linear corelation, the equation at Lumajang District y = 0.0836x + 9.4767 ($R^2$ = 0.45) with correlation coefficient (r = 0.67). For 2$^{nd}$ time sampling the equation y = 0.082x + 7.6714 ($R^2$ = 0.30) with correlation coefficient (r = 0.54).

## Probolinggo District

The relationships between the number of AMF spore with available of P in soil at Probolinggo District are presented in Figure 4 a and 4b.

At the 1st sampling time, the number of AMF spores have a linear correlation with available P, the equation y = 0.137x + 0.8191 ($R^2$ = 0.59) with correlation coefficient (r = 0.77). For 2nd sampling time the equation y = 0.0537x + 17.94 ($R^2$ = 0,51) with correlation coefficient (r = 0.72).

## Pasuruan District

The relationships between the number of AMF spores and available -P in soil at Pasuruan was presented in Figure 5a and 5b.

At the 1st time sampling the AMF spore number have a linear corelation with available P, the equation y = 0.2651x – 16.187 ($R^2$ = 0,72) with correlation coefficient (r = 0.85). For 2nd sampling time, the equation: y = 0.0531x + 26.838 ($R^2$ = 0.32) with correlation coefficient (r = 0.57).

## Malang District

The relationship between the number of AMF spores and available P in soil at Malang District are presented in Figure 6a and 6b.

At the 1st sampling time the AMF spore number have a linear correlation with available P, the equation y = 0.1084x + 2.5628 ($R^2$ = 0.49) with correlation coefficient (r = 0.71), and at 2nd time

Table 2. Numbers and kind of AMF genus and available P from six districts at the first and the second season.

| District | AMF genus | 1st season | | 2nd season | |
|---|---|---|---|---|---|
| | | AMF Spore numbers ($g^{-1}$ soil) | Available-P ($P_2O_5$) (mg $kg^{-1}$) | AMF Spore numbers ($g^{-1}$ soil) | Available-P ($P_2O_5$) (mg $kg^{-1}$) |
| Jember | *Glomus* sp. | 161 | | 130 | |
| | *Gigaspora* | 25 | | 30 | |
| | *Acaulospora* | 18 | | 22 | |
| | *Scutellospora* | 29 | | 35 | |
| | Total | 233 | 17.65 | 216 | 19.19 |
| Lumajang | *Glomus* sp. | 137 | | 151 | |
| | *Gigaspora* | 24 | | 35 | |
| | *Acaulospora* | 19 | | 25 | |
| | *Scutellospora* | 27 | | 40 | |
| | Total | 207 | 26.83 | 251 | 26.68 |
| Probolinggo | *Glomus* sp. | 117 | | 208 | |
| | *Gigaspora* | 20 | | 48 | |
| | *Acaulospora* | 17 | | 35 | |
| | *Scutellospora* | 28 | | 55 | |
| | Total | 182 | 25.21 | 346 | 36.72 |
| Pasuruan | *Glomus* sp. | 107 | | 161 | |
| | *Gigaspora* | 18 | | 38 | |
| | *Acaulospora* | 15 | | 27 | |
| | *Scutellospora* | 25 | | 43 | |
| | Total | 165 | 27.41 | 269 | 41.10 |
| Malang | *Glomus* sp. | 119 | | 145 | |
| | *Gigaspora* | 19 | | 34 | |
| | *Acaulospora* | 17 | | 24 | |
| | *Scutellospora* | 28 | | 39 | |
| | Total | 183 | 23.46 | 241 | 38.46 |
| Magetan | *Glomus* sp. | 194 | | 162 | |
| | *Gigaspora* | 32 | | 38 | |
| | *Acaulospora* | 27 | | 27 | |
| | *Scutellospora* | 45 | | 43 | |
| | Total | 298 | 28.52 | 270 | 25.28 |

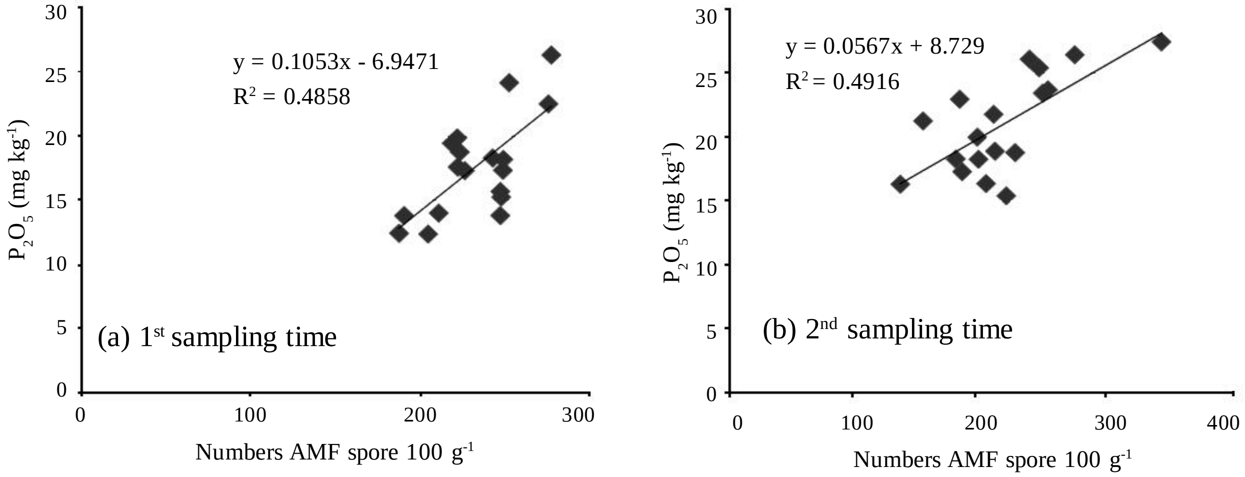


Figure 2. The relationship between the number of AMF spores and available P at (a) 1st sampling time and (b) 2nd sampling time at Jember District.

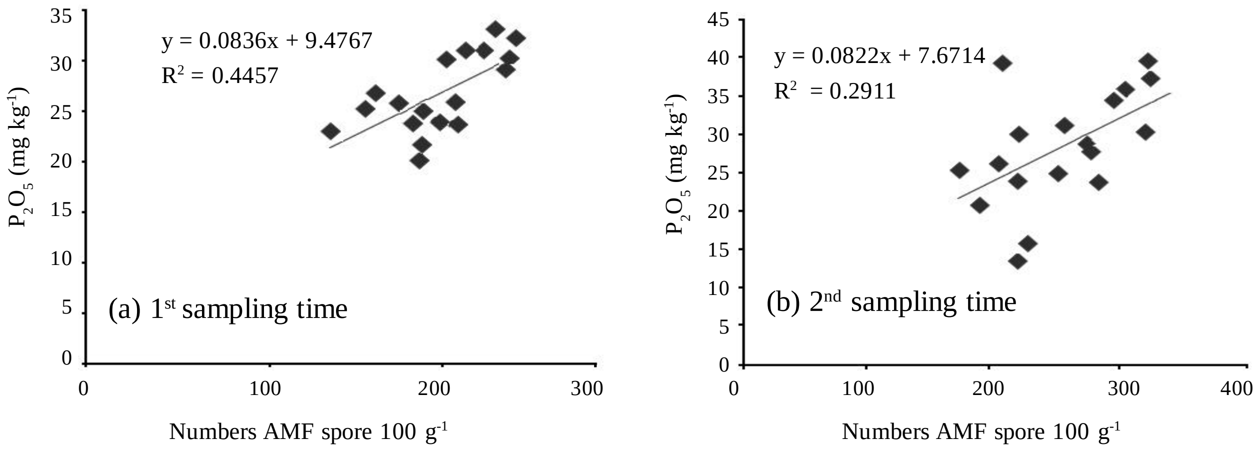


Figure 3. The relationship between the number of AMF spores and available P at (a) 1st sampling time and (b) 2nd sampling time at Lumajang District.

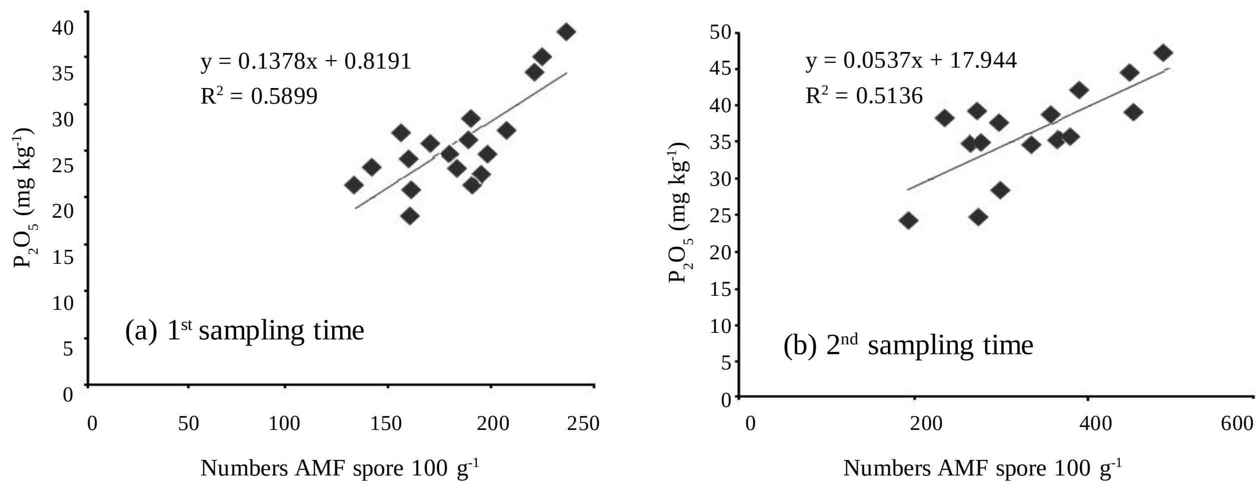


Figure 4. The relationship between the number of AMF spores and available P at (a) 1st sampling time and (b) 2nd sampling time at Probolinggo District.

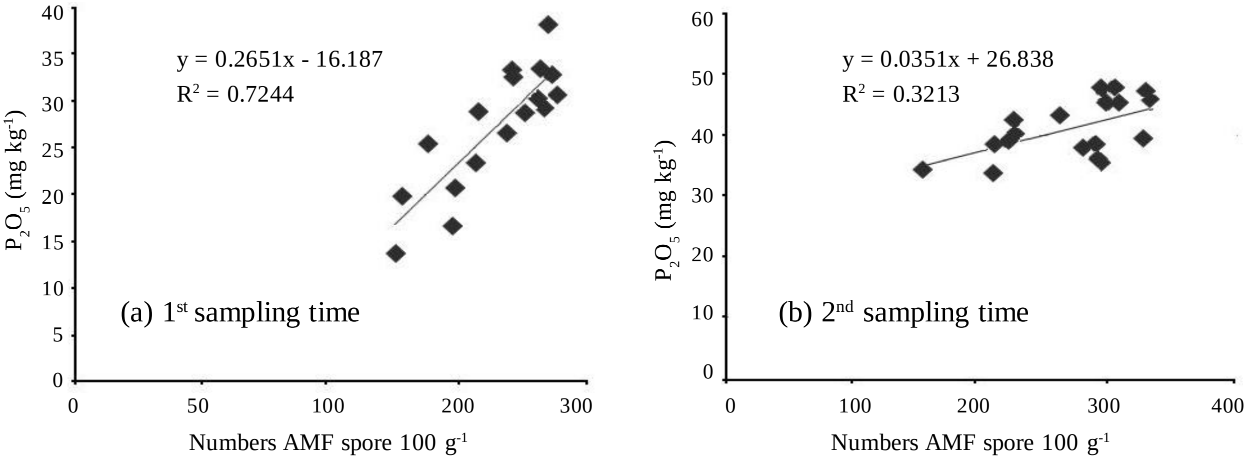


Figure 5. The relationship between the number of AMF spores and available P at (a) 1st sampling time and (b) 2nd sampling time at Pasuruan District.

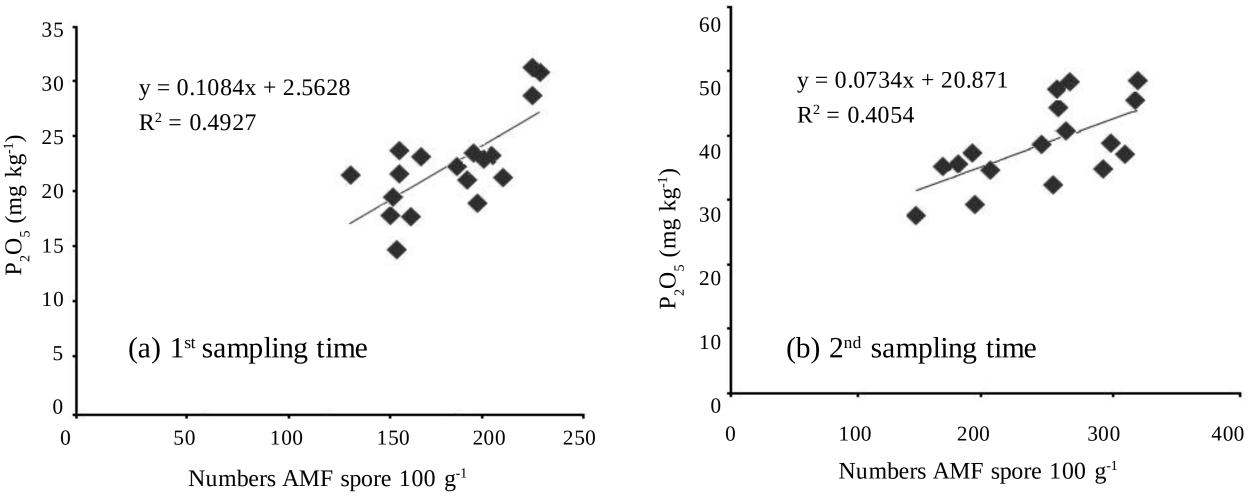


Figure 6. The relationship between the number of AMF spores and available P at (a) 1st sampling time and (b) 2nd sampling time at Malang District.

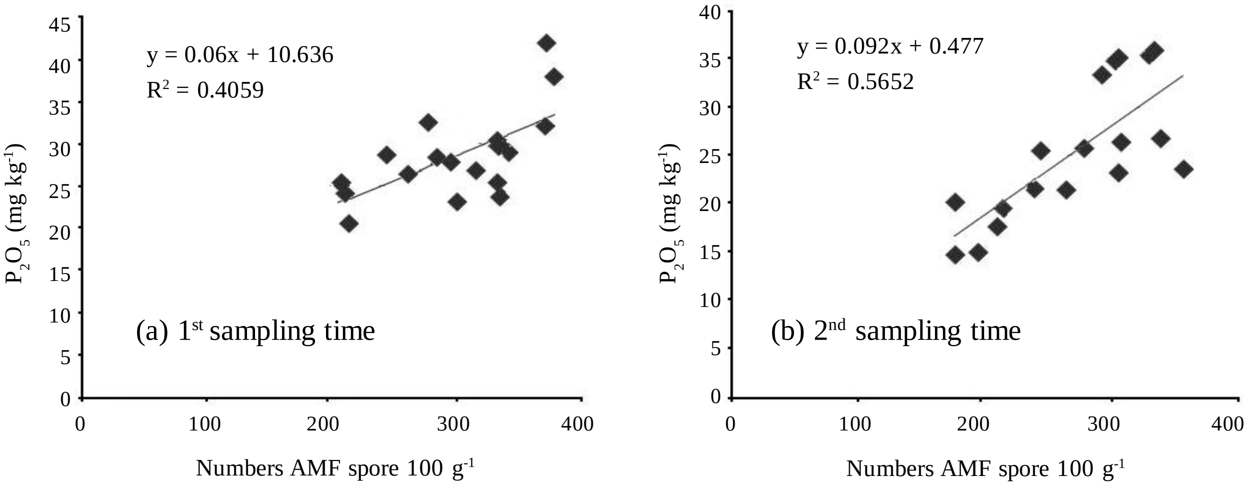


Figure 6. The relationship between the number of AMF spores and available P at (a) 1st sampling time and (b) 2nd sampling time at Magetan District.

sampling also have a linear correlation with the equation: $y = 0.0734x + 20.871$ ($R^2 = 0.41$) with correlation coefficient ($r = 0.64$).

### Magetan District

The relationships between the AMF spore number and available P in soil at Magetan District are presented in Figure 7a and 7b.

At the 1st time sampling the AMF spore number have a linear correlation with available P, the equation: $y = 0.06x + 10.636$ ($R^2 = 0.41$) with correlation coefficient ($r = 0.64$). For 2nd sampling time, the equation: $y = 0.092x + 0.477$ ( $R^2 = 0.56$) with correlation coefficient ($r = 0.75$).

### Discussion

The numbers of AMF spore in the six soil samples varied and its equivalent to $P_2O_5$ was made (Table 3). The number of AMF spores increasing linearly at the six sample locations were by followed the phosphorus content in soil P. There patterns that can be generally accepted, especially on soils such as Andisols. From the Figures 7 each soil at least has a total population of AMF 100 spores equivalent to the available P between 8-12 mg $kg^{-1}$ $P_2O_5$, or every 10 AMF spores equivalent to approximately 1 mg $P_2O_5$ $kg^{-1}$. This pattern is also likely to occur in areas with soil not like AndisolsJember planted vegetables during one year of planting. Means the existence of the number of AMF spores in soil can be used as a tool to predict quantitatively the availability of P (Barea 1991).

Phosphorous is classified as a macro nutrient and it is required by plants and a relatively fewer compared to others such as C, H, O, N and K. Because phosphorus plays an important role in plant metabolism, its small amount has been provided for soil availability of the land which contributed by mycorrhizal fungi that can ensure the needs of plants to grow well (Setijono 1996). The low availability of P in the soil, among others, due to the fixation of P by binding components in the soil. Mycorrhizae seems to reduce the fixation process and to maintain the availability of P as indicated by the increasing number of AMF spores in the soil will be followed by the increasing of available P (Bieleski 1973; Effendy *et al*. 2006).

The results of this study indicated that the function of AMF in supplying P was mainly derived from unavailable P in the soil. So the impact on the sustainability of the P availability was all times during plant growth (Effendy 2010). The arbuscular mycorrhizal fungi population was always available because of the accumulation of P in soil from P fertilizer residues which were always applied at each planting season, and it was known that only about 30% of P can be absorbed by plants, and the rest becomes solids-P (secondary mineral). The phosphorus in solids form are low in solubility, to be dissolved and to be available for plants by P solubilizing microbes such as bacteria, fungi, and mycorrhizal fungi works well as a translocator of phosphorus by hyphae in soil and roots (Barea 1991; Bieleski 1973). With this potential it is expected to reduce the amount of applied P fertilizer, so it can save the cost of managing the plant. It has been proved by research at Pujon in Malang, that the AMF spore populations can save 60 kg $P_2O_5$ $ha^{-1}$ of the optimum P fertilizer recommendation of 90 kg $P_2O_5$ $ha^{-1}$. On soil that has given doses of 30 kg $P_2O_5$ $ha^{-1}$ or equal to 100 kg SP36 enough to obtain optimum production (Effendy *et al*. 2007).

## CONCLUSIONS

The population of arbuscular mycorrhizal fungi (AMF) on six soil sampling locations varied from 135 AMF spores per 100 g of soil (small number) until 497 spores per 100 g soil (greater number). None has higher number of spores (more than 500 AMF spores per 100 g soil). While the content of $P_2O_5$ were in the range of low (14 mg $kg^{-1}$ $P_2O_5$) to very high (more than 35 mg $kg^{-1}$ $P_2O_5$).

Table 3. Distribution of AMF spore population and its equivalent to the content of $P_2O_5$ in the soil.

| District | 1st sampling time (May 2008) | | 2nd sampling time (Juli 2008) | |
|---|---|---|---|---|
| | AMF spore numbers | Equivalent to $P_2O_5$ content (mg $kg^{-1}$) | AMF spore numbers | Equivalent to $P_2O_5$ content (mg $kg^{-1}$) |
| Jember | 188-278 | 12-26 | 135-342 | 16-27 |
| Lumajang | 143-242 | 20-33 | 170-323 | 14-40 |
| Probolinggo | 132-236 | 18-38 | 198-497 | 25-47 |
| Pasuruan | 125-188 | 14-38 | 153-331 | 34-48 |
| Malang | 134-227 | 18-31 | 145-318 | 28-49 |
| Magetan | 206-376 | 21-42 | 175-356 | 15-35 |

Almost all sampling sites hade correlation between the number of AMF spores and the availability of P. AMF population equivalent to 100 spores can contribute to the provision of P between 8-12 mg kg$^{-1}$ $P_2O_5$, or 10 spores of AMF equivalent to 1 mg kg$^{-1}$ $P_2O_5$.

This result gives the sense that the availability of P might be expected by using the number of AMF spores in soil. This method gives a hope to be implied as an alternative method to be applied in prediction of available P using AMF spore population. The benefits of this method can be implementing to provide recommendations in the provision of a more efficient P fertilizer application. It is necessary to continue the research to the treatment levels of P fertilizer using corn as indicator plants which will be inoculated and un-inoculated by AMF.

## ACKNOWLEDGEMENTS

We thank to the Indonesian Directorate General of Higher Education (DGHE), Ministry of National Education Republic of Indonesia which has provided funding to conduct fundamental research for Fiscal Year 2008.

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