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The Diversity of Arbuscular Mycorrhiza Fungus (AMF) Indigenous in Peanuts (*Arachis Hypogea L)* Rhizosphere under Different Elevation

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ABSTRACT

Arbuscular Mycorrhiza Fungus (AMF) is a type of soil microorganisms with obligate symbiotic characteristic. It can associate with high-level plants at the rate of 90%. Its association level highly depends on the type of AMF and the host plant. The lack of information about the AMF diversity in an ecosystem, and the insufficient number and types of isolates available, are limiting factors for the widespread use of AMF. It was a survey and observation research. In this research AMF indigenous potentials were observed in soil and roots of peanuts. Samples were taken from area with different elevation: low, medium, and high in West Sumatra. The study reveals that the highest number of AMF indigenous spores in peanuts rhizosphere from area with different elevation: low, medium, and high are dominated by *Glomus* sp *1* (159 spores), *Acaulospora* sp*1* (110 spora) and *Glomus* sp*2* (82 spores), however AMF indigenous with the highest percentage of existence is *Glomus* sp*1* and *Acaulospora* sp*1* (100%). In addition, the infectious level of AMF indigenous on roots of peanuts are 81.1%, 64,4% and 78,9% on low, medium and high elevation, respectively. The highest number of population and infectious level are on low elevation whereas the lowest number is on high elevation. Thus, the elevation level correlates with the type of infection, the size of population, and the percentage of infection.

Keywords: Diversity, elevation, mycorrhiza, peanuts rhizosphere

ABSTRAK

Fungi Mikoriza Arbuskular (FMA) merupakan salah satu jenis mikroorganisme tanah yang bersifat simbiotik obligat. Mikoriza ini dapat berasosiasi dengan tumbuhan tingkat tinggi sekitar 90%. Hanya saja tingkat efektifitas sangat tergantung kepada jenis FMA itu sendiri dan jenis tanaman inangnya. Kurangnya informasi tentang keanekaragaman FMA pada suatu ekosistem, dan kurangnya jumlah dan jenis isolat yang tersedia, merupakan faktor pembatas penggunaan FMA secara luas. Potensi FMA *indigenous* diamati pada tanah dan akar tanaman kacang tanah, sampel diambil pada lahan dengan elevasi berbeda yaitu; dataran rendah, sedang, dan tinggi yang berada di wilayah Sumatera Barat. Hasil penelitian menunjukkan jumlah spora FMA *Indigenous* yang terbanyak pada *rizosfir* tanaman kacang tanah pada dataran rendah, dataran sedang dan dataran tinggi, didominasi oleh jenis *Glomus* sp1 (159 spora), *Acaulospora* sp1 (110 spora) dan *Glomus* sp2 (82 spora), sedang FMA Indigenous yang memiliki persentase keberadaan tertinggi adalah jenis *Glomus* sp1 dan *Acaulospora* sp1 (100%). Hasil pengamatan infeksi FMA indigenous terhadap akar tanaman kacang tanah; pada dataran rendah 81,1%, dataran sedang 64,4% dan pada dataran tinggi 78,9%. Populasi dan persentase infeksi FMA yang tertinggi terdapat pada dataran rendah sedangkan pada dataran sedang menunjukkan yang terendah. Dengan demikian elevasi yang berbeda memberikan pengaruh yang berbeda pula terhadap jenis, jumlah populasi dan persentase infeksi.

Kata kunci : Elevasi, mikoriza, keanekaragaman, kacang tanah

INTRODUCTION

Arbuscular Mycorrhiza Fungus (AMF) is a type of soil microorganism with obligate symbiotic characteristic. Mycorrhiza can associate about 90% with high level of vegetation. Its effectiveness level depends on the type of the AMF and its host plant (Muas 2006). In general, there are two types of mycorrhiza in tropical region: Mycorrhiza Vesicular-Arbuscular (MVA)/ Endomycorrhiza and Ectomycorrhiza. Generally, this type of fungus is in the group of *Ascomycetes* and *Basidiomycetes* (Pujiyanto 2001).

Arbuscular Mycorrhiza Fungus can be found almost in all types of soil (Smith and Read 1997) and quite diverse. The diversity of AMF is largely based on type of AMF in ecosystem. The lack of information about the AMF diversity, and lack of number and types of isolates available restrain the use of AMF.

The main benefit of Arbuscular Mycorrhiza Fungus is to increase land productivity and crop yield. Previous research found that AMF can help in absorbing P nutrient, improving the resistance of plants to drought, pests, and diseases, synergistic with other microbes, playing an important role in the nutrient cycle, and improving the ecosystem stability as part of sustainable agriculture.

The following are the mechanism of Arbuscular Mycorrhiza Fungus in absorbing elements of P. First AMF spores around plant roots, germinate and infect the plant roots. Then, the spores grow to form long branch hypa. The hypha network has a wider coverage area than the plant roots. This AMF hypha acts as the root of the plant in absorbing water and nutrients in the soil (Anonim 2008).

P nutrient elements have an important role in developing and increasing peanut yield. P elements together with organic fertilizers such as manure and compost are required to generate peanut pods and seed filling.The P nutrient for peanuts can be made available by exploiting *Arbuscular Mycorrhiza Fungus* (AMF) that can help in absorbing P elements.Therefore, AMF could be an alternative technology to help the growth and increase the quality and the productivity of plant especially on marginal lands that are less fertile (Pulungan 2013).

The aims of the research were: 1). To investigate the amount and types of indigenous *Arbuscular Mycorrhiza Fungus* (AMF) on peanut rhizosphere at various elevation; 2).To analysethe diversity of different types of indigenous *Arbuscular Mycorrhiza Fungus* (AMF) on peanut rhizosphere at different elevation. The research can contribute to the development of biotechnology and soil microbes, and also help the government in the provision of organic fertilizer towards sustainable agriculture.

MATERIALS AND METHODS

Study Site

The research employed survey methods and observation of mychorriza at laboratory. The substances that were observed were roots and soils in peanuts rhizosphere on different elevations from farms at West Sumatra; Kasang in Pariaman Regency (lowlands area, altitude 7 meters above sea level), the region of Purwajaya in Limapuluh Kota (plains area, altitude 513 above sea level), and Tanjung Alam Bukittinggi (highland area elevation, altitude 915 meters above sea level).

Samples of plant roots and soils around rhizhosphere area were taken at 3 plants spot randomly at each elevation. The criteria of samples was that, the poods have been formed and they were taken by digging the soil at 0-30 centimeters depths.

The observation variables covered the types of mychorriza, the number of spores in each type and the level of infected roots by AMF. The identification and determination of mycorrhiza types according to *"Manual for Identification of Mychorriza Fungi"* by Schenek and Peres (1990) were based on spore form, spore dimension, surface ornament of spore, and spore color in PVLC and Melzer's.

Extracting Methods and Calculation

The AMF was isolated by extracting spores technic in casting and straining way according to Pacioni's method in Yassir and Maulana (2006). 50 grams of rhizosphere soils were mixed in 500 ml aquades and stirred till granular soils suspended, then filtered in a set of strainer with different dimensions of 500, 250, 125, and 63 mesh. The suspended soil which filtered in 125 and 63 mesh was taken in order to continue in centrifuge technic based on Brundrett *et al.* method (1996) in Yasir and Mulyana (2006). 30 ml aquades were added to filtrate to be centrifuged in a speed 200 rpm for five minutes. Then, pellet was added in to 80% of sucrose solution 15 ml to be centrifuged once more in a speed of 200 rpm for a minute. The result of centrifuge put in petri dish in order to observe and identify the AMF type using microscope.

The number of AMF colony in roots of peanut rhizosphere was calculated according to *Most Probable Numbe /MPN* method (Porter 1979). The obtained spores from centrifuged result were observed its density by counting the spore number. The result is the number of spores found in each 50 grams of soil.

The infected root by AMF was observed by modified coloring roots method (Kormanik and Mc. Graw 1982; Nusantara *et al.* 2012). The observation was done systematically by taken pieces of fiber root in 10 cm length and colored it with 0.05% Lactofenol Trypan Blue, then it was arranged on preparation glass to observe each of them by microscope. Based on Giovannetti and Mosse (1980) in Utaminingsih (2011), each of fiber roots which are infected showed colonization signs: occurred hypae, vesicular, arbuscular, chlamydospores, and spores. The percentage of infected root was calculated with the following formula (Kormanik and McGraw 1982; Tushar and Satish 2013).

$$\text{The infected root (\%)} = \frac{\text{The number of infected root}}{\text{The number of observed root}} \times 100\%$$

The data from identification results (types, the number of types, existence and were tabulated in table form and analysed in descriptive way.

RESULTS AND DISCUSSION

The diversity of the AMF indigenous

Arbuscular Mycorrhiza Fungus Indigenous in peanuts rhizosphere on lowlands (Pariaman), plains (Limapuluh Kota), and highlands (Bukittinggi) showed diverse results (Table 1).

Three types of Arbuscular Mycorrhiza Fungus indigenous that were found in peanuts rhizosphere in the three research sites were *Glomus* (3 species), *Acaulospora* (2 species) and *Sclerocystis*. In addition, the number of spores in each type of AMF indigenous was also diverse.The type of AMF indigenous, *Glomus* sp.1,had the most number of spores, 159 spores per 50 grams soil, and also owned the highest percentage of frequency presence (100%). On the other hand, *Glomus* sp.2 and *Glomus* sp3 had less number of spores and less percentage of frequency presence than *Glomus* sp.1, 82 and 18 spores; 67% and 11% respectively. Further more, the AMF type *Acaulospora* sp.1 was found to have the second most number of spores after *Glomu* sp.1, 110 spores per 50 grams soils with the number of frequency presence reaching almost 100% rather than *Acaulospora* sp.2 (32 spores; 33%). The last type of AMF indigenous *Sclerocystis* sp just had 12 spores and the number of frequency presence was 33%.

The peanuts which were grown up in lowlands, plains, and highlands had the AMF indigenous *Glomus* sp.1 and *Acaulospora* sp1, while *Acaulospora* sp.2 was only found in plains and *Sclerocystys sp.* in highlands alone. Some literatures suggested that *Sclerocystis* sp. was already extinct yet the result said *Sclerocytis* sp. still existed and was only found in peanuts plant area at a highland. Overall, peanuts can be symbiotic with some of AMF indigenous naturally.

Based on the study results (Table 1), that the existence of *Glomus* sp was more dominant than other types of AMF indigenous. This is in accordance with the opinion of the Ragupathy and Mahadevan, (1991) who stated that *Glomus* sp is more adaptable than other genus in wide range of environmental circum stances. According to Clark (1997), the ability of a species to live in an environment is heavily influenced by the species adaptation to the environment. On the other hand Fitter *et al.* (2004) argued that AMF existence is closely connected with ecological changes, and biotic and abiotic component factors. They also stated that there is a possibility that certain species have very specific ecological niches as well as other organisms. Consequently, the diversity of the AMF communities may be very sensitive to environmental change.

Smith and Read (1997), found that the amount of species *Glomus*, *Acaulospora*, *Scutelospora* on

Table 1. The number of spores, the types of spores, and the frequency of presence of AMF indigenous at different elevations.

Type of AMF Indigenous	The Number of Spores AMF (50 gram)$^{-1}$ in Air-Dried Samples									Total of Population	The Frequency of Existence (100%)
	Lowlands			The Plains Area			The Highlands				
	1	2	3	1	2	3	1	2	3		
Glomous sp1	25	17	26	19	15	17	15	12	13	159	100
Glomous sp2	-	10	12	21	-	-	17	11	11	82	67
Glomous sp3	-	-	-	-	18	-	-	-	-	18	11
Acaulospora sp1	11	10	17	11	11	11	14	14	11	110	100
Acaulospora sp2	6	9	17	-	-	-	-	-	-	32	33
Sclerocystis sp	-	-	-	-	-	-	5	3	4	12	33
∑ Spores	42	46	72	51	44	28	51	40	39		
∑ Type	3	4	4	3	3	2	4	4	4		
Infection of The Root (%)	86,7	83,3	73,3	66,7	63,3	63,3	86,7	76,7	73,3		

critical land were more than species *Entherophospora*. It has different results on cocoa rhizosphere in West Sumatra due to different environmental conditions. The diversity of the AMF does not depend on the plant species, but much depends on other factors, such as soil characteristics. Moreover Smith and Read (1997) stated that environmental factors can affect the host plant, which will eventually affect the AMF as its symbiosis.

A comparison of the Number of Species, Spores, and Infection of the Roots

The analysis results on the number of species, the individual number and the average percentage of peanut root infection by mycorriza in each location are described in Table 2. Based on the table, highlands has the highest number of types of AMF Indigenous (4 types) followed by lowlands with 3-4 types, and the lowest number of types AMF Indigenous is on lowlands (2-3 types).

The highest average number of individual spores was found on lowlands with 159 spores, while on highlands and plains areas were found 128 and 123 spores, respectively. There is no relationship between the number of types of spores and the number of spores in each location based on the results.Many number of spore types have not necessarily been able to provide the large number of spore individuals too, and vice versa.

The difference in the number and types of AMF on the three elevation level is influenced by management practices and soil fertility. On agricultural land, aquaculture activities such as tilling, fertilizing and application of pesticides can cause excessive damage on land systems. Damage on land system leads to low diversity of species of microorganisms. Munyanziza *et al.* (1997) stated that the conversion of forest into farm land can reduce the diversity of types and the number of fungus propagules, due to the change in plant species, the amount of organic matter generated, and nutrient content.

The highest percentage of indigenous AMF infection in peanut rhizosphere occured on the lowlands with an average infection of 81.1%, while the lowest percentage of infections in peanut rhizosphere (64.4%) was found on plains area. The effectiveness of mycorrhiza differs on each plant and environmental condition (Zarate and Cruz 1995). In extend, Chalimah *et al.* (2007) and Ulfa *et al.* (2011) stated that the ability of AMF to infect root fibre depends on the level of infectivity and effectiveness from every symbiosis between the host plants and AMF.

The difference in infection level is suspected due to the difference in plant growth. Peanuts wich were grow on lowland are likely to provide enough carbohydrate as the result offotosintat for the needs of the AMF Indigenous.

Our study (Table 2) reveals that the more the number of AMF is the higher the percentage of infection on peanuts, and vice versa, the less the number of individual from different AMF is the lower the percentage of infection on root. It indicates that there is a positive relationship between the number of spores with the level of infection of AMF indigenous on peanut roots.

On lowlands, high average number of spores leads high level of root infection by AMF indigenous (160 spores and root infection of 81.1%). This phenomenon can be caused by high temperature and high humidity on lowland area that encourages AMF, as a type of fungus, to grow well compared to plain and highland areas. Temperatures are highly correlated with elevation, the lower the elevation the higher the temperature.

Atmaja and Dana (2001) stated that the growth of mycorrhiza is heavily influenced by environmental in which the relatively high temperatures will increase the activity of fungus. In wet tropical area, this is beneficial. The process of formation of the AMF germination through three phases namely Spore Germination in soil, penetration of Hypha into cells of the roots and development of the Hypha in the root context. Optimum level of temperature for spore germination varies greatly depending on the type. Some of *Gigaspora* isolated from soil in Florida (subtropical) germinate well at the temperature of 34°C, while the species *Glomus* originating from the cold region needs 20°C to germinate at optimum level. The process of penetration and germination of Hypha into roots are also sensitive to the temperature of the soil.

Table 2. The number of species, the population of infection and mychorriza in peanut root.

Sample Location	Types Number	Spores Number	Infection Average (%)
Lowlands	3-4	160	81,1
The Plains Area	2-3	123	64,4
The Highland	4	128	78,9

CONCLUSIONS

The most numbers of spores in peanut rhizosphere on three different elevations were dominated by *Glomus* sp.1, *Acaulospora* sp.1, and *Glomus* sp.2 (159, 110, and 82 spores). In addition, *Glomus* sp.1 and *Acaulospora* sp.1 have the highest percentage of existence wich is 100%. On the lowlands there are 3-4 types of AMF Indigenous with the highest individual number of spores of 160 spores, and there are 4 types of AMF Indigenouson highlands area with only 128 spores. *Acaulospora* sp.2 is only found in the lowland area mean while *Sclerocystis* sp. is found only on the highland area. AMF Indigenous on lowlands shows the best effective spores with the highest infection rateof 81.1%, whereas the lowest infection rateoccurred on the plains area (64.4%).

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