

# Root colonization by arbuscular mycorrhizal fungi (AMF) in various age classes of revegetation post-coal mine

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**Abstract.** *Salim MA, Budi RSW, Setyaningsih L, Iskandar, Wahyudi I, Kirmi H. 2020. Root colonization by arbuscular mycorrhizal fungi (AMF) in various age classes of revegetation post-coal mine. Biodiversitas 21: 5013-5022.* This study aims to evaluate the status of colonization of the roots of the host plant in various age classes of revegetation of post-coal mining land associated with AMF spores populations and soil fertility. The study was conducted in the post-mining revegetation land at PT. Berau Coal, East Kalimantan. Isolation of the spores was carried out using the wet-sieving and centrifugation method, while the colonization of the roots was done by the root coloring technique. Result showed that revegetation activities were able to increase the content of some nutrients in the soil. The AMF population showed tendency an increase along with the increasing revegetation age classes. Eight-year revegetation age classes had the highest average number of spores. Plant species and understorey have shown the existence of AMF colonization with different values for each species. AMF root colonization had a negative relationship with the number of spores in the soil. The C-organic, N-total, CEC Ca, Mg and Fe contents had a positive correlation with AMF root colonization, while available P, total P, K, and Al had a negative correlation with AMF root colonization. Mg was significantly correlated ( $r = 0.861$ ) to AMF root colonization.

**Keywords:** AMF, revegetation, root colonization, spores, soil nutrient

## INTRODUCTION

Post-mining land reclamation activities need to be carried out to restore land productivity (Mushia et al. 2016) as same as the condition before mining activities. Revegetation is one of the activities undertaken after post-mining land reclamation activities. Revegetation is an effective way to reduce soil erosion and protect the soil during the reclamation process (Sheoran et al. 2010). The success of revegetation is greatly supported by the presence of soil microorganisms, especially Arbuscular Mycorrhizal Fungi (AMF). AMF plays an important role in ecosystem sustainability, soil ecological balance, plant development, and biodiversity maintenance (Ezeokoli et al. 2019; Krüger 2017; Bi et al. 2018).

AMF is one of microorganism that has a very important role in almost every ecosystem (Chairul et al. 2019; Pinos et al. 2019), especially in post-mining lands (Krüger et al. 2017). AMF can make the relationship between the abiotic and biotic components of the ecosystem (Kumar et al. 2010). AMF is very common in disturbed land and shows a positive role both in the growth and development of plant communities. AMF is the key to the success of the restoration process of soil ecological functions (Ezeokoli et al. 2019) and rehabilitation processes (Gosling et al. 2016)

especially on degraded lands including mined land. AMF is one of the potential biotechnologies to enhance and support the success of revegetation and revegetation on degraded lands (Asmelash et al. 2016).

Generally, post-mining land has low fertility, which is characterized by poor physical, chemical, and biological properties of the soil (Mushia et al. 2016), thus causing suboptimal plant growth. The presence of AMF on coal-mining land can help in plant growth and the succession process (Sousa et al. 2014). AMF helps plant growth in absorbing water and nutrients (Goltapeh et al. 2013; Kumar et al. 2010; Polcyn et al. 2019; Wu et al. 2015), increasing plants resistance against drought stress and salinity (Bhuvaneswari et al. 2014; Doubková et al. 2011; Latef and Chaoping 2011; Porcel et al. 2015; Shi-chu et al. 2019), plant protection of root pathogens (Banuelos et al. 2014; Oyewole et al. 2017; Veresoglou and Rillig 2012; Yang et al. 2014), increasing plants in tolerating heavy metals (phytoremediation) (Cabral et al. 2015; Husna et al. 2016; Rajtor and Piotrowska-Seget 2016; Tian et al. 2013). Also, AMF can improve and stabilize soil structure through glomalin production (Matthias et al. 2010; Rillig et al. 2015), increase plant growth and plant fitness (Barea et al. 2011; Husna et al. 2019, 2020; Janoušková et al. 2017; Musyoka et al. 2020) and increase crop productivity (Sing

and Jamaluddin 2011). The symbiosis of AMF and plants can influence interactions between plants, plant structure, increase crop production, ecosystem recovery, and conservation of the ecosystem (Davison 2015). More than 80% of plant growth surveys in mining areas have been colonized by the AMF (Wang 2017). The existing plants in the post-coal mining land are associated with AMF quite well (Kumar et al. 2003), thereby increasing plant survival. Research on AMF has been conducted in various post-mining lands in Indonesia, such as in post-mining areas of gold (gold tailings) (Suharno et al. 2016, 2017; Tuheteru et al. 2019), in post-coal mining areas (Husin et al. 2017; Salim et al. 2019; Ulfa et al. 2011), and post-nickel mining areas (Setiadi and Setiawan 2011; Prayudyarningsih et al. 2018). However, there are still few reports regarding the status of AMF for various revegetation age groups on post-coal mining land. Therefore, AMF has a very important and significant role in ecological restoration (Asmelash et al. 2016), especially on ex-mining land (Wang 2017). The aim of this study is to evaluate the status of colonization of the roots of the host plant in various age classes of revegetation of post-coal mining land associated with AMF spores populations and soil fertility.

## MATERIALS AND METHODS

### Study area

The study was conducted at the post-coal mining area of the Binunga site, PT. Berau Coal, East Kalimantan, Indonesia. The samples were taken in November 2019. Soil and root samples were taken from several revegetation age classes i.e. 2, 4, 6, 8, 10, and 12 years (Table 1). Soil sampling refers to the method of Sahner et al. (2015) using a 50m x 50m plot and there were three 5m x 5m subplots. Soil samples were taken from 0-20 cm deep from each subplot, while root samples were taken from 4 trees in each subplot.

### Soil analysis

The parameters were analyzed by different methods (Table 2) based on Eviati and Sulaeman (2009). The chemical properties of the soil were analyzed by 13 parameters, while the physical properties were measured only by the soil texture.

**Table 1.** Sampling location several revegetation age classes

Revegetation age (years)	Revegetation years	Area (ha)	Coordinate	
			North	East
2	2017	5.00	01°59'20.2"	117°24'8.9"
4	2015	14.80	01°59'10.6"	117°23'34.7"
6	2013	13.27	01°58'54.1"	117°23'35.0"
8	2012	12.78	02°03'26.2"	117°26'21.2"
10	2010	16.67	02°02'51.9"	117°27'12.6"
12	2008	9.70	02°03'19.5"	117°26'23.3"

### Isolation of spores and root colonization

Isolation of AMF spores was carried out by using the wet-sieving and centrifugation methods described by Brundrett et al. (1996) with modifications. A 20 g soil sample was put into a measuring cup and water, and stirred for several minutes, then spilled into a spore filter (500, 125, and 45 µm). Repeat the process until the suspension of the soil in the measuring cup is clear enough. Soil deposition contained in a 125 and 45 µm filter was moved into a centrifuge tube with the aid of a spatula and water. After that, add glucose solution to two-thirds of the tube. The sample was centrifuged at a speed of 3000 rpm (rotation per minute) for 3 minutes. The supernatant solutions were poured into filter paper and wait a few minutes for all the solutes to drop. After that, the filtered filter paper was transferred to a petri dish to calculate the number of spores.

The technique of root staining was done using the method of Clapp et al. (1996) with modifications. The roots were washed with water until cleaned, then soaked with 20% KOH for two days (depending on plant roots). After that, the roots were washed by water, and soaked in 0.1 M HCl for 15 minutes. Subsequently, the roots were soaked again with a trypan blue dye solution for one day. The roots were washed again using water and soaked in a destaining solution overnight. Preparation begins with cutting the root ± 1 cm and was placed parallel to the object preparation. Each object preparation contained 10 pieces of root and every five pieces of the root were closed using a slipcover. Root colonization was calculated based on the appearance of AMF intraradical structure (hyphae, arbuscular, vesicles, and spores). The percentage of root colonization was determined using the formula Giovanenetti and Moose (1980). The following formula was used to calculate root colonization:

$$\text{Root colonization (\%)} = \frac{\text{The total roots area infected}}{\text{The total roots planned observed}} \times 100\%$$

Percent colonization was then classified according to O'Connor et al. (2001), namely: (i) Not colonized: 0% colonization, (ii) Low: colonization value <10%, (iii) Medium: 10-30% colonization value, (iv) High: colonization value > 30%.

**Table 2.** Parameter and soil analysis methods

Parameters	Analysis method	Unit
pH	H <sub>2</sub> O	-
C-organic	Walkley & Black	%
N-Total	Kjeldahl	%
P-available	Bray I	ppm
P-Total	HCL 25%	ppm
Ca	N NH <sub>4</sub> OAc pH 7.0	cmol <sup>(+)</sup> /kg
Mg	N NH <sub>4</sub> OAc pH 7.0	cmol <sup>(+)</sup> /kg
K	N NH <sub>4</sub> OAc pH 7.0	cmol <sup>(+)</sup> /kg
Na	N NH <sub>4</sub> OAc pH 7.0	cmol <sup>(+)</sup> /kg
CEC	N NH <sub>4</sub> OAc pH 7.0	cmol <sup>(+)</sup> /kg
Al	N KCl	cmol <sup>(+)</sup> /kg
H	N KCl	cmol <sup>(+)</sup> /kg
Fe	DTPA	ppm
Texture (sand, silt, and clay)	Metode pipet	%

### Data analysis

Regression analysis was performed to determine the relationship between the number of spores with colonization. Meanwhile, Pearson correlation analysis was performed to determine the relationship between soil chemical properties and root colonization.

## RESULTS AND DISCUSSION

### Soil properties

The results showed that revegetation activities were able to increase the content of some nutrients in the soil (Table 3). Soil pH at the revegetation age ranges from 3.80-5.67 and was classified as very acidic and slightly acidic. The 10-year revegetation age class showed the highest C-organic (3.58 h), P-total (15.57ppm), and Mg (7.80 h) values compared to other revegetation age classes. The P-available and P-total content were very high in the six-year revegetation age class. Meanwhile, other elements differed between age classes revegetation. The content of Al and Fe was found to be highest in the 12-year revegetation age class compared to other revegetation age classes. Presumably, this was because the soil pH in the 12-year revegetation age class was classified as very acid (3.80). Meanwhile, soil texture in the revegetation age class was dominated by clay fraction (34.36-43.30%), dust fraction (26.67-40.22%), and a sand fraction (16.49-36.78%).

### AMF population

Result exhibited that the number of spores was present in the soil. The increase of the age revegetation class was able to increase the number of spores although this was not consistent. In the 10-year revegetation age class, the average number of spores decreased but increased again in the 12-year age class (Figure 1). Eight-year revegetation age classes showed the highest average number of spores compared to other revegetation age classes.

### Root colonization

The types of plants in each revegetation age class indicate that they were colonized by AMF, although they differ between plant types and revegetation age classes (Table 4). *F. moluccana* and *S. siamea* were the dominant species, widely planted in the revegetation areas of the study site with an average of 32-44% and 12-20% respectively. *A. mangium* and *G. cepium* were species that grew naturally on post-coal mining land and showed 50% and 100% root colonization. *G. cepium* was the type with the highest root colonization compared to other types. The other types were insertions plant, planted in revegetation areas to increase the diversity of vegetation types and have different types of root colonization.

After observation, some of the understorey samples also showed the presence of root colonization by AMF (Table 5). Colonization of root plants was classified as moderate to high, while some species exhibited 100% root

colonization such as *C. pubescens*, *U. lobata*, *P. conjugatum*, *A. conyzoides*, *M. affine*, and *P. javanica*. This situation revealed that plants have an important role in the development of AMF in the post-coal mining land.

Root colonization was very much influenced by propagules found in the soil. spores were one of the AMF propagules that can colonize the roots of the host plant. Regression analysis results showed that the number of spores had negative effect on root colonization ( $R^2 = 0.36\%$ ) (Figure 2). Increasing the number of spores was not always able to increase root colonization by AMF.

Soil properties especially chemical properties affected root colonization by AMF. The results of the correlation analysis showed that the C-org, N-total, CEC, Ca, Mg, and Fe were positively correlated to root colonization, while available P, total P, K, and Al were negatively correlated to root colonization (Table 6). Mg was one of the elements that correlate significantly with root colonization compared to other elements.

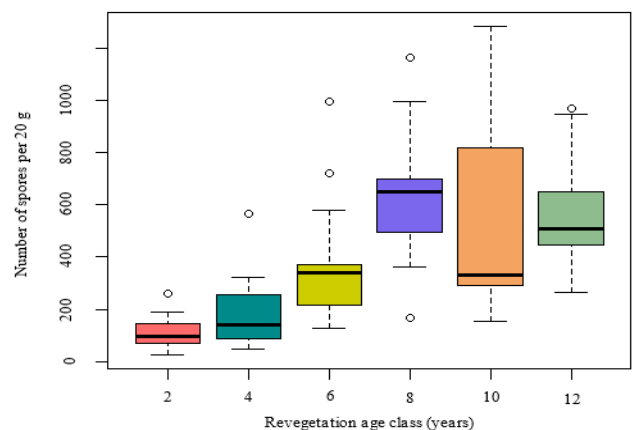


Figure 1. The number of spores in each revegetation age class

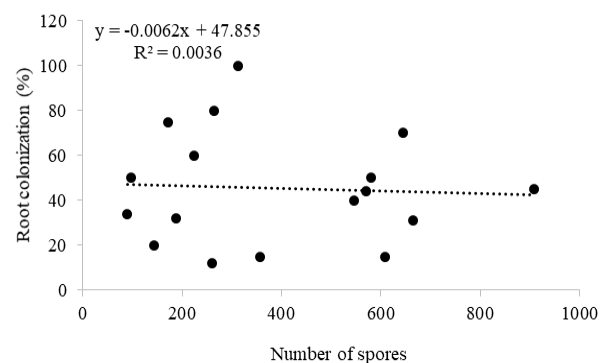


Figure 2. Relationship between number of spores and AMF root colonization

**Table 3.** Characteristics of land in various age classes post-mining land revegetation of coal

Parameters	Revegetation age class (years)					
	2	4	6	8	10	12
pH H <sub>2</sub> O	5.67ra	4.57a	4.42va	4.49a	5.31ra	3.80va
C-org (%)	1.71l	1.30l	2.94m	2.62m	3.58h	3.38h
N-total (%)	0.14l	0.14l	0.28m	0.26m	0.31m	0.32m
P-available (ppm)	1.56vl	1.74vl	304.317vh	8.60m	6.51l	5.73l
P-total (ppm)	11.00vl	8.06vl	868.54vh	10.72vl	15.57l	11.81vl
K (cmol <sup>(+)</sup> /kg)	0.45vl	0.24vl	0.88vl	0.61vl	0.59vl	0.40vl
Ca (cmol <sup>(+)</sup> /kg)	12.46h	4.66l	4.43l	2.69l	7.95m	2.20l
Mg (cmol <sup>(+)</sup> /kg)	3.41h	3.35h	2.09h	3.59h	7.80h	2.92h
CEC (cmol <sup>(+)</sup> /kg)	13.46l	15.49l	17.22m	22.62m	21.22m	17.24m
Al (cmol <sup>(+)</sup> /kg)	nm	0.64	1.59	1.60	nm	2.24
Fe (ppm)	70.43vh	80.52vh	169.70vh	140.85vh	156.43vh	477.33vh
Sand (%)	34.38	32.36	32.02	16.49	36.78	37.75
Silt (%)	26.67	32.75	30.18	40.22	28.86	28.22
Clay (%)	38.95	34.89	37.81	43.30	34.36	34.02

Note: CEC (cation exchange capacity); ra (rather acid); a (acid); va (very acid); l (low); vl (very low); m (medium); h (high); vh (very high); nm (not measurable). The criteria for soil properties are based on Eviati and Sulaeman (2009)

**Table 4.** The average root colonization of plant in various age classes of revegetation

Revegetation age class (years)	Species	Average colonization (%)	Criteria
2	<i>Falcataria moluccana</i>	34	High
	<i>Senna siamea</i>	25	Medium
	<i>Swietenia macrophylla</i>	50	High
4	<i>Falcataria moluccana</i>	32	High
	<i>Senna siamea</i>	15	Medium
	<i>Acacia mangium</i>	75	High
6	<i>Senna siamea</i>	22.86	Medium
	<i>Shorea leprosula</i>	50	High
	<i>Senna siamea</i>	14.55	Medium
8	<i>Acacia mangium</i>	45	High
	<i>Senna siamea</i>	31.11	High
	<i>Shorea leprosula</i>	70	High
10	<i>Enterolobium cylocarpum</i>	60	High
	<i>Gliricidia sepium</i>	100	High
	<i>Falcataria moluccana</i>	44	High
12	<i>Vitex cofasus</i>	80	High
	<i>Neolamarcia cadamba</i>	40	High
	<i>Dryobalanops lanceolata</i>	20	Medium

Note: Colonization criteria based on O'Connor et al. (2002)

**Table 5.** The average root colonization of understorey in various age classes of revegetation

Revegetation age class (years)	Species	Average colonization (%)	Criteria
2	<i>Centrosema pubescens</i>	100	High
	<i>Mucuna mucronata</i>	90	High
	<i>Urena lobata</i>	100	High
4	<i>Paspalum conjugatum</i>	70	High
	<i>Ageratum conyzoides</i>	75	High
	<i>Paspalum conjugatum</i>	100	High
6	<i>Rhyncospora corymbosa</i>	60	High
	<i>Ageratum conyzoides</i>	100	High
	<i>Melastoma affine</i>	70	High
8	<i>Melastoma affine</i>	100	High
	<i>Nephrolepis biserrata</i>	20	Medium
	<i>Melastoma malabatricum</i>	60	High
10	<i>Chromolaena odorata</i>	60	High
	<i>Pueraria javanica</i>	100	High
	<i>Paspalum conjugatum</i>	100	High
12	<i>Ageratum conyzoides</i>	40	High
	<i>Digitaria bicornis</i>	10	Medium
	<i>Nephrolepis biserrata</i>	20	Medium
12	<i>Melastoma affine</i>	20	Medium
	<i>Ageratum conyzoides</i>	100	High

Note: Colonization criteria based on O'Connor et al. (2002)

## Discussion

At the beginning of the age class of revegetation, soil fertility was relatively low, as indicated by several nutrients that were relatively low. The variation of soil properties on post-mining land was strongly influenced by origin condition of the mine soil itself (Zipper et al. 2011).

Soil pH affects the availability of some nutrients in the soil. Ezeokoli et al. (2019) also reported that the average soil pH on reclaimed land after coal mining has acidic (4.03-4.89). Acidic soil pH to very acidic in post-mining land was strongly influenced by the condition of host rock that makes up the soil itself. The pH of acid soils on post-

mining coal also due to concentrations of pyrite (FeS<sub>2</sub>) that were significantly higher in the soil (Rai and Paul 2011). Mine soil pH can change quickly when the rock was split and oxidized (Sheoran et al. 2010). High rainfall can lead to the leaching of the alkaline cation (K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>) that contribute to soil acidity (Seguel et al. 2013). Besides, the coal reacting with rainwater was able to produce Fe, Al and Mn (heavy toxic metals) which generate quite high acidity (Agus et al. 2016; Sheoran et al. 2010). Fe content was very high in all revegetation age classes (Kusmana et al. 2013) reported that the Al and Fe content were quite high in post-coal mining land in South Kalimantan.

**Tabel 6.** Correlation of soil chemical properties with root colonization

Parameters	Root colonization
C-organic	0.533
N-total	0.438
P-available	-0.333
P-total	-0.331
K	-0.157
Ca	0.160
Mg	0.861*
CEC	0.272
Al	-0.934
Fe	0.231

Note: \*: correlation is significant at the 0.05 level

The content of C-organic increases in revegetation age classes, especially in the eight and 12 years revegetation classes. This was due to new input of carbon content released by the plant and decomposition of many litters (Ahirwal et al. 2017). Soil organic carbon has strongly affected by litter decomposition, crop productivity, and revegetation age (Singh et al. 2004). Ahirwal et al. (2017) reported that soil organic carbon increased after eight years of revegetation. Soil organic carbon and nitrogen have increased along with the increasing age of reclamation after coal mining (Wick et al. 2009; Reynolds and Reddy 2012). The availability of nitrogen in the soil has greatly influenced by the presence of organic matter (litter) which decomposed and mixed with the soil (Chaubey et al. 2012).

The P-available and P-total content in the revegetation age class was much higher in the six-year revegetation age class. Salim et al. (2019) reported that the content of P-total at some revegetation age class is high to very high. The P-available content is strongly influenced by the content of organic matter, the higher the organic material will increase the availability of P-available (Chaubey et al. 2012). The high P-available content was due to the high activity of acid phosphatase in the soil, so that insoluble phosphorus in the soil became available for plants (Bi et al. 2018).

Soil texture in various revegetation age classes was dominated by clay fraction. This result follows the research of Agus et al. (2018) who reported that land on coal mining areas in East Kalimantan was dominated by clay (32.4–39.44%), sand (24.7–37.3%) and dust (28.8–36.5%). Singh et al. (2004) reported that the lowest clay content was around 11% and maximum sand was 80%. Post-mining land conditions can recover along with increasing age of revegetation (Hazarika et al. 2006; Chandra 2014).

The AMF population increased along with increasing of revegetation, but the increase was still inconsistent. Casazza et al. (2017) stated that the composition and diversity of AMF was different for each location. The differences between host plant, location, and root rhizosphere can influence the AMF population in the soil (Husna et al. 2015; Rahim et al. 2016). Soil properties that varied in each revegetation age classes and plant types (also affected different AMF spores. The availability of nutrients in the soil can change the abundance and diversity of AMF (Camenzind et al. 2014; Lekberg et al. 2007). The

formation of spores was also influenced by the physical and chemical properties of the soil (Chandra 2014). Revegetation activities can facilitate the development of AMF on post-mining land (Li 2006). Several studies indicate that there was a change in the composition of the AMF community during ecosystem development (Sikes et al. 2009; Martínez-García et al. 2015). The presence of AMF populations plays an important role in the formation and growth of plants, especially in post-coal fields have low soil fertility (Singh and Jamaluddin 2011).

Each plant in each revegetation age class shows that it has been colonized by AMF (although it varies.. Plant species found above ground level can be the host for the survival of AMF (Ong et al. 2012). Host plants provide a habitat and food supply for soil microbes that live around the rhizosphere (Akib et al. 2018). According to Becklin et al. (2012), trees were indirectly able to interact with another plant influencing the composition and diversity of AMF in the soil. Each type of plant can produce different levels of colonization by AMF (Berruti et al. 2016; Bi et al. 2018).

Some of the dominant species and understorey plants on post-coal mining land were those of Fabaceae or Leguminosae such as *F. moluccana*, *A. mangium*, *S. Siamea*, *C. pubescens*, *P. Javanica*, and *M. Mucronata*. Wulandari et al. (2016) reported that the *Paraserianthes falcataria* plant had 59% root colonization which was planted in the post-coal field. Legume plants were responsive to associations with AMF especially in soils with low P availability, which will affect the performance of the plant growth (Kumar et al. 2010).

The average percentage of understorey AMF root colonization was higher than that of staple. Roumet et al. (2006) stated that the root system greatly affects the percentage of root colonization and affects dependence on mycorrhizae. Closa and Goicoechea (2011) reported that the colonization percentage of annual plant roots (*Meehringia trinervia*) was lower than that of grass species (*B. pinnatum* and *F. rubra*) which grew in disturbed forests. However, in some cases, the colonization rate of annual plant roots was higher than that of perennial plants (Roumet et al. 2006). Cheeke et al. (2019) reported that grassland plant species in late succession were very responsive to AMF and showed quite strong sensitivity.

Spores were one of the AMF propagules that can colonize the roots of the host plant. The source of inoculum which can cause root infection by AMF consists of hyphae, and infected parts of the root (Smith and Read 2008; Silvana et al. 2018). The presence of spores greatly affects the colonization of roots. Increased spore production in the soil can increase the root colonization of AMF (Cuenca and Lovera 2010). The regression analysis showed that the number of spores had a negative relationship with root colonization. The percentage of root colonization was inversely proportional to the AMF spore population in some ecosystems (Moreira et al. 2006). Zangaro et al. (2012) reported that increasing the number of spores can increase root colonization. Husna et al. (2015) reported that spore density was not correlated with AMF colonization.

The analysis showed that root colonization by AMF had a positive correlation with carbon organic, N-total, CEC, and Fe, while it was negatively correlated with available P, total P, K, and Al. Carbon organic was able to trigger the presence of AMF in the soil and become a food source for soil microorganisms (Thirkell et al. 2016). The organic carbon has a strong correlation with the number of spores (Bath et al. 2014). The percentage of AMF colonization was positively correlated with soil organic matter (Silvana et al. 2018; Silvana et al. 2018). According to Melo et al. (2019) root colonization by AMF was influenced by soil pH, available P, K and Mg. The level of root colonization by AMF was also influenced by the level of soil fertility (Martinez and Johnson 2010; Nyamwange et al. 2018). t AMF root colonization had a positive correlation with tanh pH, Ca, K and Ca/Mg ratio in serpentine soil has been reported by Doubková et al. (2011). Mg was one of the elements that have a significant positive correlation. Mg was a nutrient capable of developing mycorrhizae in the roots (Saleh Rastin 2001). Ardestani et al. (2011) reported that Mg was able to increase root colonization by AMF to a certain concentration (up to 7.2 meq/l), but could reduce the AMF colonization when the concentration of Mg is too high. In *in vitro*, 1.5 mM Mg was able to inhibit the growth of *Glomus claroideum* hyphae, but did not affect root colonization. (Malcová et al. 2002). Zhang et al. (2015) reported that root colonization by AMF was significantly higher in Mg deficiency conditions, this shows that root colonization by AMF has a negative correlation with Mg concentration.

P-available and P-total have negative correlation with root colonization by AMF. Lakshmipathy et al. (2012) reported that AMF activity has a negative correlation between P-available and P-total. High availability of P in the soil can reduce root colonization by AMF and spore production (Gosling et al. 2013; Kowalska et al. 2015; Schmidt et al. 2010; Smith and Smith 2011). The reduction of phosphorus elements in the soil can increase root colonization by *Rhizopagus irregularis* (Bonneau et al. 2013). Soka et al. (2015) also reported that increasing P was able to reduce hyphal density. Besides, the number of spores was also high at the low P-available conditions (Birhane et al. 2010; Tian et al. 2011). The results of Kowalska and Konieczny's (2015) report that high P concentrations (140 mg dm<sup>-3</sup>) have a negative impact on the development of mycorrhizal structures, but P concentration (70 mg dm<sup>-3</sup>) have a positive on the development of mycorrhizal structures. However, the results of Zangaro et al. (2013) reported that root colonization by AMF had a positive correlation with P in the soil.

Potassium (K) also has a negative correlation with the percentage of root colonization by AMF. These results are consistent with several studies which show that the potassium in the soil has a negative correlation with root colonization by AMF (Ardestani et al. 2011; Melo et al. 2019; Panwar et al. 2011). However, in some cases potassium can stimulate and increase the rate of root colonization by AMF (Melo et al. 2019; Zhang et al. 2017). Potassium can increase root colonization by AMF in

drought conditions (Melo et al. 2019; Zhao et al. 2017). Potassium was not able to increase the rate of AMF root colonization, but was only able to increase the length of the roots colonized by AMF. (El-Mesbahi et al. 2012). Each plant has a different AMF response to root colonization. AMF spore population which is pretty much not always able to increase root colonization. Soil chemical properties can affect the root colonization by AMF, especially Mg.

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