

The Role of Indigenous Mycorrhizae of Corn Plants in Various Soil Types in Gunung Kidul, Indonesia

DOI: [10.18196/pt.v10i1.11428](https://doi.org/10.18196/pt.v10i1.11428)

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ABSTRACT

Indigenous Vesicular-Arbuscular Mycorrhizae (VAM) are natural mycorrhizae from specific areas that have good environmental adaptability. This study, conducted from January to November 2020 at the Faculty of Agriculture, Universitas Gadjah Mada, aimed to isolate the vesicular-arbuscular mycorrhizal fungus so that it can be used as information on the type and role of VAM on Gunung Kidul soil. The research was arranged in a Completely Randomized Design (CRD) with three factors. The first factor was soil type from Gunung Kidul Regency (Inceptisol, Mollisol, and Alfisol), the second factor was sterilization (sterilized soil and unsterilized soil), and the third factor was corn variety (local and hybrid). Analysis of soil and plant growth was performed by using Analysis of Variance (ANOVA) and Tukey's Honestly Significant Difference (Tukey's HSD) Test. Genetic detection of root infecting VAM was performed by using Terminal Restriction Fragment Length Polymorphism (T-RFLP) method with FAM AML1-AML2 labeled primers. The VAM detected in the roots of hybrid variety included *Acaulospora* sp., *Gigaspora* sp., and *Septoglomus* sp., and those in the roots of local variety were *Acaulospora* sp., *Gigaspora* sp., and *Funelisformis* sp. The results showed that the role of VAM could be seen through unsterilized soil so that there was no VAM elimination in the soil. Unsterilized soil showed the best results of root infection, leaf fresh and dry weight, leaf phosphor (P) content, and leaf P uptake. Meanwhile, Alfisol showed the best result of root infection, fresh weight, dry weight, leaf P content, and leaf P uptake. The treatment of plant varieties showed that the varieties did not significantly affect the root infection, fresh weight, dry weight, leaf P content, and leaf P uptake.

Keywords: Corn variety, Indigenous mycorrhiza, Soil, Sterilization,

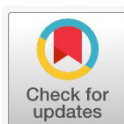
ABSTRAK

Mikoriza arbuskular vesikular (VAM) indigenous adalah mikoriza alami unggul dari wilayah spesifik yang memiliki daya adaptasi lingkungan yang baik. Penelitian ini, yang dilakukan pada Januari-November 2020 di Fakultas Pertanian Universitas Gadjah Mada bertujuan untuk mengisolasi cendawan Mikoriza vesikular arbuskular sehingga dapat digunakan sebagai informasi jenis serta peran VAM pada 3 tanah Gunung Kidul. Metode penelitian menggunakan Rancangan Acak Lengkap 3 Faktor. Faktor pertama Jenis Tanah terdiri atas Inceptisol, Mollisol, Alfisol. Faktor kedua adalah Jenis Sterilisasi terdiri atas tanah steril dan tanah tanpa steril. Faktor ketiga jenis Varietas, terdiri atas varietas lokal dan hibrida. Analisis data parameter tanah dan tanaman menggunakan Analisis Varians (ANOVA) dengan Uji Beda Nyata Jujur (HSD Tukey). Pendeteksian genetik Mikoriza penginfeksi akar menggunakan metode Terminal Restriction Fragment Length Polymorphism (T-RFLP) dengan primer berlabel FAM AML1-AML2. Mikoriza vesikular arbuskular yang terdeteksi pada akar varietas hibrida antara lain *Acaulospora* sp., *Gigaspora* sp., dan *Septoglomus* sp., kemudian pada akar varietas lokal adalah *Acaulospora* sp., *Gigaspora* sp., dan *Funelisformis* sp. Peran VAM dapat dilihat melalui tanah yang tidak steril sehingga tidak terjadi eliminasi VAM di dalam tanah. Tanah yang tidak disterilkan menunjukkan hasil infeksi akar terbaik, berat segar dan kering daun, kandungan fosfor (P) daun, dan serapan P daun. Perlakuan jenis tanah menunjukkan bahwa Alfisol menunjukkan hasil infeksi akar, bobot segar, bobot kering, kandungan P daun, dan serapan P daun yang paling baik. Varietas tidak berpengaruh nyata terhadap hasil infeksi akar, bobot segar, bobot kering, kandungan P daun, dan serapan P daun terbaik.

Kata kunci: Mikoriza indigenous, Tanah, Sterilisasi, Varietas jagung

INTRODUCTION

Gunung Kidul is an area with carcass as a parent material with several soil orders formed on it. Some examples of soil orders with soil development rates include young soils, such as Inceptisols, Alfisols, and Mollisols. According to Abdurachman et al. (2008), Inceptisols with dry land in hilly areas (15-30%) generally have low soil fertility, steep slopes, and shallow solum. Meanwhile, Alfisol has the characteristics of an argillic horizon, relatively easily weathered minerals, and alkaline saturation > 3% at a depth of 180 cm from the soil surface or 125 below the upper limit of the argillic horizon. Mollisols are soils that are generally deep soluble, dark in color, and rich in bases, and the alkaline



Article History
Received: 05 Apr 2021
Accepted: 25 Jan 2022



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saturation is > 50% in Mollisols whose solum is > 1 m (Rachim, 2007).

Soils formed above the karst environment cause frequent drought in the Gunung Kidul area. This is because the carcass structure causes the water retention capacity to be very low. Low water retention capacity during the dry season will affect plant growth and production. Dry soil will have a dense texture, inhibiting the absorption of water and nutrients in plants. Dense soil is also less favorable for plant growth because root growth and penetration will be limited and also has a low percentage of pores and aeration. The plant growth and production are affected by soil fertility, including physical, chemical, and biological properties of the soil. Soil biological property is a determining factor of soil quality due to the symbiosis between soil microorganisms and plants. One of the well-known soil microorganisms to have a great defense system in extreme conditions supporting dryland farming is Arbuscular Mycorrhizae.

Vesicular-Arbuscular Mycorrhizal is microorganism of the fungal group. According to Aguzen (2009), VAM can have a mutualistic symbiosis with higher plants. The external hyphae of mycorrhizal fungi, which are longer and finer than root hairs, can expand the surface area of root absorption. This would increase nutrient and water uptake, especially in critical soil conditions. Bukovská et al. (2018), in their research, stated that VAM could significantly contribute to nitrogen (N) absorption from complex organic sources. The plants in which VAM grew were 6.4 times larger, accumulating 15 N derived from organic labeled sources 20.3 times higher compared to non-VAM plants. Praharasti et al. (2012), in their research, stated that VAM could increase the growth and productivity of plants such as corn.

The results of research by Alguacil et al. (2016) showed that VAM had associations with soil rhizo-

sphere and root habitats. Soil type also determines the distribution of VAM communities in the soil, and this effect cannot be attributed to a single soil characteristic. This is because at least three soil properties are related to microbial activity, namely pH and levels of two nutrients (Mn and Zn) that play an important role in triggering the VAM population. The pH value affects the ability of spores to germinate, and micronutrients, such as Mn and Zn, are essential nutrients for plant metabolism.

The results of research by Oehl et al. (2010) showed that soil characteristics (soil type and pH) were more important in regulating the composition of VAM species. Several VAM species appear to be specialists in existence. For instance, *Pacispora* in that study was generally absent in Inceptisol and was never found at pH <6.0. His research concluded that there were many differences in VAM species in the observed differences in soil types. However, the land-use intensity had a greater impact on the VAM population and species composition than the soil type did.

Vesicular-arbuscular mycorrhizae form a mutualism symbiosis in most plant roots. This symbiosis is of great relevance to sustainable agriculture because of its ability to increase productivity, nutrient uptake (Carballar-Hernandez et al., 2018), soil aggregation, and crop protection. Endophytic and symbiotic VAM directly interact with live host plants. Corn plants are usually associated with VAM because their root system supports the fungi's growth, and corn plants supply carbon in exchange for nutrients, especially phosphorus.

Therefore, understanding the community and the abundance of VAM in soil types in the karst environment, which are identical to drought, is necessary. Vesicular-arbuscular mycorrhizae are detected using the T-RFLP technique. This technique is used to determine the diversity, structure, and dynamics of the microbial community of an

environment. This technique is often used because of the production and analysis of data that is accurate, fast, and effective in differentiating microbial communities (Kitts, 2001). The advantages of the T-FRLP technique compared to other techniques are that it provides the same replication, higher resolution, and is more sensitive (Osborn et al., 2000). The information can be used to develop appropriate management strategies, thus, optimizing the role of VAM in achieving sustainable agriculture. Astuti et al. (2017) have identified the indigenous Mediterranean VAM in Gunung Kidul using the trapping method. They found the type of *Glomus* sp. and tested its effectiveness on the growth of cassava plants including root length, plant height and plant dry weight. Thus, this study aimed to determine the role of indigenous vesicular-arbuscular mycorrhizal (VAM) and root infection by VAM on nutrient uptake and plant development.

MATERIALS AND METHODS

Time and Location of the Research

There were three types of soil used in this study, including Mollisol and Inceptisol obtained from Tahura Bunder Gunung Kidul and Alfisol from the Mulo area, Gunung Kidul. This research was conducted from January to November 2020. Vesicular-arbuscular mycorrhizae's DNA and soil analysis were carried out at the Laboratory of Microbiology and Soil Science of the Faculty of Agriculture UGM. Other materials included hybrid (Bisi 18) and local (Guluk-guluk) corn varieties. Basic fertilizers used were urea 300 kg / ha (0.75 g / polybag), KCl 100 kg / ha (0.25 g / polybag) (Isrun, 2006), and rock phosphate 300 kg / ha (0.75 g / polybag) (Wahyudin et al., 2017).

Experimental Design and Data Collection

The experimental design applied was a completely randomized design with three treatment

factors. The first factor was soil type (T) (Inceptisol (T1), Mollisol (T2), and Alfisol (T3)), the second factor was soil sterilization (S) (with soil sterilization (S1) and without soil sterilization (S2)), and the third factor was corn Varieties (V) (local (V1) and Bisi 18 hybrid (V2) variety).

The planting media were prepared by sieving the soils, which then were separated into two parts. The first part of the soil was sterilized, and the second one was not sterilized. The method of sterilization was formaldehyde sterilization with a cover. The soil was put in a bucket, given 2% formaldehyde at a dose of 2 l / ft², given water to field capacity, and then incubated by covering it with plastic for seven days. The soil was then drained and air-dried for three weeks before planting (Cahyani, 2009; Lawrence, 1956). The variables observed were the percentage of mycorrhizal infections, plant height, plant fresh and dry weight, leaf P, plant nutrient P uptake, and mycorrhizal species diversity.

Root infection was observed by taking small roots with good morphology taken as samples collected in plastic bags that can be sealed and then stored at a temperature of - 4 °C until further processing (Boeraeve et al., 2019). The VAM's DNA in the roots was extracted by taking 0.5 g of fine corn roots, which were then crushed with liquid nitrogen then continued with the CTAB isolation method (Doyle and Doyle, 1990; Khan et al., 2007). The next stage, namely DNA amplification, was performed by filling the PCR tube using 25 microliters of the PCR reaction mixture for T-RFLP using a PCR thermocycler machine for 40 cycles, then optimizing the temperature during annealing. The primer pairs used for the T-RFLP annealing stage were AML1 (ATCAACTTTC-GATGGTAGGATAGA) labeled FAM and AML2 (GAACCCAAACACTTTGGTTTCC) (Desah and Widada, 2014). The first and second denaturation, annealing process, extension process, and the final

extension was performed at a temperature of 95 °C for two minutes, 95 °C for 30 seconds, 55.9 °C for 30 seconds, 72 °C for a minute, and 72 °C for five minutes, respectively. Visualization of T-RFLP DNA amplification results was viewed by gel electrophoresis using agarose. After the DNA bands were visible on the - / + 800 base pairs column, then the T-RFLP amplified DNA was cut using the restriction enzyme MspI (5'- CC ^ GG-3 ') by mixing all the reagents to be incubated at 37 °C for three hours and continued to the fragment analysis stage.

Statistical Analysis

Data from the analysis of soil and plant growth were analyzed using analysis of variance (ANOVA), continued with Tukey's test (HSD) to find out the significant differences between treatments. Data from laboratory analysis in the form of T-RFLP were collected from the database available at NCBI. From the collected data, fragments of each species were cut with NEB cutter at neb.com. The species found were matched from each peak formed during the fragment analysis.

In this study, Inceptisol had a sandy clay loam texture, consisting of 52.41% sand, 22.43% silt, and 25.16% clay (Table 1). The Inceptisol soil before sterilization was slightly alkaline (pH H₂O of 7.73), while after being sterilized, the value of pH H₂O was 7.44 (neutral). This result is due to the content of CaCO₃, which is the dominant constituent of the parent material of limestone. The CO₃²⁻ ion dissociating from CaCO₃ in the water system would hydrolyze the water, thereby releasing OH⁻ into the soil solution and increasing the pH (Hanudin et al., 2012).

The CEC value of the Inceptisol soil, both before and after being sterilized, was in the high category (34.37 and 33.83 [Cmol (+).kg⁻¹], respectively). Soils that have higher clay/colloid content and/or higher organic matter content have a higher CEC than soils that have low clay and organic matter content (sandy soil), as well as the soils that have low organic matter content. CEC value is also influenced by the clay type. The soil with a clay type of 2: 1 (montmorillonite) will have a higher CEC compared to that with a clay type of 1: 1 (kaolinite) or 2: 1: 1 (chlorite) (Winarso, 2005).

RESULTS AND DISCUSSION

Table 1. Chemical and physical properties of Inceptisol soil in Gunung Kidul

Parameter	Unit	Unsterilized Inceptisol		Sterilized Inceptisol	
		Value	Category	Value	Category
Texture					
Sand	%	52.41	Sandy clay loam		
Silt	%	22.43			
Clay	%	25.16			
pH H ₂ O	-	7.73	Slightly alkaline	7.44	Neutral
CEC	[Cmol (+).kg ⁻¹]	34.37	High	33.83	High
Organic C	%	2.33	Medium	2.24	Medium
Total N	%	0.94	Extremely high	0.75	Extremely Low
NH ₄ ⁺	%	0.01	-	0.01	-
NO ₃ ⁻	%	0.01	-	0.01	-
Available P	ppm	0.74	Extremely low	0.74	Extremely low
Total P (bray)	ppm	3.68	Extremely low	4.14	Extremely low
Available K	[Cmol (+).kg ⁻¹]	0.40	Medium	0.54	Medium
Available Na	[Cmol (+).kg ⁻¹]	0.59	Medium	0.53	Medium
Available Ca	[Cmol (+).kg ⁻¹]	7.59	Medium	7.68	Medium
Available Mg	[Cmol (+).kg ⁻¹]	1.59	Medium	1.64	Medium

Table 2. Chemical and physical properties of Mollisol soil in Gunung Kidul

Parameter	Unit	Unsterilized Mollisol		Sterilized Mollisol	
		Value	Category	Value	Category
Texture					
Sand	%	10.35			
Silt	%	27.08	Clay		
Clay	%	62.57			
pH H ₂ O	-	6.81	Neutral	6.42	Slightly acidic
CEC	[Cmol (+).kg ⁻¹]	27.72	High	29.06	High
Organic C	%	4.35	High	4.76	High
Total N	%	2.64	Extremely high	2.45	Extremely high
NH ₄ ⁺	%	0.01		0.01	-
NO ₃ ⁻	%	0.01		0.01	-
Available P	ppm	0.46	Extremely low	0.67	Extremely low
Total P	ppm	3.88	Extremely low	3.22	Extremely low
Available K	[Cmol (+).kg ⁻¹]	0.12	Low	0.24	Low
Available Na	[Cmol (+).kg ⁻¹]	0.30	Low	0.38	Low
Available Ca	[Cmol (+).kg ⁻¹]	4.85	Low	4.81	Low
Available Mg	[Cmol (+).kg ⁻¹]	1.94	Medium	1.96	Medium

The organic C content of the Inceptisol soil in this study was in the medium category, both before and after the sterilization process, with a value of 2.33 and 2.24%, respectively. Organic matter can improve the chemical, physical, and biological properties of the soil, which have irreplaceable functions. Meanwhile, the total N of the Inceptisol soil, before and after being sterilized, was in the extremely high category, with a value of 0.94 and 0.75%, consecutively. The NH₄⁺ and NO₃⁻ content before and after sterilization were 0.01 and 0.01% and 0.01 and 0.01%, respectively. Meanwhile, the available P and total P of the Inceptisol soil were extremely low. This result could happen because the P element is fixed or retained by base cations such as Ca. Thus, its availability can be very low (Carreira et al., 2006). The total P and available P of the Inceptisol soil before and after sterilization were 3.68 and 4.14 ppm and 0.74 and 0.74 ppm, consecutively.

All of the alkaline cations (K, Ca, Na, and Mg) of the Inceptisol soil in this study, both before and after the sterilization process, were in the medium category. The available K of the Inceptisol soil before and after the sterilization process was 0.399 and 0.54 [Cmol (+).Kg⁻¹], respectively.

Meanwhile, the available Ca before and after the sterilization process was 7.59 and 7.68 [Cmol (+).kg⁻¹], consecutively. On the soils developing from base parent materials with soil development that is not classified as old soils, Ca becomes a cation dominating the 70-90% exchange complex of the land exchange site (Winarso, 2005). This is in line with the Ca of the Inceptisol soil in this study, which was in the medium category (7.59 and 7.68 [Cmol (+). kg⁻¹]), showing the highest percentage value of other cations. Meanwhile, the available Na was 0.59 and 0.53 [Cmol (+).kg⁻¹], and the available Mg was 1.59 and 1.64 [Cmol (+).kg⁻¹].

Based on the results of the initial soil analysis (Table 2), the Mollisol soil in this study had a clay texture, with a percentage of 10.35% sand, 27.08% silt, and 62.57% clay. The value of pH H₂O was 6.81. Based on the category set by Balai Penelitian Tanah (2009), the pH value was in the neutral category, and after sterilization, the pH of H₂O changed to 6.42, which is slightly acidic. The CEC of the mollisol soil in this study both before or after sterilization was categorized in the high category (27.72 and 29.06 [Cmol (+).kg⁻¹]). Organic C of the mollisol soil before and after sterilization was 4.35 and 4.76%, respectively, categorized in the

Table 3. Chemical and physical properties of Alfisol soil in Gunung Kidul

Parameter	Unit	Unsterilized Alfisol		Sterilized Alfisol	
		Value	Category	Value	Category
Texture					
Sand	%	6.65			
Silt	%	68.93	Silt loam		
Clay	%	24.42			
pH H ₂ O	-	6.53	Acidic	6.50	Acidic
CEC	[Cmol (+).kg-1]	27.16	High	28.96	High
Organic C	%	3.63	High	3.68	High
Total N	%	1.79	Extremely high	1.74	Extremely high
NH ₄ ⁺	%	0.01	-	0.02	-
NO ₃ ⁻	%	0.01	-	0.01	-
Available P (olsen)	ppm	0.65	Extremely low	0.97	Extremely low
Total P (bray)	ppm	7.32	Low	7.40	Low
Available K	[Cmol (+).kg-1]	0.24	Low	0.29	Low
Available Na	[Cmol (+).kg-1]	0.20	Low	0.24	Low
Available Ca	[Cmol (+).kg-1]	3.61	Low	3.67	Low
Available Mg	[Cmol (+).kg-1]	1.51	Medium	1.59	Medium

high category. The nitrogen element in mollisol soil was analyzed for either total N or NH₄⁺ and NO₃⁻. The mollisol in this study had a very high total N value both before and after sterilization, namely 2.64 and 2.45%, respectively. Meanwhile, before and after sterilization, the content of NH₄⁺ was 0.01 and 0.01%, respectively, and NO₃⁻ was 0.01 and 0.01%, consecutively.

The alkaline cations (K, Na, Ca, and Mg) of the mollisol soil in this study were also tested. The content of available K before and after sterilization was 0.12 and 0.24 [Cmol (+). kg-1], respectively, categorized in the low category. Na content was 0.30 and 0.38 [Cmol (+). kg-1], categorized in the low category. The content of Ca was 4.85 and 4.81 [Cmol (+). kg-1], categorized in the low category. Meanwhile, the Mg content Mg was 1.94 and 1.96 [Cmol (+). kg-1], categorized in the medium category (Balai Penelitian Tanah, 2009).

The Alfisol soil in this study had a silt loam texture, consisting of 6.65% sand, 68.93% silt, and 24.42% clay (Table 3). Soil reaction (soil pH) is a term used to describe acid-base reactions in the soil. The pH H₂O of the sterilized and unsterilized Alfisol soil in this study was 6.53 and 6.50, respectively, categorized as acidic according to Balai

Penelitian Tanah (2009). Cation exchange capacity (CEC) is the ability of the soil to absorb and exchange cations. The CEC of Alfisol soil in this study before and after sterilization was 27.16 and 28.96 [Cmol (+) kg-1], consecutively, categorized in the high category. The greater the value of the CEC, the greater the power of cation exchange in the soil. Several factors affecting the CEC are the amount of clay, the type of clay minerals, and organic matter content since organic matter can increase the negative charge (Darlita et al., 2017).

Soil organic matter can be determined by measuring the level of organic carbon in the soil. The organic matter content of Alfisol before and after sterilization was 3.63% and 3.68%, respectively, categorized in the high category (Balai Penelitian Tanah, 2009).

Nitrogen is an essential macro element for plant growth due to its function to increase chlorophyll content that plays an important role in the photosynthesis process. The N content of the sterilized and unsterilized Alfisol soil in this study was 1.79% and 1.74%, respectively, categorized in the very high category (Balai Penelitian Tanah, 2009).

Phosphorus is an essential macro element closely related to plant growth due to its irreplace-

able roles in plants. Phosphorus is mostly absorbed by plants in the form of primary orthophosphate ions (H_2PO_4^-). Meanwhile, its small amount is absorbed in the form of secondary orthophosphate ions (HPO_4^{2-}). Phosphorus plays an essential role in photosynthesis, respiration and energy transfer and storage, cell division, and enlargement. The total P content of Alfisol soil in this study before and after sterilization was 7.32 ppm and 7.40 ppm, consecutively, categorized in the low category. Meanwhile, the available P of the soil before and after sterilization was 0.65 ppm and 0.97 ppm, respectively (very low) (Balai Penelitian Tanah, 2009).

K, Ca, Na, and Mg elements are types of alkaline cations adsorbed by the soil. The available K before and after sterilization was 0.24 and 0.29 [Cmol (+).kg-1], respectively, categorized in the low category. Na, as one of the basic cations, is an element from mineral leaching in the soil usually absorbed by plants in the form of Na^{2+} . The available Na was low, both before and after sterilization, at a value of 0.20 [Cmol (+).kg-1] and 0.24 [Cmol (+).kg-1], consecutively. The available Ca content in the soil is strongly influenced by the soil parent material. Soils containing limestone source rock tend to have higher Ca levels. However, Ca levels will generally decrease as the soil depth decreases. This is because the soil is getting away from the parent

material rich in CaCO_3 (Hanudin et al., 2012). The available Ca in Alfisol in this study was low, which was 3.61 and 3.67 cmol (+).kg-1 before and after sterilization, respectively. The low available Ca as a base cation in Alfisol in this study correlates with the acidic soil conditions. The available Mg was in the medium category, both before and after sterilization, namely 1.51 and 1.59 cmol (+).kg-1). Magnesium in the soil can come from weathering rocks that contain Mg. The main source of Mg for plants is from soil solutions and the sorption complex. Magnesium can be absorbed by plants in the form of Mg^{2+} cations.

Root Infection

Based on the ANOVA results (Table 4), there was an interaction effect of soil type and sterilization on the root infection. The Alfisol soil without sterilization showed the highest root infection of 89.44 (%) compared to other treatments. The effect of soil sterilization using formaldehyde showed a decrease in the percentage of root infections. However, infected roots were still found after sterilization with formaldehyde, indicating that there were strains resistant to formalin (Hayman, 1970). Hu et al. (2020) mention that sterilization reduces colonization to less than 0.1% as well as decreases the germination rate and survival rate

Table 4. Effects of soil types and sterilization on the root infection

Soil types	Sterilization		Mean (%)
	S1	S2	
Inceptisol	6.11 c	60.92 b	33.52
Mollisol	1.67 c	56.66 b	29.17
Alfisol	3.33 c	89.44 a	46.39
	3.70	69.01	(+)

Remarks: Values followed by the same letters in the same column are not significantly different according to Tukey's test (HSD) at 5 %.

Table 5. Effects of corn plant varieties on the root infection

Corn plant varieties	Mean (%)
Local variety	35.56 a
Hybrid variety	37.16 a

Remarks: Values followed by the same letters in the same column are not significantly different according to Tukey's test (HSD) at 5 %.

of VAM. The high percentage of VAM infections is also caused by the P content that is not high. It causes a balanced mutualism symbiosis between VAM and plants. The low P content in the soil establishes a good symbiosis between VAM and plants. Vesicular-arbuscular mycorrhizae help translocate P from the soil to plants, while plants would provide carbohydrates for VAM growth (Bao et al., 2019); Correa et al., 2012). In contrast, Jasper et al. (1979) stated that in general, plants rich in P lacked carbohydrates, thereby reducing VAM colonization.

Based on ANOVA analysis (Table 5), there was no significant effect of the corn plant varieties on the root infection. The root infections in the local and hybrid corn varieties were 35.56% and 37.158%, respectively. The treatment of varieties was not significant for root infection because the

exudate produced could be the same amount. This is in accordance with research by Nursyamsi (2009), reporting that the average root exudate was not significantly different between corn varieties. Root exudates would appear significantly different at different stages of plant growth. Mc. Cully (1989) in Carrenho et al. (2007) states that exudates are important because root infection occurs when plants emit a signal in the form of root exudates to invite VAM to germinate and penetrate into plant roots.

Plant Height

Figure 1 shows the observation data of the corn plant height every week from one to six weeks after planting. It illustrates that the corn plant height observed once every seven days appears to increase every week. The observation was performed for six weeks (until the stage of maximum vegetative

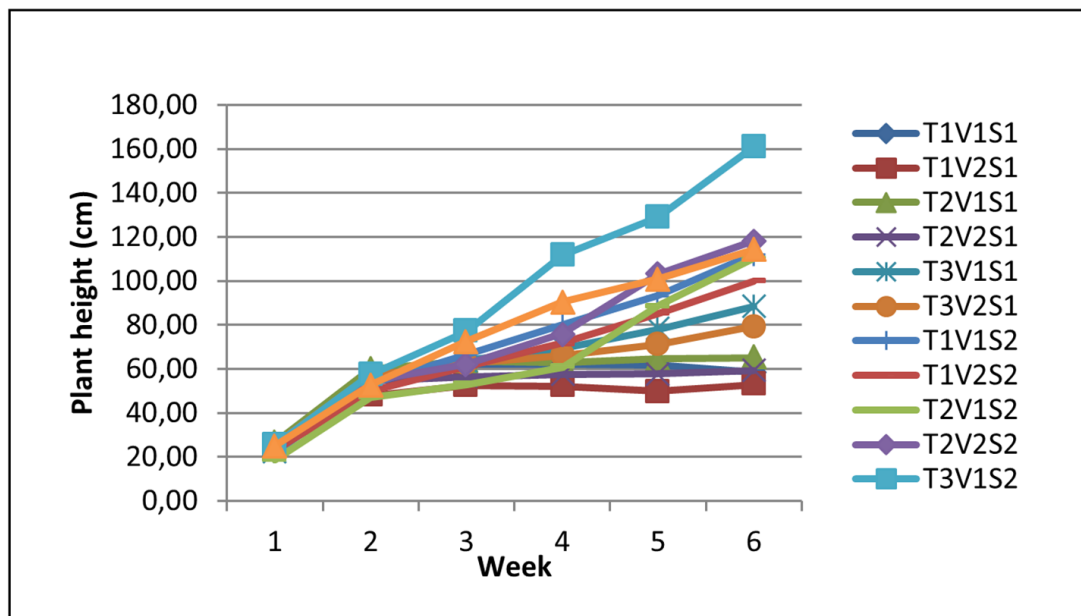


Figure 1. Effects of soil types, corn varieties, and soil sterilization on the corn plant height

growth).

Based on Figure 1, each treatment showed varied average plant height each week. The treatment resulting in the highest plant height was T3V1S2 (Alfisol, local corn variety, unsterilized soil). Mean-

while, the lowest plant height was found in the T1V2S1 treatment (Inceptisol, hybrid corn variety, sterilized soil). The T3V1S2 treatment resulted in the highest plant height because, in this treatment, the Alfisol soil was not sterilized. This result can

also be attributed to the presence of high VAM in the Alfisol soil compared to other soil types.

Ortas et al. (2018) state that plant height growth is influenced by unsterilized soil due to the indigenous VAM infecting the roots so that plants can grow well. Sterilized soil would kill all indigenous VAP so that plants growing on sterile soil conditions do not grow well compared to those growing on non-sterile soils. Ortas (2012) and Ortas et al.

(2002) showed that, without the presence of VAM, plants grew better on unsterilized soils than on sterilized soils, which could be attributed to the effectiveness of indigenous VAM.

Leaf Fresh Weight

Fresh weight is one of the parameters that represent the growth of a plant. The leaf fresh weight is the fresh weight of the leaf after harvest before

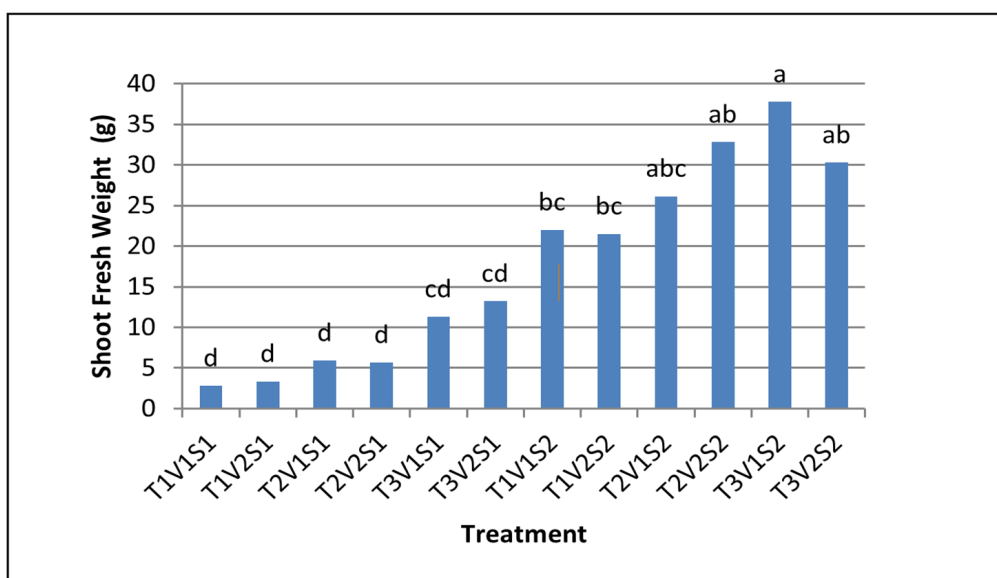


Figure 2. Effect of soil types, corn varieties, and soil sterilization on the leaf fresh weight

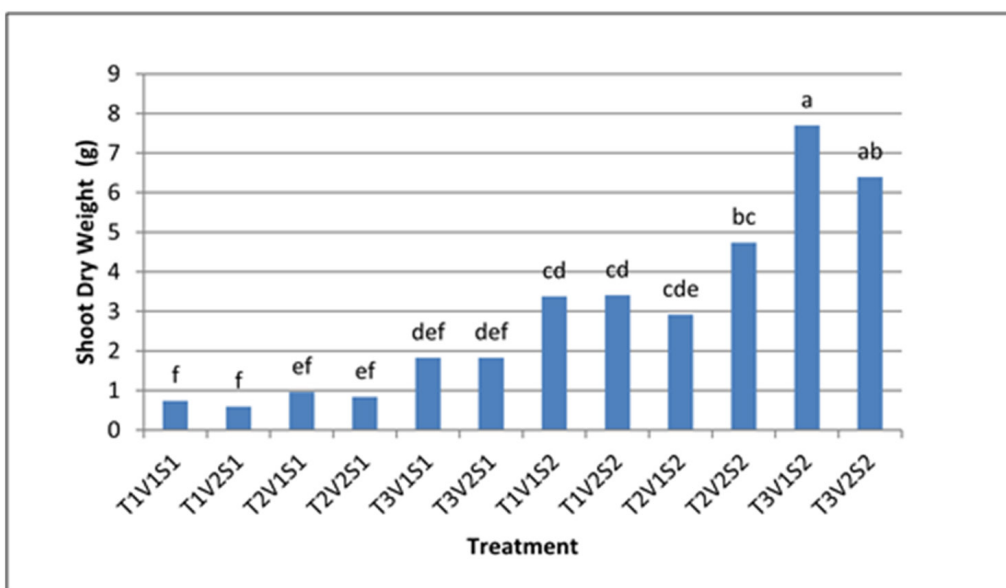


Figure 3. Effect of soil types, corn varieties, and soil sterilization on the leaf dry weight

being oven-dried. The data of the fresh weight of the corn plant leaves are presented in Figure 2.

Based on Figure 2, the treatment of unsterilized Alfisol and local corn variety (T3V1S2) resulted in the highest value of leaf fresh weight compared to other treatments. Meanwhile, the lowest value of leaf fresh weight was found in the treatment of sterilized Inceptisol with local corn variety (T1V1S1). The highest leaf fresh weight was found in the unsterilized Alfisol treatment because the soil had the highest VAM infection rate compared to other soil types; this was related to environmental characteristics that supported the development of VAM such as pH and soil texture. In this treatment, no soil sterilization was carried out so that it did not eliminate VAM (Ortas et al., 2018).

Leaf Dry Weight

Plant dry weight is the weight of plant biomass after all the water content contained in the biomass is removed. Leaf dry weight is the net result of the photosynthesis process produced from the tip of the plant to the base of the plant stem (Samanhudi et al., 2018). This part is formed from the accumulation of carbohydrates and plant metabolism. The data of the leaf dry weight of corn plants are presented in Figure 3.

The local corn variety (T3V1S2) grown on the unsterilized Alfisol (Figure 3) resulted in the highest value of leaf dry weight compared to other

treatments. Meanwhile, the lowest dry weight was found in the hybrid corn variety (T1V2S1) grown on sterilized Inceptisol.

Ortas et al. (2018) state that in general, plants grown on unsterilized soils grow better than those on sterile ones, which is due to the presence of VAM on the unsterilized soils that help provide nutrients. This is consistent with the results of their research showing that the presence of VAM significantly increased biomass uptake. Mawarni et al. (2013) state that VAM would infect plant roots so that the nutrient absorption process supporting photosynthesis would be used for preparing organic matters, thereby improving plant growth and dry weight. Whereas on sterilized soils, plant growth is reduced due to the elimination of VAM that have an essential role in plant growth (Ortas et al., 2016). Grant et al. (2005) state that the increase in plant biomass production by the presence of VAM can be triggered by plants requiring P since the beginning of their growth period.

Leaf P Content

The effects of the soil types, corn plant varieties and soil sterilization on the leaf P content are presented in Table 6 and Table 7.

Based on Table 6, there was an interaction effect of soil types and corn plant varieties on the leaf P content. The combination of Alfisol soil and hybrid variety showed the highest leaf P content

Table 6. Effects of soil types and corn plant varieties on the leaf P content

Soil types	Corn plant varieties		Mean
	Local variety	Hybrid variety	
Inceptisol	0.013 ab	0.013 ab	0.013
Mollisol	0.013 ab	0.005 b	0.009
Alfisol	0.013 ab	0.017 a	0.015
	0.013	0.012	(+)

Remarks: Values followed by the same letters in the same column are not significantly different according to Tukey's test (HSD) at 5 %.

Table 7. Effects of soil sterilization on the leaf P content.

Soil sterilization	Mean
S1	0.008 b
S2	0.017 a

Remarks: Values followed by the same letters in the same column are not significantly different according to Tukey's test (HSD) at 5 %.

compared to other treatments, namely 0.017. This result could be because, in the initial soil analysis, Alfisol had a lower available Ca content than other soil types but with the same available P content categorized in the low category, making Alfisol result in a higher leaf P content due to the lower content of Ca. Thus, the presence of Ca-P is not as high as in other soil types.

Based on Table 7, soil sterilization significantly affected the leaf P content. The highest leaf P content was in the treatment of soil without sterilization with a value of 0.017, while the lowest one was 0.008 in the sterilized soil treatment. The higher value of leaf P content in the treatment without soil sterilization is due to the presence of microorganisms, one of which is indigenous VAM. Vesicular-arbuscular mycorrhizae have fungal mycelium in the soil, which can absorb nutrients beyond the reach of effective absorption by widely reaching the soil (Syamsiyah et al., 2012). The effect of indigenous VAM colonization on P nutrients is often greater, which has an indirect effect on plant metabolism so that it has a symbiotic effect on other nutrients. Besides, the high content of P in plants with VAM is due to the ability of VAM to release phosphatase enzymes that can release AL-P, Fe-P, and Ca-P bonds (Bao et al., 2019).

Leaf P Uptake

Phosphorus is an essential nutrient, which is

absorbed by plants in the form of monovalent phosphate anion ($H_2PO_4^-$) widely available at $pH < 7$ and is absorbed more slowly in the form of divalent anion (HPO_4^{2-}) widely available at $pH > 7$ (Sanjaya et al., 2013). Phosphorus plays an essential role in plant growth, such as photosynthesis, respiration, energy transfer and storage, and cell division and enlargement (Winarso, 2005). P is also essential for development of reproductive parts such as fruit and seed (Havlin et al., 2005). The values of leaf P uptake as affected by soil types, corn plant varieties, and soil sterilization are presented in Table 8 and 9.

There was an interaction effect of soil type and sterilization on the leaf P uptake (Table 8). Meanwhile, corn plant varieties did not significantly affect the leaf P uptake (Table 9). The treatment of un-sterilized Alfisol resulted in the highest leaf P uptake of 0.135 mg/plant compared to other treatments. Ortas et al. (2018) state that generally, plants grown on unsterilized soils showed a higher P content (%) than those grown in sterilized ones, and the presence of VAM was noted to produce a higher P content (%). Smith and Read (2010) state that the amount of P uptake through mycorrhizae (mycorrhizal pathways) could be higher than through host roots. Bao et al. (2019) stated that the mechanism of P distribution to plants was detected by the discovery of the transporter genes *pf P OsPT11* and *Gint PT* (VAM pathway). Both were detected in all VAM infected plant samples

Table 8. Effects of soil types and soil sterilization on the leaf P uptake

Soil types	Soil sterilization				Mean (mg/plant)
	S1		S2		
Inceptisol	0.006	c	0.061	b	0.034
Mollisol	0.005	c	0.046	bc	0.025
Alfisol	0.019	bc	0.135	a	0.077
	0.010		0.081		(+)

Remarks: Values followed by the same letters in the same column are not significantly different according to Tukey's test (HSD) at 5 %.

Table 9. Effects of corn plant varieties on the leaf P uptake

Corn plant varieties	Mean
Local variety	0.045 a
Hybrid variety	0.046 a

Remarks: Values followed by the same letters in the same column are not significantly different according to Tukey's test (HSD) at 5 %.

Table 10. The types of arbuscular mycorrhizae found in each corn variety based on the comparison of the fragment size with the sequence in the database

Treatments	Size (bp)	Types of Mycorrhizae	Acc. Number
T1V2S2	29	Acaulospora mellea	JN687473.1
		Gigaspora margarita	KX879062
		Septoglomus constrictum	MG253627.1
	49	Acaulospora sp	MT860453.1
		Acaulospora spinosa	JX461238
	79	Unidentified	-
	137	Acaulospora rugosa	LN881564.1
	198	Unidentified	-
	222	Unidentified	-
285	Unidentified	-	
T1V1S2	27	Gigaspora margarita	KY024214.1
		Acaulospora sp.	MT860453.1
	49	Acaulospora sp	MT860453.1
		Funneliformis sp.	MT860454
	64	Gigaspora margarita	KC666029
	138	Gigaspora gigantean	AY919834
		Unidentified	-
	223	Unidentified	-
	279	Unidentified	-

but not in non-VAM roots.

Chen et al. (2014) reported that the main effect of VAM on plants was increasing the P uptake. Meanwhile, Astiko et al. (2019) state that the uptake of P and several other elements can be carried out by VAM from both soil and organic fertilizer residues even though the plants are not fertilized. George et al. (1995) mention that the length ratio of VAM hyphae to roots in the soil can be up to 100: 1 or greater, and with external hyphae, VAM roots can reach a wider area.

Leaf P uptake was not significantly affected by corn plant varieties. The leaf P uptake in local and hybrid varieties was 0.045 and 0.046 mg/plant, respectively. Khairiyah et al. (2017) stated that corn varieties did not have a significant difference in the growth when they were in the vegetative period. This could be caused by the effect of biofertilizers and the small number of microbial population density so that some of the functional characters of microbes in dissolving P from limited sources did not work optimally during the vegetative period.

Based on Table 10, the base pairs matched in the NCBI GenBank database, showing several VAM species in both treatments. Vesicular-arbuscular

mycorrhizae identified in both treatments included *Acaulospora* sp., *Funelisformis* sp., *Gigaspora* sp., and *Septoglomus* sp. Ulfa (2011) mentions in his research that VAM have their own characteristics to adapt to changes that occur in the environment. It was stated that in the case of post-mining land, the genus of *Gigaspora* sp. and *Acaulospora* sp. are not very adaptive. Hadianur (2016) in his research also showed that the type of VAM fungi had a very significant effect on plant growth, especially *Gigaspora* sp., which can increase the growth of tomato plants, such as fresh root weight in vegetative phase, fresh root weight in vegetative phase, dry root weight in vegetative phase, dry root weight in vegetative phase and root length in vegetative phase and have a significant effect on nutrient uptake.

As this study showed that the presence of VAM could increase plant growth, such as increasing best results of root infection, leaf fresh and dry weight, leaf P content, and leaf P uptake, consequently, the use of VAM as a plant growth promoter can build sustainable agriculture. In addition, VAM can increase not only P but also other nutrients, thereby indicating the need to analyze different growth variables to evaluate plant response to VAM. It is

also possible to further investigate the specific VAM species in each soil type and the role of species in the growth of plant species. This study showed a positive influence on plant growth.

CONCLUSION

The vesicular-arbuscular mycorrhizal detected in the roots of hybrid variety included *Acaulospora* sp., *Gigaspora* sp., and *Septoglomus* sp., and in the roots of local variety were *Acaulospora* sp., *Gigaspora* sp., and *Funelisiformis* sp. The role of VAM can be seen through unsterilized soil so that there is no VAM elimination in the soil. The results showed that unsterilized soil showed the best results of root infection, leaf fresh and dry weight, leaf P content, and leaf P uptake. Soil type treatment showed that Alfisol showed the best result of root infection, fresh weight, dry weight, leaf P content, and leaf P uptake. The treatment of plant varieties showed that the varieties did not significantly affect the result of root infection, fresh weight, dry weight, leaf P content, and the best leaf P uptake.

ACKNOWLEDGMENT

Special thanks to Gadjah Mada University for the financial support to Fani Aulia Diannastiti through the final assignment review program (RTA), during her postgraduate program. The authors thank the comments and suggestions provided by reviewers.

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