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Spore reproduction, glomalin content, and maize growth on mycorrhizal pot culture using acid mineral soil-based media

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ABSTRACT

Arbuscular mycorrhiza (AM) is known as multifunctional fungi for plant helpers under adverse conditions. However, studies that focused on the production strategy of AM biofertilizers with specific targets related to the soil limitations are limited. This study aimed to examine AM inocula from several sources using various compositions of acid mineral soil-based media and maize hosts in pot cultures to obtain effective AM inocula to handle the phosphor (P) limitations in acid mineral soils. Zeolite and Inceptisols were used as comparing media. The study utilized a completely randomized factorial design with two factors, namely C = media composition (C0: zeolite; C1: representative media of Alfisols; C2: typical media of a mixture of Alfisols, Oxisols, and Ultisols; C3: typical media with the addition of Bio-RP nutrition; C4: Inceptisols) and I = AM inoculum source (I0: without inoculum; I1: inoculum from Alfisols; I2: mixed inoculum from Alfisols, Ultisols, and Oxisols; I3: mixed inoculum from eight soil types), and six replications per treatment combination. The AM cultures on acid mineral soil-based media, which yielded the highest mycorrhizal infection, spore reproduction, and glomalin content, were C1I2 and C3I2, while the highest maize growth and P concentration were obtained with C1I1, CII2, C2I1, and C3I2. Compared to all the treatments, C1I1 and C1I2 are the superior AM cultures. Further study is necessary to confirm the effectiveness of AM cultures.

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1. INTRODUCTION

Acid mineral soils are dominated by three soil orders, namely Alfisols, Ultisols, and Oxisols (Kamran et al., 2018), which occupy approximately 50% of the world's plains (Kochian et al., 2004). The distribution of the three soil orders in Indonesia is around 108.8 million ha with the widest distribution in the Sumatra, Kalimantan, and Papua regions (Mulyani & Sarwani, 2013). Acid mineral soil types are characterized by their low pH (Pan et al., 2019), high exchangeable aluminum content (Seguel et al., 2016), low nutrient content, especially phosphorus (Antoniadis et al., 2016), low cation exchange capacity and base saturation, alkaline saturation (Shi et al., 2017), and low soil organic matter (Sarma et al., 2017). The availability of P is known to be one of the main limiting factors for plant growth in acid mineral soils (Goulding, 2016).

Several studies indicate that the application of arbuscular mycorrhiza (AM) inoculum as a biofertilizer can be an alternative to overcome problems in acid mineral soils, especially regarding the fixation of different P forms, which contribute to their unavailability for plant growth (Mora et al., 2019). AM fungi colonize nearly 78% of plant species and form a symbiosis with the host in exchange for nutrients and water by energy in the form of carbonated compounds (Brundrett & Tedersoo, 2018). The important roles of AM symbiosis include increasing nutrient uptake, especially P (Smith & Smith, 2011), raising water uptake (Santander et al., 2017), improving soil aggregation (Zhang et al., 2019), protecting crops from pests and diseases (Vos et al., 2013), and increasing plant tolerance to the toxicity of heavy metals (Crossay et al., 2020). In the interaction with the host, AM fungi produce a hydrophobic glycoprotein called "glomalin," which has crucial functions, including protecting AM hyphae from the loss of nutrients and water due to erosion (Wu et al., 2014), stabilizing soil aggregates and increasing carbon sequestration (Peng et al., 2013), and raising organic nitrogen in soils (Wright et al., 1998). The glomalin content has also

been used as an indicator of mycorrhizal activity (Lovelock et al., 2004).

AM inoculum has been commonly applied in the forms of spores (Salgado et al., 2016), infected root fragments and/or pot/trap culture containing sand and infected roots (El Maaloum et al., 2020), extramatrical hyphae (Friese & Allen, 1991; He et al., 2020), soil mixed with AM spore propagules and fungal hyphae (Suri & Choudhary, 2013, 2014); Suri & Choudhary, 2014), AM culture mixed with fine sand (Laxminarayana, 2016), and commercial inoculum containing propagules (spores, mycelium, and colonized root fragments in zeolite as medium) (Lopes et al., 2022; Lopes et al., 2021). Many studies have reported the utilization of AM inoculum to improve plant growth and yield. However, information regarding the production strategy of AM inoculum with specific effectiveness targeted to particular crops, soil types, or soil conditions is still limited.

Cahyani, Alfin, et al. (2019) and Cahyani, Suryanti, et al. (2019) emphasized the importance of test-screening the functional capability of different AM inocula under targeted conditions to obtain an effective AM biofertilizer. Evaluating media selection for AM biofertilizer production by considering the characteristics of the target soil type is also crucial.

The present study aimed to investigate the interaction between media composition and mycorrhizal sources in the formulation of AM cultures to determine the high effectiveness of AM cultures, which were targeted to be applied on acid mineral soils. This study is the first to screen AM isolates from various sources using different compositions of acid mineral soil-based media to obtain the effective AM inoculum in handling P constraints in tropical acid mineral soil. Three compositions of acid mineral soil-based media were examined. The first media comprised an Alfisols as one representative type of acid mineral soils; the second media was a mixture of Alfisols, Oxisols, and Ultisols or some typical acid mineral soils; the third media had the same composition as the second media, which comprised a mixture of Alfisols, Oxisols, and Ultisols with additional Bio-RP nutrition. In addition, zeolite and Inceptisols were used for comparison media. Zeolite is the representative of commercial media in producing AM biofertilizers, whereas Inceptisols is the representative of a soil-media type with a minimal limitation for P availability.

2. MATERIALS AND METHODS

2.1. Soil Sampling

Rhizospheric soils of healthy maize and elephant grass from eight soil types (Alfisols, Oxisols, Ultisols, Inceptisols, Andisols, Histosols, Vertisols, and Entisols), distributed across 10 locations in Central and West Java, Indonesia (Table 1) were collected from December 2019 to January 2020 to obtain AM inoculum sources. AM spores were isolated by wet filtration and precipitation methods (Gerdemann & Nicolson, 1963) with glucose solution and centrifuged (Ianson & Allen, 1986). The counting of AM spores under a stereoscopic microscope according to the method of He et al. (2010) and the characterization of AM spores were conducted at the genus level (Souza, 2015).

Non-rhizospheric soils from four soil types (Alfisols, Oxisols, Ultisols, and Inceptisols) (Table 1) were taken in January 2020 for pot culture media. The soil samples were then air-dried, sieved at a diameter size of 2 mm, and prepared for pot culture media. In addition to the four soil types, zeolite was used as a comparison media. Furthermore, zeolite was added to all soil-based media with a 20% w/w zeolite proportion of the total weight of media/pot to ensure media porosity. The chemical characteristics of each media type are presented in Table 2.

Table 1. Soil sampling locations for rhizospheric soil for mycorrhizal arbuscular inoculum sources and non-rhizospheric soil for pot culture media

Sampling Location	Soil Type	Latitude & Longitude	Diversity Mycorrhizal Genera	Spore density (spores/100 g soil)
Jumantono, Karanganyar, CJ	Alfisols	07°37'47"S; 110°56'51"E	*Glomus, Acaulospora*	703 ± 25
Bawen, Semarang, CJ	Oxisols	07°15'58"S; 110°27'06"E	*Glomus, Gigaspora*	91 ± 16
Kentrong, Lebak, WJ	Ultisols	06°29'01"S; 106°28'01"E	*Acaulospora, Glomus*	537 ± 21
Ceper, Klaten, CJ	Inceptisols	07°39'20"S; 110°44'21"E	*Gigaspora, Acaulospora, Glomus*	1105 ± 93
Tengaran, Semarang, CJ	Andisols	07°15'51"S; 110°26'57"E	*Glomus, Acaulospora*	2273 ± 35
Tawangmangu, Karanganyar, CJ	Andisols	07°39'52"S; 111°10'45"E	*Glomus, Gigaspora, Acaulospora*	4912 ± 13
Banyubiru, Semarang, CJ	Histosols	07°18'23"S; 110°25'21"E	*Glomus, Acaulospora*	685 ± 17
Bawen, Semarang, CJ	Histosols	07°15'48"S; 110°27'00"E	*Scutellospora, Gigaspora, Glomus*	650 ± 10
Gondangrejo, Karanganyar, CJ	Vertisols	07°31'06"S; 110°56'51"E	*Acaulospora,Glomus, Gigaspora, Scutellospora*	489 ± 20
Pracimantoro, Wonogiri, CJ	Entisols	07°51'46"S; 110°54'26"E	*Glomus, Acaulospora*	251 ± 16

Remarks: CJ = Central Java Province and WJ = West Java Province, Indonesia

Table 2. Chemical characteristics of non-rhizospheric soil and zeolite used for pot culture media

Chemical Analysis	Media Types				
	Alfisols	Oxisols	Ultisols	Inceptisols	Zeolite
pH H_2O	6.1	5.7	4.5	6.45	7.5
pH KCl	5.0	4.8	3.3	5.88	7.3
Organic C (%)	1.6	0.3	1.7	1.8	0.1
Total N (%)	0.1	0.04	0.28	0.25	0.02
Available P ($mg.kg^{-1}$)	1.03	0.83	1.65	2.16	0.57
Total P (%)	0.024	0.037	0.074	0.081	0.009
Al-dd ($cmol(+).kg^{-1}$)	1.32	2.61	15.83	0.53	0.00

2.2. Greenhouse Pot Experiment

A pot experiment was performed at the greenhouse of the Faculty of Agriculture, Sebelas Maret University, Surakarta, Central Java, Indonesia, from February to June 2020.

The greenhouse experiment was conducted using a completely randomized factorial design with two factors, namely media composition (C) and AM inoculum source (I), which comprised five and four levels, respectively. All treatment combinations were set up for six replications. The first factor of media composition (C) comprised the following: C0: zeolite media; C1: representative media of Alfisols; C2: typical media of a mixture of Alfisols, Oxisols, and Ultisols; C3: typical media with additional Bio-RP nutrition; C4: Inceptisols media. The second factor of AM inoculum source (I) comprised the following: I0: without inoculum; I1: inoculum from Alfisols; I2: mixed inoculum from Alfisols, Oxisols, and Ultisols; I3: mixed inoculum from eight soil types (Alfisols, Oxisols, Ultisols, Inceptisols, Andisols, Histosols, Vertisols, and Entisols). Each pot culture with a diameter and depth of 8 and 12 cm, respectively, was prepared by adding 300 g media, which comprised 80% (w/w) of a mixture of soil compositions according to treatments (C1, C2, C3, and C4) and 20% (w/w) of zeolite. The treatment of C0 contained 100% zeolite. Bio-RP nutrition, which was added to media C3, contained a mixture of liquid cultures of N-fixing and phosphate-solubilizing bacteria at a dosage of 10 mL/pot and 0.04 g/pot of rock phosphate. Maize BISI II variety was planted in two seeds/pot. Seven days after planting (DAP), the AM inoculum was inoculated in the rhizospheric area at a dosage of 30 spores/pot comprising AM sources according to each treatment. The moisture content of pot culture media was maintained at around 70% of field capacity. The plants were harvested on 70 DAP at the maximum vegetative period for three replications of the treatments. The plant and root samples and the media were then subjected to analysis of plant growth parameters, mycorrhizal infectivity, and soil/media chemical characteristics. For the three other replications, pot cultures were extended for 30 days with no additional watering or allowing the plant to dry until 100 DAP, and then the media was subjected to the AM spore reproduction analysis.

2.3. Analysis of Pot Culture Media, Arbuscular Mycorrhiza (AM) Colonization, and Plant Growth

The chemical characteristics of each media (soil and zeolite) were analyzed considering pH (H_2O) and (KCl) using a digital pH meter (1:2.5; soil:solution), organic C (Walkley and Black method), total N (Kjeldahl method), and available P (Olsen method) (Pansu & Gautheyrou, 2006).

The AM colonization parameter was observed from the AM infectivity, spore density (SD), and the content of glomalin-related soil protein (GRSP). The AM infectivity was measured on the basis of the root staining method (Giovannetti & Mosse, 1980; Phillips & Hayman, 1970). The SD, which was observed on 70 and 100 DAP, was measured on the basis of the sequential spore isolation procedures by using the wet filtration and precipitation methods (Gerdemann & Nicolson, 1963)) and the centrifugation method with glucose solution (Ianson & Allen, 1986). AM spores were continuously counted under a stereoscopic microscope (He et al., 2010). The GRSP was measured as easily extractable glomalin-related soil protein (EE-GRSP) and total glomalin-related soil protein (T-GRSP), which were both extracted with 50 mM sodium citrate (pH 8.0) and then autoclaved for 30 and 90 min, respectively, according to Wright et al. (1998) as modified by Syamsiyah et al. (2014). The supernatant from each extract was collected by centrifugation and was then subjected to determining GRSP using the Bradford assay with bovine serum albumin as the standard (Bradford, 1976).

Plant growth parameters, including plant height, root fresh and dry weight, plant fresh and dry weight, chlorophyll content, and P concentration, were observed. The chlorophyll content was measured using a spectrophotometer (Hendry & Grime, 1993). The P concentration content was measured from the leaf samples by using the acid digestion method with mixtures of nitric and perchloric acids (Wheal et al., 2011; Zarcinas et al., 1987).

2.4. Statistical Analysis

All obtained data were analyzed by ANOVA (*Analysis of Variance*), DMRT (*Duncan Multiple Range* 5% Test), and Pearson correlation analysis. Statistical analysis was performed using the IBM SPSS 23.0 application on Windows 10.

3. RESULTS

3.1. Effects of the Treatments on Soil Chemical Characteristics

The interaction between the treatments of media composition and AM inoculum source (C × I) had a very significant effect ($p < 0.01$) on pH (H_2O), organic C, total N, and available P but no significant effect ($p > 0.05$) on pH (KCl) (Table 3). Among the acid mineral soil-based media (C1, C2, and C3), on the representative media of Alfisols (C1), the inoculation treatment of I2 resulted in the highest available P with an increase of 160% compared with the corresponding control (C1I0).

Table 3. Effect of the treatment on soil chemical characteristics

	pH H_2O	pH KCl	Organic Carbon (%)	Total N (%)	Available P (mg kg^{-1})
F values					
C	122**	116**	52.9**	62.1**	69.7**
I	8**	3.6**	121**	144**	89**
C × I	2.4**	1.2ns	19.7**	18.1**	8.5**
Media Composition (C) × Mycorrhizal Arbuscular Inoculum (I)					
C0I0	8.7 ± 0.19l	6.8 ± 0.34	0.3 ± 0.05a	0.08 ± 0.02a	0.46 ± 0.03a
C0I1	8.3 ± 0.11jk	7.1 ± 0.29	2.2 ± 0.04fg	0.21 ± 0.01b	2.92 ± 0.08cdef
C0I2	8.0 ± 0.35hijk	6.8 ± 0.23	1.6 ± 0.05cde	0.29 ± 0.01de	1.99 ± 0.14b
C0I3	8.3 ± 0.20jk	6.8 ± 0.16	1.3 ± 0.06c	0.35 ± 0.01f	2.68 ± 0.04bcde
C1I0	7.2 ± 0.04cde	6.4 ± 0.40	1.7 ± 0.02de	0.17 ± 0.00b	2.36 ± 0.32bc
C1I1	7.4 ± 0.22efg	6.6 ± 0.24	3.1 ± 0.04j	0.49 ± 0.01h	4.22 ± 0.10gh
C1I2	7.2 ± 0.05cdef	6.8 ± 0.14	2.3 ± 0.02gh	0.28 ± 0.01de	6.14 ± 0.09k
C1I3	7.6 ± 0.15fghi	6.5 ± 0.35	1.9 ± 0.02ef	0.37 ± 0.00g	3.37 ± 0.09defg
C2I0	6.2 ± 0.11a	4.3 ± 0.62	0.9 ± 0.01b	0.11 ± 0.01a	2.48 ± 0.03bcd
C2I1	6.6 ± 0.26ab	4.6 ± 0.71	1.5 ± 0.05cd	0.27 ± 0.00cd	3.80 ± 0.12fg
C2I2	6.4 ± 0.23a	4.8 ± 0.76	2.4 ± 0.04gh	0.33 ± 0.01ef	4.86 ± 1.38hij
C2I3	6.9 ± 0.14bcd	4.5 ± 0.20	2.8 ± 0.32ij	0.19 ± 0.02b	5.48 ± 0.13ijk
C3I0	6.4 ± 0.24a	4.5 ± 0.70	1.6 ± 0.45cde	0.22 ± 0.06bc	3.41 ± 0.87efg
C3I1	6.6 ± 0.14ab	5.0 ± 0.41	1.7 ± 0.11de	0.28 ± 0.01d	3.83 ± 0.08fg
C3I2	6.6 ± 0.19ab	4.7 ± 0.28	2.9 ± 0.25ij	0.36 ± 0.04f	5.70 ± 0.07jk
C3I3	6.9 ± 0.38bc	4.7 ± 0.26	2.5 ± 0.60ghi	0.43 ± 0.10g	5.18 ± 1.41ij
C4I0	7.4 ± 0.31def	6.0 ± 0.17	1.5 ± 0.04cd	0.26 ± 0.00cd	3.21 ± 0.12cdef
C4I1	7.6 ± 0.20efgh	6.4 ± 0.15	2.4 ± 0.08gh	0.34 ± 0.01ef	4.77 ± 0.03hi
C4I2	7.8 ± 0.41ghij	6.3 ± 0.04	3.0 ± 0.03j	0.41 ± 0.01g	7.59 ± 0.07l
C4I3	8.1 ± 0.38ijk	6.7 ± 0.10	2.7 ± 0.03hi	0.46 ± 0.00gh	5.17 ± 0.06ij

Remarks: Significant levels: ns = no significant, * = $p < 0.05$, ** = $p < 0.01$. Means followed by same letter (s) are not significantly different at 5% level by DMRT.

In the typical media of C2, the treatments of I2 and I3 gave significant increases in available P at 96% and 121%, respectively, compared with C2I0. In the typical + Bio-RP nutrition media of C3, the significant increase in available P as indicated by C3I2 and C3I3 was at 67% and 52%, respectively, compared with C3I0 (Table 3). Among those treatments that yielded the highest increase in available P on the three acid mineral soil-based media (C1, C2, and C3), by comparing with the respective corresponding control, namely C1I2 with C1I0, C2I3 with C2I0, and C3I2 with C3I0, it was revealed that C1I2 yielded the highest increase in available P to the level of 6.14 $mg.kg^{-1}$.

The addition of Bio-RP nutrition on C3 media significantly increased organic C and total N. Thus, comparing C3I0 and C2I0, the organic C and total N of C3I0 were 78% and 100% higher than C2I0, respectively.

The status of available P on zeolite media without mycorrhizal inoculation (C0I0) was at 0.46 mg kg^{-1} or approximately 19.5% of the available P level on Alfisols media (C1I0) (Table 3). However, on the C0 media, the inoculation of mycorrhizal with the treatment of I1, I2, and I3 increased available P by 535%, 332%, and 482% compared with I0 (Table 3), respectively. Table 3 shows that the highest increase in available P resulted in 2.92 mg kg^{-1} on C0I1.

For the Inceptisol media (C4), the level of available P on C4I0 was 3.21 mg kg^{-1} or 136% of the available P level on Alfisols media (C1I0) (Table 3). The mycorrhizal inoculation treatments of I1, I2, and I3 on the Inceptisol media resulted in an increase in available P by 49%, 136%, and 61%, respectively, compared with C4I0 (Table 3). This finding indicates a similar percentage of the increase as found on acid mineral soil-based media.

3.2. Effects of Treatments on Mycorrhizal Colonization

The ANOVA results revealed the very significant effect ($p < 0.01$) of the interaction of the treatments (C × I) in increasing mycorrhizal infectivity (MI), SD at 70 and 100 DAP, EE-GRSP concentration, and T-GRSP (Table 4). By comparing the treatments on acid mineral soil-based media (C1, C2, and C3) with the corresponding controls that produced the highest MI, SD at 100 DAP, EE-GRSP, and T-GRSP, C1I2 compared with C1I0, C2I3 with C2I0, C3I2 and C3I0 showed the following: an increase of 507%, 248%, and 340% for MI, respectively; an increase of 86%, 98%, and 81% for SD at 100 DAP, respectively; an increase of 175%, 86%, and 115% for EE-GRSP, respectively; and an increase of 108%, 52%, and 54% for T-GRSP, respectively. Thus, C1I2, which had MI of 91%, SD 697 spores/100 g of media, EE-GRSP of 0.77%, and T-GRSP of 0.81%, showed the highest increase in MI, SD, EE-GRSP, and T- GRSP among acid mineral soil-based media.

On zeolite media (C0), the highest MI was exhibited by the treatment combination C0I3, whereas on Inceptisol media (C4), the highest MI was found in the treatment combination C4I2. As for the variable SD, the treatment without inoculum (I0) on zeolite media (C0) showed no mycorrhizal spores. On zeolite media, the highest spore production at 100 DAP was obtained by the treatment C0I1 (341 spores/100 g of media), followed by C0I3 (282 spores/100 g of media) and C0I2 (250

spores/100 g of media). These spore productions on zeolite showed significantly lower levels compared with those on acid mineral soil-based media. Notably, among the treatments without mycorrhizal inoculation on acid mineral soil-based media (C1I0, C2I0, and C3I0), the lowest SD was yielded by the C2I0 treatment (275 spores/100 g media) (Table 4). On Inceptisol media (C4), the highest SD was found in the treatment C4I2. As represented by EE-GRSP and T-GRSP, the highest glomalin content was obtained by the C0I1 treatment on zeolite and C4I2 on Inceptisols media.

3.3. Effects of Treatments on Plant Growth

The interaction of the treatments (C × I) had a very significant effect ($p < 0.01$) in increasing P concentration, chlorophyll content, plant height, root fresh and dry weight, and plant fresh and dry weight. The DMRT results revealed that on mineral acid soil-based media (C1, C2, and C3), the highest increases in plant fresh and dry weights on media C1 were obtained by the two treatments of C1I1 and C1I2. Compared with C1I0, the increase in plant fresh weight was 19.2% and 18.7% and the increase in plant dry weight was 80.8% and 59.1%, respectively. On media C2, the highest plant fresh and dry weights were obtained by C2I1; compared with C2I0, the increase was 75.5% and 55.9%, respectively. On media C3, the highest plant fresh and dry weights were obtained by C3I2; compared with C3I0, the increase was 15.8% and 12.3%, respectively (Table 5).

The measurement of root fresh and dry weights showed that the highest level on media C1 was obtained by the treatment of C1I1 (5.22 and 2.85 g plant^{-1}) and followed by C1I2 (3.71 and 1.95 g plant^{-1}); on media C2, the highest level was obtained by the treatment C2I1 (5.09 and 2.35 g plant^{-1}); on media C3, the highest level was obtained by the treatment C3I2 (5.28 and 2.16 g plant^{-1}) (Table 5).

The variable of P concentration in shoot tissues, which was the main indicator to examine the functional capability of AM cultures in handling the P limitation on acid mineral soils in the present study, showed varying results for each type of media composition.

The highest P concentrations on each composition of acid mineral soil-based media (C1, C2, and C3) were obtained by C1I1 (0.21%), C1I2 (0.2%), C2I1 (0.16%), and C3I2 (0.19%) (Table 5). Compared with the corresponding controls (C1I0, C2I0, and C3I0), the increase in P concentration obtained by treatments C1I1, C1I2, C2I1, and C3I2 were 200%, 186%,167%, and 136%, respectively.

Table 4. Effect of the treatment on arbuscular mycorrhizal parameters

	MI (%)	SD 70 DAP (spore/100 g media)	SD 100 DAP (spore/100 g media)	EE-GRSP (%)	T-GRSP (%)
F values					
C	5.4**	3233**	1899**	5415**	29931**
I	75.3**	3828**	2521**	2891**	14765**
C × I	8.6**	438**	266**	579**	2141**
Media Composition (C) × Mycorrhizal Arbuscular Inoculum (I)					
C0I0	0 ± 0a	0 ± 0a	0 ± 0a	0.14 ± 0a	0.14 ± 0a
C0I1	61 ± 10.07gh	291 ± 6e	341 ± 14.2d	0.32 ± 0.001e	0.56 ± 0.002h
C0I2	32 ± 4bcde	191 ± 7.6b	250 ± 6.2b	0.21 ± 0.001b	0.37 ± 0.003b
C0I3	52 ± 10.58fg	251 ± 14.2c	282 ± 10.8c	0.26 ± 0.003c	0.44 ± 0.002d
C1I0	15 ± 6.11ab	350 ± 4.6g	374 ± 10.5e	0.28 ± 0.001d	0.39 ± 0.005c
C1I1	56 ± 16fgh	465 ± 12k	518 ± 7.6i	0.46 ± 0.003h	0.65 ± 0.001k
C1I2	91 ± 4.62i	651 ± 6q	697 ± 6.2o	0.77 ± 0.004n	0.81 ± 0.002q
C1I3	29 ± 8.33bcd	376 ± 4.6h	405 ± 12f	0.36 ± 0.003f	0.46 ± 0.001e
C2I0	21 ± 6.11bc	264 ± 6d	275 ± 8.7c	0.35 ± 0.003f	0.50 ± 0.003g
C2I1	40 ± 4cdef	346 ± 4.6g	403 ± 12.1f	0.45 ± 0.003gh	0.61 ± 0.002j
C2I2	64 ± 4gh	433 ± 9.2j	465 ± 13.1h	0.52 ± 0.003i	0.68 ± 0.003 m
C2I3	73 ± 28.94hi	517 ± 7.6l	547 ± 7.6j	0.65 ± 0.027 m	0.76 ± 0.002o
C3I0	20 ± 12b	313 ± 9.2f	370 ± 8.7e	0.26 ± 0.003c	0.48 ± 0.003f
C3I1	32 ± 12bcde	407 ± 6.2i	428 ± 13.9g	0.36 ± 0.002f	0.58 ± 0.004i
C3I2	88 ± 8i	606 ± 6o	671 ± 9.6 n	0.56 ± 0.003j	0.74 ± 0.003n
C3I3	47 ± 8.33defg	543 ± 5.2 m	613 ± 12.1l	0.44 ± 0.002g	0.66 ± 0.003l
C4I0	24 ± 4bc	263 ± 4.6d	272 ± 3.5c	0.59 ± 0.004k	0.77 ± 0.003p
C4I1	51 ± 10.07efg	322 ± 4.6f	368 ± 9.2e	0.63 ± 0.004lm	0.85 ± 0.003r
C4I2	87 ± 8.33i	646 ± 7.6p	665 ± 8.7 m	0.77 ± 0.001n	1.01 ± 0.002t
C4I3	61 ± 6.11gh	556 ± 7.6n	609 ± 7.9k	0.63 ± 0.013l	0.92 ± 0.001s

Remarks: MI: Mycorrhizal Infectivity; SD 70 DAP: Spore Density 70 Days After Planting; SD 100 DAP: Spore Density 100 Days After Planting; E-GRSP: Extractable Glomalin-Related Soil Protein; T-GRSP: Total Glomalin-Related Soil Protein. Significant levels: ns = no significant, * = $p < 0.05$, ** = $p < 0.01$. Means followed by the same letter (s) are not significantly different at 5% level by DMRT.

Table 5. Effect of the treatment on plant growth: plant height, root fresh and dry weight, plant fresh and dry weight, chlorophyll, and P concentration

	Plant Height (cm)	Root Fresh Weight (g)	Root Dry Weight (g)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Chlorophyll	P concentration (%)
C	340**	12**	9**	171**	67**	9**	1718**
I	196**	3**	3ns	41**	19**	20**	5254**
C × I	43**	58**	15**	30**	15**	5**	653**
C0I0	23.6 ± 0.25a	1.54 ± 0.02a	0.67 ± 0.05a	2.28 ± 0.06a	1.00 ± 1a	0.16 ± 0.002a	0.04 ± 0.002a
C0I1	26.7 ± 0.98b	1.80 ± 0.06a	0.74 ± 0.03a	2.64 ± 0.06a	1.09 ± 1.09a	0.33 ± 0.004bc	0.06 ± 0.003c
C0I2	23.9 ± 1.30a	2.33 ± 0.48b	0.86 ± 0.02ab	3.79 ± 0.68b	1.26 ± 1.26a	0.39 ± 0.005cdef	0.08 ± 0.001e
C0I3	36.4 ± 0.78d	3.11 ± 0.15d	1.24 ± 0.12bc	3.97 ± 0.11b	1.72 ± 1.72b	0.44 ± 0.005def	0.10 ± 0.006g
C1I0	40.2 ± 2.14e	3.19 ± 0.06d	1.23 ± 0.01bc	5.73 ± 0.24ef	2.08 ± 2.08cd	0.29 ± 0.008b	0.07 ± 0.004d
C1I1	47.6 ± 1.21g	5.22 ± 0.17h	2.85 ± 0.05h	6.83 ± 0.30g	3.76 ± 3.76h	0.48 ± 0.002f	0.21 ± 0.002n
C1I2	54.4 ± 0.85h	3.71 ± 0.07ef	1.95 ± 0.14ef	6.80 ± 0.18g	3.31 ± 2.31h	0.51 ± 0.011g	0.20 ± 0.003 m
C1I3	40.3 ± 1.21e	3.47 ± 0.27def	1.53 ± 0.04cde	5.59 ± 0.21e	2.30 ± 2.30cde	0.41 ± 0.013cdef	0.09 ± 0.003f
C2I0	30.0 ± 2.79c	2.74 ± 0.28c	1.52 ± 0.15cde	4.21 ± 0.65bcd	1.86 ± 1.86bc	0.38 ± 0.112bcde	0.06 ± 0.001c
C2I1	41.2 ± 0.81e	5.09 ± 0.07h	2.35 ± 0.34g	7.39 ± 0.37h	2.90 ± 2.90g	0.39 ± 0.023cdef	0.16 ± 0.001k
C2I2	32.3 ± 1.01c	3.27 ± 0.07d	1.69 ± 0.23de	4.68 ± 0.44cd	2.33 ± 2.33de	0.38 ± 0.128bcd	0.15 ± 0.002j
C2I3	47.1 ± 1.21g	2.61 ± 0.23bc	1.55 ± 0.02cde	4.12 ± 0.18bc	2.17 ± 2.17de	0.34 ± 0.028bcd	0.12 ± 0.001h
C3I0	40.5 ± 2.59e	4.15 ± 0.13g	1.86 ± 0.56ef	5.90 ± 0.36ef	2.52 ± 2.52def	0.37 ± 0.021bcd	0.08 ± 0.002e
C3I1	40.5 ± 1.57e	3.67 ± 0.25ef	1.48 ± 0.25cde	5.93 ± 0.27ef	2.25 ± 2.25cde	0.38 ± 0.013bcde	0.13 ± 0.003i
C3I2	48.8 ± 0.81g	5.28 ± 0.17h	2.16 ± 0.13fg	6.83 ± 0.23g	2.83 ± 2.79g	0.41 ± 0.059cdef	0.19 ± 0.004l
C3I3	54.4 ± 1.16h	3.48 ± 0.33def	1.51 ± 0.15cde	5.53 ± 0.42e	2.54 ± 2.54fg	0.35 ± 0.066bcd	0.15 ± 0.001j
C4I0	40.0 ± 1.20e	3.38 ± 0.35de	1.41 ± 0.51cd	4.79 ± 0.29d	2.06 ± 2.06bcd	0.33 ± 0.029bc	0.05 ± 0.002b
C4I1	44.1 ± 1.69f	3.68 ± 0.22ef	1.48 ± 0.04cde	6.28 ± 0.70fg	2.26 ± 2.26cde	0.39 ± 0.052cdef	0.15 ± 0.003j
C4I2	40.2 ± 1.25e	3.80 ± 0.01f	1.53 ± 0.11cde	5.45 ± 0.23e	2.27 ± 2.27cde	0.41 ± 0.014cdef	0.20 ± 0.003 m
C4I3	56.4 ± 1.01h	5.35 ± 0.05h	2.48 ± 0.21g	8.72 ± 0.22i	3.61 ± 3.61h	0.47 ± 0.055ef	0.22 ± 0o

Remarks: Significant levels: ns = no significant, * = $p < 0.05$, ** = $p < 0.01$. Means followed by same letter (s) are not significantly different at 5% level by DMRT.

Based on the DMRT result, the highest chlorophyll content was indicated by the treatment of C1I2 (0.51 mg g^{-1}), followed by C1I1 (0.48 mg g^{-1}), C3I2 (0.41 mg g^{-1}), C1I3 (0.41 mg g^{-1}), and C1I3 (0.41 mg g^{-1}) (Table 5), whereas the chlorophyll content on other acid mineral soil media was at the slightly low level at the range of 0.29–0.38 mg/g (Table 5). On zeolite (C0) and Inceptisols (C4) media, AM cultures I3 (C0I3 and C4I3) showed the highest effectiveness on all plant growth variables compared to I1 and I2.

4. DISCUSSION

AM inoculation significantly increased the pH (H_2O) on all media, except for zeolite media, wherein a decrease was observed. Although the effect of AM inoculation on pH (H_2O) showed a different direction on zeolite (C0) compared with the other media (C1, C2, C3, and C4), they exhibited the same effect in increasing the available P. Arraudah et al. (2020) reported the same trend on acid mineral soil; the application of AM independently or in interaction with humic acid increased the pH of Ultisols, wherein the maximum increase in pH value from pH 4.4 to 6.1 was obtained by the interaction of AM inoculant with a dose of 5 g $plant^{-1}$ and humic acid with a concentration of 45 mL L^{-1}. The decrease in pH on zeolite media due to AM inoculation in the present study was supported by El Maaloum et al. (2020), who reported that the additional AM in combination with phospho-compost and phosphate-solubilizing bacteria on alkaline soils with pH 8.0 resulted in considerable effects in decreasing soil pH to 7.5 after 60 days compared with the treatment without AM which exhibited no change in pH value. An interesting phenomenon was found in the present study: AM inoculation increased pH on acid mineral soil-based media and decreased pH on zeolite media, which might statistically indicate that no significant correlation ($p > 0.05$) existed between pH H_2O and available P in the media. Based on the present findings, AM is crucial in supporting buffering in soil pH that might be mediated by the indirect effect through the organic exudates released from the host roots. This mechanism was supported by the data of organic C and total N obtained in the present study, which significantly increased due to AM inoculation.

The two variables had positive and significant correlation with soil available P ($r = 0.838$; $p < 0.01$ and $r = 0.579$; $p < 0.01$, respectively). The different levels of the effects in increasing organic C, total N, and available P given by each AM inoculum source (I1, I2, and I3) on all media that were identified in the present study were supported by Ma et al. (2022), who reported that different mycorrhizal have various effects on the accumulation and distribution of nutrients at different sites in plant tissues and different contents of organic acids in root exudates. The increase in available P on acid mineral soils could be explained by the potential Al exposure reduction by AM fungi (Aguilera et al., 2017; Kumar et al., 2014) and nutrient mobilization improvement through intensive expansion of the AM hyphae (Mehta et al., 2019). The inoculum I2 (a mixture of inoculum from Alfisols, Oxisols, and Ultisols) was the most effective AM on acid mineral soil-based media due to the highest increase in soil available P, especially on media C1 (Alfisols media).

The total SD at 100 DAP was higher than that at 70 DAP, proving that water stress treatment stimulated the reproduction of mycorrhizal spores. This study revealed that the inoculum I2 (a mixture of inoculum from Alfisols, Oxisols, and Ultisols) exhibited superiority in increasing SD compared with I3 (a mixture of inoculum from Alfisols, Oxisols, Ultisols, Inceptisols, Andisols, Histosols, Entisols, and Vertisols) and I1 (a single inoculum from Alfisols). This finding indicated that the inoculum containing a mixture of AM propagules originating from the same typical acid mineral soils with similar characteristics resulted in higher spore production compared with that containing a mixture of AM propagules from various soil types or a single soil type. Based on the study on cucumber seedlings, Chen et al. (2017) reported that the inoculation of inoculum containing various AM genera (*Claroideoglomus* sp., *Funneliformis* sp., *Diversispora* sp., *Glomus* sp., and *RhizophagusHas* sp.) highly increased mycorrhizal colonization compared with that of inoculum containing several species from the same genus (*Glomus intraradices*, G. *microageregatum* BEG, and G. *claroideum* BEG 210) and a single inoculum (*Funneliformis mosseae*).

Compared with acid mineral soil-based media, the highest SD on zeolite and Inceptisol media were obtained from different mycorrhizal sources. The highest SD on zeolite was indicated by I1 (C0I1), whereas that on Inceptisol was indicated by I2 (C4I2). The present study revealed that propagation of mycorrhizal propagules from the same sources using zeolite as media in pot culture yielded significantly different effects on spore reproduction compared with acid mineral soil-based media. The SD is the main indicator to determine the quality of the mycorrhizal biofertilizer product. Zeolite is known as common media in commercial products of mycorrhizal biofertilizers in the agricultural market. The results of this study estimated that the high SD in the mycorrhizal biofertilizer product using zeolite or other media as carriers does not always result in high infectivity, spore reproduction, and effectiveness of plant growth in the application to the targeted soil. On the contrary, a low SD of the mycorrhizal biofertilizer product may be highly effective for plant growth.

Based on the measurements of all variables of AM parameters, the inoculum I2 showed superiority in increasing MI, SD, EE-GRSP, and T-GRSP on acid mineral soil-based media (C1, C2, and C3) compared with inoculum I3 and I1. Inoculum I2, which contained a mixture of isolates from several types of acid mineral soils (typical soils), was considered to be more adaptable and had multifunctional capabilities to handle the limitations of acid mineral soils compared with isolates I3 and I1.

The present study also showed a strong correlation between MI and other mycorrhizal parameters (SD, EE-GRSP, and T-GRSP), in which the increase in MI level was followed by the increase in SD and GRSP in the rhizosphere soil. The results of EE-GRSP and T-GRSP on acid mineral-based media (C1, C2, and C3) and Inceptisol media (C4) showed a similar pattern to MI and SD, in which the inoculum treatments of I2 and I3 showed higher EE-GRSP and T-GRSP than I1. The EE-GRSP and T-GRSP showed a significantly positive correlation with MI ($r = 0.722$; $p < 0.01$ and $r = 0.716$; $p < 0.01$) and SD 100 DAP ($r = 0.756$; $p < 0.01$ and $r = 0.776$; $p < 0.01$). The functional contributions of mycorrhizal in increasing nutrients status in media were also reflected from the EE-GRSP and T-GRSP contents, which showed positive correlations with the soil organic C ($r = 0.0686$; $p < 0.01$ and $r = 0.751$; $p < 0.01$), with total N ($r = 0.415$; $p < 0.01$ and $r = 0.526$; $p < 0.01$) and available P ($r = 0.836$; $p < 0.01$ and $r = 0.875$; $p < 0.01$). Several studies have also shown a positive correlation between SD, MI, and GRSP (Ji et al., 2019; Reyes et al., 2019). Mycorrhizal colonization stimulates the production of GRSP through the formation of hyphae (Peng et al., 2013) because most GRSP (>80%) is produced by mycorrhizal hyphae (Driver et al., 2005); therefore, if mycorrhizal activity decreases, then the production of GRSP will also decline (Steinberg & Rillig, 2003).

The T-GRSP was significantly higher than the EE-GRSP under all treatment combinations (Table 4). This finding could be explained by the extended duration in autoclaving of 90 min, which results in a significant recovery of the extracted T-GRSP compared with the 30 min duration in autoclaving to extract EE-GRSP. The present findings are supported by Syamsiyah et al. (2014), who reported a significantly higher T-GRSP compared with EE-GRSP by the same method.

Compared with the results on acid mineral soil-based media, a different finding was found in zeolite media (C0) where inoculum I1 resulted in higher EE-GRSP and T-GRSP compared with inoculum I2 and I3. On zeolite media with no inoculation (C0I0), although no indication of mycorrhizal infection was found in maize roots and mycorrhizal spores in the rhizosphere, EE-GRSP and T-GRSP were still detected at low levels (0.14%). These EE-GRSP and T-GRSP contents on C0I0 were significantly lower compared with all treatments without AM inoculation on acid mineral soil-based media (C1I0, C2I0, and C3I0), which were at the range of 0.26%–0.35% and 0.39%–0.50%, respectively. The existence of indigenous AM fungi in each media (C1, C2, and C3) based on the MI and SD contributed to determining the content of GRSP. No indigenous AM was found on zeolite media as indicated by the absence of mycorrhizal infection and spores in the treatment of C0I0. Thus, these findings, wherein EE-GRSP and T-GRSP were detected despite the absence of AM

infection and spores on the treatment C0I0, showed that GRSP was produced by nonmycorrhizal plants, even though at a significantly lower level (0.14%) compared with mycorrhizal plants. These results are consistent with the study of He et al. (2020), who also found GRSP with the absence of AM colonization and hyphae in the treatment without mycorrhizal inoculation on *Trifoliate orange* plants on Xanthi-Udic Ferralsols in the chambers. Moreover, He et al. (2020) reported that the treatment of three types of mycorrhizal (*Rhizoglomus intraradices, Diversispora epigaea,* and *Paraglomus occultum*) in a chamber with root + hyphae source of inoculants showed a 30%–42% higher T-GRSP compared with those without mycorrhizal, whereas that in a chamber with hyphae as the inoculant source showed a 34%–49% higher T-GRSP than without mycorrhizal. Compared with the results of He et al. (2020), the present study showed that mycorrhizal inoculation treatment C0I1 on zeolite media yielded 129% and 300% higher EE-GRSP and T-GRSP compared with the treatment C0I0 without mycorrhizal infection at all. By contrast, the treatment of C1I2 on acid mineral soil-based media yielded 175% and 108% higher EE-GRSP and T-GRSP compared with the treatment without inoculation (C1I0).

GRSPs are glycoproteins abundantly produced by AM fungi in roots and soil, and the factors that influence their formation are as follows: the addition of organic matter (Bertagnoli et al., 2020), soil physical conditions (Xiao et al., 2019), soil management processes (Singh et al., 2013), fire, and tillage (Sharifi et al., 2018). The present findings confirmed that the EE-GRSP and T-GRSP contents can be valuable indicators for the existence, the abundance and the functional activity of mycorrhizal in their plant symbionts, especially in supporting the mechanisms of P availability in soil and P concentration in plant tissues.

Among all the treatments on acid mineral soil-based media, the treatments of C1I1, C1I2, C2I1, and C3I2 yielded the highest level of plant fresh and dry weights and root fresh and dry weights. Notably, all treatments of pot cultures for AM inoculum production in the present study had a higher root biomass proportion (>60%) compared with shoot biomass in the total maize biomass. The dominance of root biomass compared to shoot biomass in AM pot culture is a crucial factor to determine the quality of AM biofertilizer products because roots containing mycorrhizal structures can be used as the source of mycorrhizal propagules. Some studies reported that the effect of each AM species varied on the shoot and root growth. Zhang et al. (2019) showed that the highest shoot biomass of seedling growth of *Toona sinensis* was produced by the inoculation treatment of *Funneliformis mosseae* (4.6 g) while the highest root biomass was produced by that of *Rhizophagus intraradices* (2.7 g). Different results were found in the study of Ortas et al. (2011), wherein *Glomus etunicatum* inoculation treatment produced the highest shoot dry and root dry weights, namely 19.3 and 4.1 g, respectively, compared with other treatments.

On all media compositions, mycorrhizal inoculation treatments of I1, I2, and I3 resulted in higher chlorophyll content and P concentration in shoot tissues compared with the treatments without mycorrhizal inoculation (I0). This result is consistent with the report of Andrade et al. (2018) that the mycorrhizal inoculation increased P concentration and P uptake of soybean on Oxisols. Gheisari Zardak et al. (2017) found that the application of AM increased the accumulation of P in plant tissues and simultaneously stimulated plant growth. Increasing chlorophyll content due to AM inoculation was supported by Zare-Maivan et al. (2017). Notably, all treatments that indicated the highest plant fresh and dry weights did not always exhibit the highest content of chlorophyll and P concentration. Functional contributions to other plant nutrition by mycorrhizal resulted in the high plant growth, and these potential contributions were not covered in the plant growth variables in this study; for example, the influences of AM in increasing the uptake of other nutrients, such as N (Zhu et al., 2016), Ca and Zn (Salgado et al., 2016), and Mg (Zare-Maivan et al., 2017), as well as Mn, Cu, and Zn (Lombardo et al., 2021), and some other benefits of AM.

The mechanism of mycorrhiza to support plant growth was determined from mycorrhizal parameters. Among the treatments on acid mineral soil-based media, the highest MI, SD, EE-GRSP, and T-GRSP were obtained by C1I2 and C3I2. The mycorrhizal contributions were revealed by the positive significant correlations between all plant growth and mycorrhizal parameters: for example, the main variables of plant dry weight and P concentration that significantly correlated with MI ($r = 0.303$; $0.01 < p < 0.05$ and $r = 0.732$; $p < 0.1$), with SD ($r = 0.629$; $p < 0.01$ and $r = 0.803$; $p < 0.01$) and T-GRSP ($r = 0.533$; $p < 0.01$ and $r = 0.732$; $p < 0.01$). In addition, all variables of the plant growth parameters were significantly correlated with the soil chemical variables of organic C, total N, and available P. As the main variables of plant growth, the plant dry weight and P concentration showed significant positive correlation with organic C ($r = 0.574$; $p < 0.01$ and 0.826; $p < 0.01$), total N ($r = 0.632$; $p < 0.01$ and $r = 0.680$; $p < 0.01$), and available P ($r = 0.539$; $p < 0.01$ and $r = 0.784$; $p < 0.01$). All the correlations among the mycorrhizal parameter–soil/media parameter–plant growth parameter showed the support mechanisms of mycorrhizal on plant growth in the soil/media. The effect of mycorrhizal on plant growth in the present study was considered to be an effective indicator of the inoculum to play as a micro-symbiont to the growth of the plant symbiont in the pot culture.

On the zeolite (C0) and Inceptisols (C4) media, the mixture inoculum from eight soil types (I3) indicated the highest effectiveness on plant growth as presented by all plant growth variables on C0I3 and C4I3. These results were different from those of mycorrhizal variables because the highest level of all mycorrhizal variables on zeolite media was indicated by C0I1 followed by C0I3, whereas that on Inceptisols media was indicated by C4I2 followed by C4I3. Thus, the effectiveness of each inoculum (I1, I2, and I3) toward plant growth on zeolite and Inceptisol was different from the results obtained on acid mineral soil-based media. The present findings confirmed that a series of assessments is required to find AM cultures with superior specific functional capability, starting with the exploration of the potential AM propagules and assessments of the compatibility of AM propagules with the formulation of pot culture media as a

carrier. Evaluating the effectiveness of the AM cultures on the target utilization considering host compatibility and soil-media character is necessary for further study, and a variety of agricultural cultivation management methods is also tested.

5. CONCLUSION

The type and composition of pot culture media are the main factors in the propagation of mycorrhizal inoculum. Based on the mycorrhizal parameter, inoculum I2 on the acid mineral soil-based media C1 and C3, inoculum I1 on zeolite media (C0), and inoculum I2 on Inceptisol media (C4) showed the highest values of MI, SD, EE-GRSP, and T-GRSP. Inocula I1 and I2 on media C1, C2, and C3, inoculum I3 on media C0, and inoculum I3 on media C4 exhibited the highest performance based on the plant growth parameter. Among all the treatments, C1I1 and C1I2 were found to be the superior AM cultures with MI 56% and 91%, SD 518 and 697 spores/100 g of media, EE-GRSP 0.46% and 0.77%, and T-GRSP 0.65% and 0.81% and yielded high effectiveness for maize growth with PFW 6.83 and 6.8 g plant^{-1}, PDW 3.76 and 3.31 g plant^{-1}, and P concentration 0.21% and 0.2%. Further study is necessary to reveal the effectiveness of AM inocula on the target conditions.

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Declaration of Competing Interest

The authors declare no competing financial or personal interests that may appear and influence the work reported in this paper.

References

Aguilera, P., Marín, C., Oehl, F., Godoy, R., Borie, F., & Cornejo, P. (2017). Selection of aluminum tolerant cereal genotypes strongly influences the arbuscular mycorrhizal fungal communities in an acidic Andosol. *Agriculture, Ecosystems & Environment*, *246*, 86-93. https://doi.org/10.1016/j.agee.2017.05.031

Andrade, F. R., Nóbrega, J. C. A., Nóbrega, R. S. A., Lustosa Filho, J. F., Zuffo, A. M., & Moreira, F. M. d. S. (2018). Mycorrhization stimulant in soybean associated with phosphate fertilization in oxisols. *Revista Caatinga*, *31*, 823-831. https://doi.org/10.1590/1983-21252018v31n404rc

Antoniadis, V., Koliniati, R., Efstratiou, E., Golia, E., & Petropoulos, S. (2016). Effect of soils with varying degree of weathering and pH values on phosphorus sorption. *CATENA*, *139*, 214-219. https://doi.org/10.1016/j.catena.2016.01.008

Arraudah, R., Bertham, Y. H., Pujiwati, H., Murcitro, B. G., & Sukarjo, E. I. (2020). Application of Humic Acid and Arbuscular Mycorrhizal Fungi to Increase Growth and Yields of Soybean in Ultisol. *TERRA: Journal of Land Restoration*, *3*(2), 56-64. https://doi.org/10.31186/terra.3.2.56-64

Bertagnoli, B. G. P., Oliveira, J. F., Barbosa, G. M. C., & Colozzi Filho, A. (2020). Poultry litter and liquid swine slurry applications stimulate glomalin, extraradicular mycelium production, and aggregation in soils. *Soil and Tillage Research*, *202*, 104657. https://doi.org/10.1016/j.still.2020.104657

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Brundrett, M. C., & Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist*, *220*(4), 1108-1115. https://doi.org/https://doi.org/10.1111/nph.14976

Cahyani, V. R., Alfin, M. R., & Hanifah, N. (2019). Screening of Arbuscular Mycorrhiza Isolated from Rhizosphere of Elephant Grass from Seven Soil Types for Biofertilizer in Zeolite Pot Culture. *Bulgarian Journal of Agricultural Science*, *25*(4), 724-731. https://www.agrojournal.org/25/04-15.pdf

Cahyani, V. R., Suryanti, Setiawan, D. F., Suntoro, Minardi, S., Purwanto, & Rahayu. (2019). Examination of mycorrhizal inoculum for improving maize tolerance to water stress in pot culture using zeolite and Andisol. *IOP Conference Series: Earth and Environmental Science*, *393*(1), 012018. https://doi.org/10.1088/1755-1315/393/1/012018

Chen, S., Zhao, H., Zou, C., Li, Y., Chen, Y., Wang, Z., . . . Ahammed, G. J. (2017). Combined Inoculation with Multiple Arbuscular Mycorrhizal Fungi Improves Growth, Nutrient Uptake and Photosynthesis in Cucumber Seedlings [Original Research]. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.02516

Crossay, T., Cavaloc, Y., Majorel, C., Redecker, D., Medevielle, V., & Amir, H. (2020). Combinations of different arbuscular mycorrhizal fungi improve fitness and metal tolerance of sorghum in ultramafic soil. *Rhizosphere*, *14*, 100204. https://doi.org/10.1016/j.rhisph.2020.100204

Driver, J. D., Holben, W. E., & Rillig, M. C. (2005). Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, *37*(1), 101-106. https://doi.org/10.1016/j.soilbio.2004.06.011

El Maaloum, S., Elabed, A., Alaoui-Talibi, Z. E., Meddich, A., Filali-Maltouf, A., Douira, A., . . . El Modafar, C. (2020). Effect of Arbuscular Mycorrhizal Fungi and Phosphate-Solubilizing Bacteria Consortia Associated with Phospho-Compost on Phosphorus Solubilization and Growth of Tomato Seedlings (Solanum lycopersicum L.). *Communications in Soil Science and Plant Analysis*, *51*(5), 622-634. https://doi.org/10.1080/00103624.2020.1729376

Friese, C. F., & Allen, M. F. (1991). The Spread of Va Mycorrhizal Fungal Hyphae in the Soil: Inoculum Types

and External Hyphal Architecture. *Mycologia*, *83*(4), 409-418. https://doi.org/10.1080/00275514.1991.12026030

Gerdemann, J. W., & Nicolson, T. H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*, *46*(2), 235-244. https://doi.org/10.1016/S0007-1536(63)80079-0

Gheisari Zardak, S., Movahhedi Dehnavi, M., Salehi, A., & Gholamhoseini, M. (2017). Responses of field grown fennel (Foeniculum vulgare Mill.) to different mycorrhiza species under varying intensities of drought stress. *Journal of Applied Research on Medicinal and Aromatic Plants*, *5*, 16-25. https://doi.org/10.1016/j.jarmap.2016.09.004

Giovannetti, M., & Mosse, B. (1980). An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots. *New Phytologist*, *84*(3), 489-500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Goulding, K. W. T. (2016). Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. *Soil Use and Management*, *32*(3), 390-399. https://doi.org/10.1111/sum.12270

He, J.-D., Chi, G.-G., Zou, Y.-N., Shu, B., Wu, Q.-S., Srivastava, A. K., & Kuča, K. (2020). Contribution of glomalin-related soil proteins to soil organic carbon in trifoliate orange. *Applied Soil Ecology*, *154*, 103592. https://doi.org/10.1016/j.apsoil.2020.103592

He, X., Li, Y., & Zhao, L. (2010). Dynamics of arbuscular mycorrhizal fungi and glomalin in the rhizosphere of Artemisia ordosica Krasch. in Mu Us sandland, China. *Soil Biology and Biochemistry*, *42*(8), 1313-1319. https://doi.org/10.1016/j.soilbio.2010.03.022

Hendry, G. A. F., & Grime, J. P. (1993). *Methods in Comparative Plant Ecology*. Springer Dordrecht. https://doi.org/10.1007/978-94-011-1494-3

Ianson, D. C., & Allen, M. F. (1986). The Effects of Soil Texture on Extraction of Vesicular-Arbuscular Mycorrhizal Fungal Spores from Arid Sites. *Mycologia*, *78*(2), 164-168. https://doi.org/10.1080/00275514.1986.12025227

Ji, L., Tan, W., & Chen, X. (2019). Arbuscular mycorrhizal mycelial networks and glomalin-related soil protein increase soil aggregation in Calcaric Regosol under well-watered and drought stress conditions. *Soil and Tillage Research*, *185*, 1-8. https://doi.org/10.1016/j.still.2018.08.010

Kamran, M. A., Jiang, J., Li, J.-y., Shi, R.-y., Mehmood, K., Baquy, M. A.-A., & Xu, R.-k. (2018). Amelioration of soil acidity, Olsen-P, and phosphatase activity by manure- and peat-derived biochars in different acidic soils. *Arabian Journal of Geosciences*, *11*(11), 272. https://doi.org/10.1007/s12517-018-3616-1

Kochian, L. V., Hoekenga, O. A., & Piñeros, M. A. (2004). How Do Crop Plants Tolerate Acid Soils? Mechanisms of Aluminum Tolerance and Phosphorous Efficiency. *Annual Review of Plant Biology*, *55*(1), 459-493. https://doi.org/10.1146/annurev.arplant.55.031903.141655

Kumar, A., Suri, V. K., & Choudhary, A. K. (2014). Influence of Inorganic Phosphorus, VAM Fungi, and Irrigation Regimes on Crop Productivity and Phosphorus Transformations in Okra (Abelmoschus esculentus L.)–Pea (Pisum sativum L.) Cropping System in an Acid Alfisol. *Communications in Soil Science and Plant Analysis*, *45*(7), 953-967. https://doi.org/10.1080/00103624.2013.874025

Laxminarayana, K. (2016). Response of Mycorrhiza, Organic Sources, Secondary and Micro Nutrients on Soil Microbial Activities and Yield Performance of Colocasia (Colocasia esculenta L.) in Alfisols. *Communications in Soil Science and Plant Analysis*, *47*(6), 775-786. https://doi.org/10.1080/00103624.2016.1146893

Lombardo, S., Scavo, A., Abbate, C., Pandino, G., Parisi, B., & Mauromicale, G. (2021). Mycorrhizal Inoculation Improves Mineral Content of Organic Potatoes Grown under Calcareous Soil. *Agriculture*, *11*(4), 333. https://doi.org/10.3390/agriculture11040333

Lopes, J. I., Arrobas, M., Raimundo, S., Gonçalves, A., Brito, C., Martins, S., . . . Rodrigues, M. Â. (2022). Photosynthesis, Yield, Nutrient Availability and Soil Properties after Biochar, Zeolites or Mycorrhizal Inoculum Application to a Mature Rainfed Olive Orchard. *Agriculture*, *12*(2), 171. https://doi.org/10.3390/agriculture12020171

Lopes, J. I., Correia, C. M., Gonçalves, A., Silva, E., Martins, S., Arrobas, M., & Rodrigues, M. Â. (2021). Arbuscular Mycorrhizal Fungi Inoculation Reduced the Growth of Pre-Rooted Olive Cuttings in a Greenhouse. *Soil Systems*, *5*(2), 30. https://doi.org/10.3390/soilsystems5020030

Lovelock, C. E., Wright, S. F., & Nichols, K. A. (2004). Using glomalin as an indicator for arbuscular mycorrhizal hyphal growth: an example from a tropical rain forest soil. *Soil Biology and Biochemistry*, *36*(6), 1009-1012. https://doi.org/10.1016/j.soilbio.2004.02.010

Ma, J., Wang, W., Yang, J., Qin, S., Yang, Y., Sun, C., . . . Huang, J. (2022). Mycorrhizal symbiosis promotes the nutrient content accumulation and affects the root exudates in maize. *BMC Plant Biology*, *22*(1), 64. https://doi.org/10.1186/s12870-021-03370-2

Mehta, P., Sharma, R., Putatunda, C., & Walia, A. (2019). Endophytic Fungi: Role in Phosphate Solubilization. In B. P. Singh (Ed.), *Advances in Endophytic Fungal Research: Present Status and Future Challenges* (pp. 183-209). Springer International Publishing. https://doi.org/10.1007/978-3-030-03589-1_9

Mora, E., Lopez-Hernández, D., & Toro, M. (2019). Arbuscular Mycorrhizae and PGPR Applications in Tropical Savannas. In D. Zúñiga-Dávila, F. González-Andrés, & E. Ormeño-Orrillo (Eds.), *Microbial Probiotics for Agricultural Systems: Advances in Agronomic Use* (pp. 169-177). Springer International Publishing. https://doi.org/10.1007/978-3-030-17597-9_11

Mulyani, A., & Sarwani, M. (2013). Karakteristik dan potensi lahan sub optimal untuk pengembangan pertanian di Indonesia. *Jurnal Sumberdaya Lahan*, *7*(1), 47-55. http://ejurnal.litbang.pertanian.go.id/index.php/jsl/article/view/6429

Ortas, I., Sari, N., Akpinar, Ç., & Yetisir, H. (2011). Screening mycorrhiza species for plant growth, P and Zn uptake in pepper seedling grown under greenhouse conditions. *Scientia Horticulturae*, *128*(2), 92-98. https://doi.org/10.1016/j.scienta.2010.12.014

Pan, X.-Y., Li, J.-Y., Deng, K.-Y., Xu, R.-K., & Shen, R.-F. (2019). Four-year effects of soil acidity amelioration on the yields of canola seeds and sweet potato and N fertilizer efficiency in an ultisol. *Field Crops Research*, *237*, 1-11. https://doi.org/10.1016/j.fcr.2019.03.019

Pansu, M., & Gautheyrou, J. (2006). *Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods*. https://doi.org/10.1007/978-3-540-31211-6

Peng, S., Guo, T., & Liu, G. (2013). The effects of arbuscular mycorrhizal hyphal networks on soil aggregations of purple soil in southwest China. *Soil Biology and Biochemistry*, *57*, 411-417. https://doi.org/10.1016/j.soilbio.2012.10.026

Phillips, J. M., & Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, *55*(1), 158-IN118. https://doi.org/10.1016/S0007-1536(70)80110-3

Reyes, H. A., Ferreira, P. F. A., Silva, L. C., da Costa, M. G., Nobre, C. P., & Gehring, C. (2019). Arbuscular mycorrhizal fungi along secondary forest succession at the eastern periphery of Amazonia: Seasonal variability and impacts of soil fertility. *Applied Soil Ecology*, *136*, 1-10. https://doi.org/10.1016/j.apsoil.2018.12.013

Salgado, F. H. M., Moreira, F. M. d. S., Paulino, H. B., Siqueira, J. O., & Carneiro, M. A. C. (2016). Arbuscular mycorrhizal fungi and mycorrhizal stimulant affect dry matter and nutrient accumulation in bean and soybean plants. *Pesquisa Agropecuária Tropical*, *46*(4), 367-373. https://doi.org/10.1590/1983-40632016v4640282

Santander, C., Aroca, R., Ruiz-Lozano, J. M., Olave, J., Cartes, P., Borie, F., & Cornejo, P. (2017). Arbuscular mycorrhiza effects on plant performance under osmotic stress. *Mycorrhiza*, *27*(7), 639-657. https://doi.org/10.1007/s00572-017-0784-x

Sarma, B., Borkotoki, B., Narzari, R., Kataki, R., & Gogoi, N. (2017). Organic amendments: Effect on carbon mineralization and crop productivity in acidic soil. *Journal of Cleaner Production*, *152*, 157-166. https://doi.org/10.1016/j.jclepro.2017.03.124

Seguel, A., Cumming, J., Cornejo, P., & Borie, F. (2016). Aluminum tolerance of wheat cultivars and relation to arbuscular mycorrhizal colonization in a non-limed and limed Andisol. *Applied Soil Ecology*, *108*, 228-237. https://doi.org/10.1016/j.apsoil.2016.08.014

Sharifi, Z., Azadi, N., Rahimi, S., & Certini, G. (2018). The response of glomalin-related soil proteins to fire or tillage. *Geoderma*, *329*, 65-72. https://doi.org/10.1016/j.geoderma.2018.05.008

Shi, R.-y., Hong, Z.-n., Li, J.-y., Jiang, J., Baquy, M. A.-A., Xu, R.-k., & Qian, W. (2017). Mechanisms for Increasing the pH Buffering Capacity of an Acidic Ultisol by Crop Residue-Derived Biochars. *Journal of Agricultural and Food Chemistry*, *65*(37), 8111-8119. https://doi.org/10.1021/acs.jafc.7b02266

Singh, P. K., Singh, M., & Tripathi, B. N. (2013). Glomalin: an arbuscular mycorrhizal fungal soil protein. *Protoplasma*, *250*(3), 663-669. https://doi.org/10.1007/s00709-012-0453-z

Smith, S. E., & Smith, F. A. (2011). Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales. *Annual Review of Plant Biology*, *62*(1), 227-250. https://doi.org/10.1146/annurev-arplant-042110-103846

Souza, T. (2015). *Handbook of arbuscular mycorrhizal fungi*. Springer Cham. https://doi.org/10.1007/978-3-319-24850-9

Steinberg, P. D., & Rillig, M. C. (2003). Differential decomposition of arbuscular mycorrhizal fungal hyphae and glomalin. *Soil Biology and Biochemistry*, *35*(1), 191-194. https://doi.org/10.1016/S0038-0717(02)00249-3

Suri, V. K., & Choudhary, A. K. (2013). Effect of Vesicular Arbuscular–Mycorrhizal Fungi and Phosphorus Application through Soil-Test Crop Response Precision Model on Crop Productivity, Nutrient Dynamics, and Soil Fertility in Soybean–Wheat–Soybean Crop Sequence in an Acidic Alfisol. *Communications in Soil Science and Plant Analysis*, *44*(13), 2032-2041. https://doi.org/10.1080/00103624.2013.783921

Suri, V. K., & Choudhary, A. K. (2014). Comparative Performance of Geographical Isolates of Glomus mosseae in Field Crops under Low-Input Intensive P-Deficient Acid Alfisol. *Communications in Soil Science and Plant Analysis*, *45*(1), 101-110. https://doi.org/10.1080/00103624.2013.849726

Syamsiyah, J., Sunarminto, B. H., Hanudin, E., & Widada, J. (2014). Pengaruh Inokulasi Jamur Mikoriza Arbuskula terhadap Glomalin, Pertumbuhan dan Hasil Padi [Glomalin; Mycorrhizae; nutrient uptake; rice yield]. *Sains Tanah - Journal of Soil Science and Agroclimatology*, *11*(1), 39-46. https://doi.org/10.15608/stjssa.v11i1.214

Vos, C., Schouteden, N., van Tuinen, D., Chatagnier, O., Elsen, A., De Waele, D., . . . Gianinazzi-Pearson, V. (2013). Mycorrhiza-induced resistance against the root–knot nematode Meloidogyne incognita involves priming of defense gene responses in tomato. *Soil Biology and Biochemistry*, *60*, 45-54. https://doi.org/10.1016/j.soilbio.2013.01.013

Wheal, M. S., Fowles, T. O., & Palmer, L. T. (2011). A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma

optical emission spectrometry (ICP-OES) analysis of plant essential elements [10.1039/C1AY05430A]. *Analytical Methods*, *3*(12), 2854-2863. https://doi.org/10.1039/C1AY05430A

Wright, S. F., Upadhyaya, A., & Buyer, J. S. (1998). Comparison of N-linked oligosaccharides of glomalin from arbuscular mycorrhizal fungi and soils by capillary electrophoresis. *Soil Biology and Biochemistry*, *30*(13), 1853-1857. https://doi.org/10.1016/S0038-0717(98)00047-9

Wu, Q.-S., Cao, M.-Q., Zou, Y.-N., & He, X.-h. (2014). Direct and indirect effects of glomalin, mycorrhizal hyphae and roots on aggregate stability in rhizosphere of trifoliate orange. *Scientific Reports*, *4*(1), 5823. https://doi.org/10.1038/srep05823

Xiao, L., Zhang, Y., Li, P., Xu, G., Shi, P., & Zhang, Y. (2019). Effects of freeze-thaw cycles on aggregate-associated organic carbon and glomalin-related soil protein in natural-succession grassland and Chinese pine forest on the Loess Plateau. *Geoderma*, *334*, 1-8. https://doi.org/10.1016/j.geoderma.2018.07.043

Zarcinas, B. A., Cartwright, B., & Spouncer, L. R. (1987). Nitric acid digestion and multi ‐ element analysis of plant material by inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis*, *18*(1), 131-146. https://doi.org/10.1080/00103628709367806

Zare-Maivan, H., Khanpour-Ardestani, N., & Ghanati, F. (2017). Influence of mycorrhizal fungi on growth, chlorophyll content, and potassium and magnesium uptake in maize. *Journal of Plant Nutrition*, *40*(14), 2026-2032. https://doi.org/10.1080/01904167.2017.1346119

Zhang, Z., Mallik, A., Zhang, J., Huang, Y., & Zhou, L. (2019). Effects of arbuscular mycorrhizal fungi on inoculated seedling growth and rhizosphere soil aggregates. *Soil and Tillage Research*, *194*, 104340. https://doi.org/10.1016/j.still.2019.104340

Zhu, X., Song, F., Liu, S., & Liu, F. (2016). Arbuscular mycorrhiza improve growth, nitrogen uptake, and nitrogen use efficiency in wheat grown under elevated CO2. *Mycorrhiza*, *26*(2), 133-140. https://doi.org/10.1007/s00572-015-0654-3