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Screening of arbuscular mycorrhiza isolated from rhizosphere of elephant grass from seven soil types for biofertilizer in zeolite pot culture

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Abstract

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Arbuscular mycorrhiza (AM) has been proven having important roles to support plant growth in a variety of environmental and land constraints. The present study aimed at elucidating the potential of AM propagules obtained from rhizosphere of elephant grass from 7 (seven) soil types in around area of Solo city and Semarang city, the province of Jawa Tengah, Indonesia, to be screened as a biofertilizer. The first step, exploration and analysis of rhizospheric soil of elephant grass from drylands of Alfisol Jumantono, Histosol Ambarawa, Oxisol Tuntang, Andisol Tengaran, Inceptisol Delanggu, Vertisol Jatikuwung and Entisol Pracimantoro for the existence of AM which consisted of spore density and diversity. The second step, pot cultural experiment was conducted for comparing the infectivity and effectivity of AM spores from 7 (seven) soil types in the same media of zeolite and using maize as host. Based on the results of exploration in natural conditions, the rhizospheric soil of elephant grass from Alfisol Jumantono (AJ) and Entisol Pracimantoro (EP) were categorized having low spore density, and based on the pot cultural experiment using zeolite, these two inoculums of AJ and EP also showed low AM infectivity, but resulted in the highest AM effectivity on plant growth as indicated by the highest plant biomass. Based on the result of exploration in natural conditions, the rhizospheric soil from Andisol Tengaran (ATe) indicated the highest spore density (450 spores/100 g soil), however based on the pot culture experiment the inoculum ATe showed the lowest AM effectivity on plant dry weight. The inoculum sources from EP and AJ showed the highest value of mycorrhizal dependency (MD) and growth response (GR), whereas the inoculum of ATe showed the lowest MD and GR in zeolite pot culture. The continued examinations for the functional capability of AM in target conditions are needed for obtaining effective AM biofertilizer.

Keywords: arbuscular mycorrhiza; biofertilizer; elephant grass; zeolite; pot culture; screening; soil type

Introduction

Arbuscular mycorrhiza (AM) is one of the symbiotic soil microorganisms with plant root. AM has been proven having important roles to support plant growth in a variety of environmental and land constraints. AM protect plant roots from pathogen (Wehner et al., 2010; Veresoglou & Rillig, 2011), increase plant tolerant to acidity, toxicity (Cahyani, 2009), increased P uptake via mycorrhizal pathway (Smith et al., 2004), by exploiting a greater volume of soil, extending away from root and translocating P as far away as 8 cm, and adding surface area to the absorptive system (Jarstfer & Sylvia, 1993), increasing plant tolerance to drought stress (Vyn & Boomsma, 2008), salinity (Ruiz-Lozano & Azcón, 2000), and increasing plant growth regulators (Fitze et al., 2005).

AM is widespread inhabit and has diverse characteristic in natural environments. AM exploration is important to obtain superior indigenous mycorrhizal spores that have potential effec-

tivity to increase plant growth and plant production. The present study focused on explorations in the rhizosphere of elephant grass. Grass is known as survival plant that versatile to exist in a wide variety of extreme conditions especially nutritional deficiency and drought stress. The capability of grass to exist in such extreme conditions due to the roles of soil functional biota, one of them is AM. Castillo et al. (2006) reported that the presence of AM on grassland is more abundant than forest.

This present study aimed to examine the potential of AM isolated from elephant grass rhizosphere of seven soil types in around area of Solo city and Semarang city, Central Java Indonesia to be used as biological fertilizer. The seven soil types used in the present study consisted of Andisol Tengaran, Entisol Pracimantoro, Alfisol Jumantono, Oxisol Tuntang, Inceptisol Delanggu, Histosol Ambarawa and Vertisol Jatikuwung. The first step of research was conducted by explorations of the existence of AM in rhizosphere of elephant grass from seven soil types by taking rhizosphere soil, isolation of AM spores, and continued with calculating of spore density and spore identification. The second step was conducted by pot cultural experiment using zeolite as media and maize as host. Zeolite is a potential absorbent media for potted plant production as zeolite has unique structure with micro-porosity (Pickerring et al., 2002). The information obtained from the present research is expected to serve the mapping of potential AM spores isolated from seven soil types for biofertilizer.

Materials and Methods

Exploration of arbuscular mycorrhiza

Exploration sites and sampling rhizosphere soil

The exploration of AM from rhizosphere of elephant grass were carried out at seven different sites around Solo city and Semarang city, Central Java, Indonesia based on the soil types. The rhizosphere soil of elephant grass was randomly taken ± 500 g at 0-20 cm depth from each soil type, with 3 replications.

Isolation and identification of arbuscular mycorrhizal spores

Soil samples were used for analysis of pH (H_2O), available P (Olsen), spore density analysis, spore identification. AM isolation using wet filter method and centrifugation with an addition of sugar solution. Soil samples were taken ± 100 g and put into 250 ml of distilled water until homogeneous. The soil solution was filtered sequentially using three different filter pore sizes of 250 µ, 90 µ and 45µ. The filtrate suspension was transferred to centrifugation tubes and 60% sugar solution was added and centrifuged at 5000 rotation per minute for 5 min. The supernatan was filtered using what- man filter paper grade 42 and washed using distilled water, and then the filter paper was transferred into a petri dish. The AM spores retained on the filter paper in the petri dish were observed for spore density of the spores using a binocular microscope with a magnification of 400x. Spore density was calculated from 100 g of the rhizosphere of elephant grass. AM identification was done by preparing AM spore into a microscope slide with a drop of Melzer solution then covered using a cover glass, observation of AM identification using a set of microscope and optilab. Identification of spore morphology was made using the description of International Culture Collection of (Vesicular) Arbuscular Mycorrhizal (IN- VAM, 2018). Data of single treatment with three replications of soil pH, available P, and mycorrhizal spore density were analyzed using the SPSS for ANOVA and DMRT.

Pot cultural experiment using zeolite for testing of arbuscular mycorrhizal spore production and arbuscular mycorrhizal effectivity

At this stage, for testing the AM spore production and AM effectivity was conducted in pot cultural experiment using the inoculum sources from rhizosphere soil of elephant grass was taken from 7 soil types that sampled in the first step of the research. Complete Randomized Design was used for pot cultural experiment. The single factor treatment of AM inoculum sources consisted of 8 levels, as follows : Z (Zeolite without mycorrhiza), ATe (Andisol Tengaran), EP (Entisol Pracimantoro), AJ (Alfisol Jumantono), OTu (Oxisol Tuntang), ID (Inceptisol Delanggu), HA (Histosol Ambarawa), VJ (Vertisol Jatikuwung), each treatment was repeated 6 times. The first 3 replications were harvested at maximal vegetative (60 days after planting) and the second 3 replications were harvested after the additional 30 days for drying process (90 days after plating) (Table 1).

Table 1. Description of the exploration sites

Soil type	Location	Treatment Code	Latitude	Longitude
Andisol	Tengaran	ATe	07° 15′51″ S	110° 26′57″ E
Entisol	Pracimantoro	EP	08° 02′14″ S	110° 46′60″ E
Alfisol	Jumantono	AJ	07° 37′47″ S	110° 56′51″ E
Oxisol	Tuntang	OTu	07° 15′51″ S	110° 26′57″ E
Inceptisol	Delanggu	ID	07° 39′20″ S	110° 44′21″ E
Histosol	Ambarawa	HA	07° 15′48″ S	110° 27′00″ E
Vertisol	Jatikuwung	VJ	07° 31′06″ S	110° 50′44″ E

Pot culture was prepared by adding 200 g zeolite to plastic pot (volume 300 ml). The method for pot culture was conducted according to the method of Brundrett et al. (1996) with modification of nutrient sources. AM spores were inoculated at a dosage of 50 spores/pot at a depth of ± 3cm using funnel method (Gerdemann, 1955). Maize sprout at the age of seven days after germination on seedling tray was planted and positioned above AM spores that had been inoculated then covered the root with zeolite. During plant growth period, nutrient "Growmore (32:10:10)" was added with concentration 7.5g L^{-1} and given once per two days. The parameters that were analyzed including mycorrhizal infectivity (percentage of root infectivity (Philip & Hayman, 1970; Trouvelot et al., 1986) and spore density), mycorrhizal effectivity (plant height, plant fresh and plant dry weight, P concentration and P uptake), mycorrhizal dependency (Plenchette et al., 1983) and growth response (Hetrick et al., 1992).

Mycorrhizal dependency (MD) indexed calculated from this formula:

$$\text{Mycorrhizal dependency (\%)} = \frac{\text{(dry weight of mycorrhizal plant – dry weight of non mycorrhizal plant)}}{\text{dry weight of mycorrhizal plant}} \times 100.$$

Growth response (GR) indexed calculated from this formula:

$$\text{Growth response (\%)} = \frac{\text{(dry weight of mycorrhizal plant – dry weight of non mycorrhizal plant)}}{\text{dry weight of non mycorrhizal plant}} \times 100.$$

Statistical analysis was calculated using the SPSS (Statistical Package for the Social Sciences) program for analysis of variance (ANOVA) and Duncan multiple range test (DMRT). Correlation analysis was calculated using statistical program Minitab version 18.

Results and Discussion

The results of exploration of mycorrhizal existence in rhizosphere of elephant grass from seven soil types

The results of analysis of isolation of AM spores from rhizosphere of elephant grass from seven soil types are presented in Table 2.

The highest AM spore density was indicated by Andisol Tengaran (ATe) in which calculated 450±7.94 spores/100g soil and consisted of *Glomus* and *Acaulospora*. The lowest AM density in the present study was indicated by Inceptisol Delanggu (ID) and Entisol Pracimantoro (EP) with the common genera of *Glomus* and *Gigaspora*, and specific existence of *Acaulospora* for ID. Similar results of AM existence in Inceptisol was found in previous study (Cahyani et al., 2017) that AM spores density in sugarcane rhizosphere was calculated of 114 spores/100g soil and consisted of *Glomus, Gigaspora* and *Acaulospora.*

Fig. 1 shows the values of soil pH H_20 and available P. According to classification of soil pH from Natural Resources Conservation Service of USDA (1998) the soil pH of Histosol Ambarawa (5.18) was categorized as strongly acid, soil pH of Oxisol Tuntang (5.75) and Alfisol Jumantono (6.21) were categorized as moderately soil. The soil pH of Inceptisol Delanggu (7.43) and Vertisol Jumantono (7.5) were categorized as neutral soil and soil pH of Entisol Pracimantoro (8.2) was categorized as alkaline soil. Based on the statistical

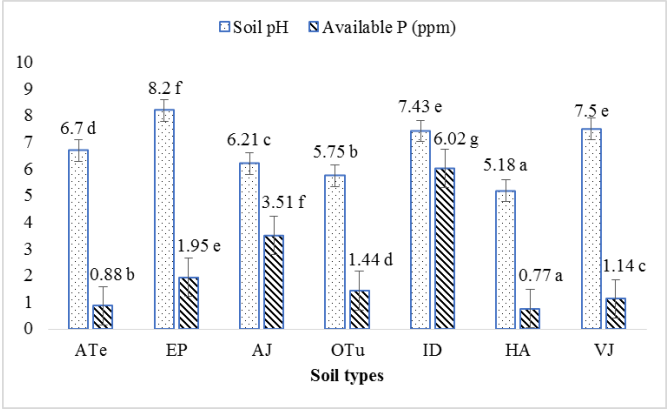


Fig. 1. pH H_20 and available P from seven soil types. Means followed by the same letter (s) are not significantly different at 5% level by DMRT

Table 2. Analysis of AM spores isolated from rhizosphere of elephant grass from seven soil types

	ATe	EP	AJ	OTu	ID	HA	VJ
Spore density (spores /100g soil)	450±7.94 d	50±1.44 a	89±3.08 ab	133±4.61 b	49±1.41 a	318±11.02 c	71±2.05 ab
Mycorrhizal diversity	Glomus – Acaulospora –	Glomus Gigaspora – –	Glomus – Acaulospora Scutellospora	Glomus – – –	Glomus Gigaspora Acaulospora –	Glomus Gigaspora – –	Glomus – Acaulospora –

Means followed by the same letter (s) are not significantly different at 5% level by DMRT

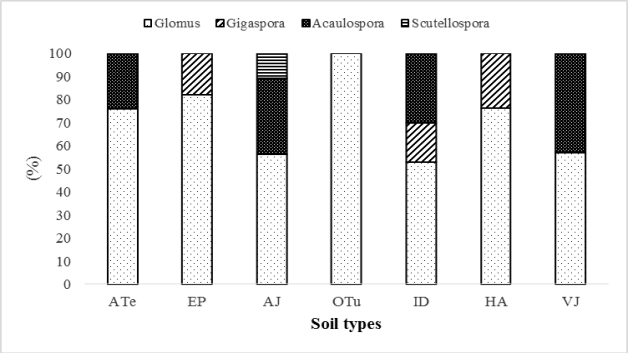
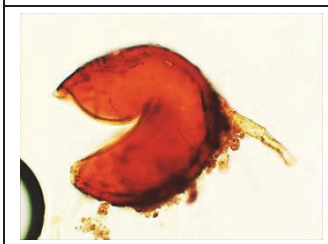
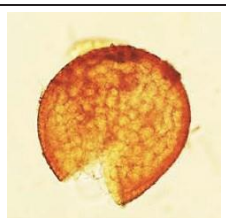
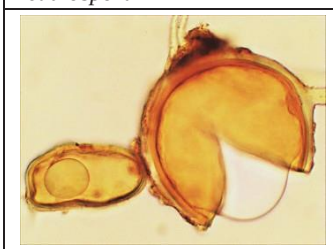
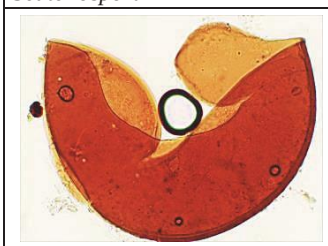


Fig. 2. Percentage distribution of arbuscular mycorrhizal genera from seven soil types

analysis, it was found that between soil pH and spore density and between soil available P and spore density were significantly negatively correlated with $r = -0.495$, $p < 0.005$ and $r = -0.550$, $p < 0.05$, respectively.

Fig. 2 shows that percentage of distribution of mycorrhizal genera from seven soil types. The results showed that *Glomus* dominated in all seven soil types, indicated higher proportion comparing with the other genera for mycorrhizal diversity. Genus *Glomus* from ATe was in amount of 342 spores (76%), from EP 41 spores (81%), from AJ 50 spores (56.3%), from OTu 133 spores (100%), from ID 26 spores (53%), from HA 242 spores (76.3%) and from VJ

Table 3. Mycorrhizal spore morphology from the rhizosphere of elephant grass (magnification 400x)

Photo	Shape	Color	Spore Surface Texture	Special Characteristic	Reaction with melzer
	Globose	Yellow-brown	Smooth	Subtending hyphae	No reaction
Glomus					
	Subglobose	Yellowish hyaline – light brown	Rough	Have an ornament	Change from yellowish hyaline to light brown
Acaulospora					
	Globose	Yellowish hyaline – light brown	Smooth	Have a germination shield	Slight change from yellowish hyaline to light brown
Scutellospora					
	Globose	Light brown – reddish brown	Rough	Have an ornament	Change from light brown to reddish brown
Gigaspora					

13 spores (57%). Astuti et al., (2018) reported that genus *Glomus* was found from wide range of soil pH and wide range of altitude.

Table 3 shows that rhizosphere of elephant grass consisted of four mycorrhizal genera, namely *Glomus*, *Acaulospora*, *Scutellospora* and *Gigaspora*. Representative *Glomus* of the elephant grass rhizosphere was Globose, yellow-brown, smooth surface spore texture with subtending hyphae, no reaction with Melzer reagent. Representative *Acaulospora* of the elephant grass rhizosphere was Subglobose, yellowish hyaline, rough surface spore texture with specific ornament, and changed from yellowish hyaline to light brown with Melzer reagent. Representative *Scutellospora* of the elephant grass rhizosphere was globose, yellowish hyaline, smooth surface spore texture with germination shield and changed from yellowish hyaline to light brown with Melzer reagent. Representative *Gigaspora* of the elephant grass rhizosphere was globose, light brown, rough surface spore texture with specific ornament, and changed from light brown to reddish brown with Melzer reagent. The present study indicated the presence of genus *Scutellospora* besides *Glomus*, *Gigaspora* and *Acaulospora* in rhizosphere of elephant grass in Alfisol Jumantono was similar as found in the rhizosphere of sugarcane, however there is no found *Scutellospora* in rhizosphere of grass (Cahyani et al., 2017).

The result of pot cultural experiment using zeolite

Fig. 3 and Fig. 4 show that among of seven inoculum sources, the highest infectivity and spore productivity was indicated by the inoculum sources of ID. From these two figures indicated that there was no correlation between root infectivity and spore density, in other words the high root infectivity was not always parallel with the high spore productivity. The inoculum source which indicated the lowest root infectivity was the inoculum AJ, whereas the lowest spore density was indicated by the inoculum source EP. The infection rate is determined by the adaptability of mycorrhizal plants, the environment, planting media and compounds produced by plants (Anderson, 1992; Cibichakravarthy et al., 2014).

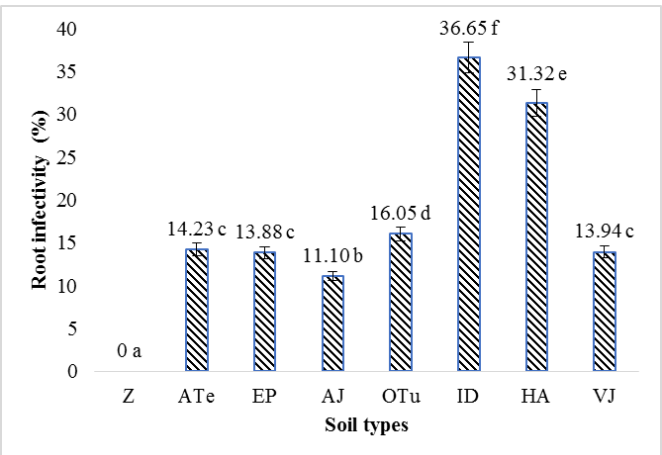


Fig. 3. The effect of inoculum sources of arbuscular mycorrhiza on root infectivity. Means followed by the same letter (s) are not significantly different at 5% level by DMRT

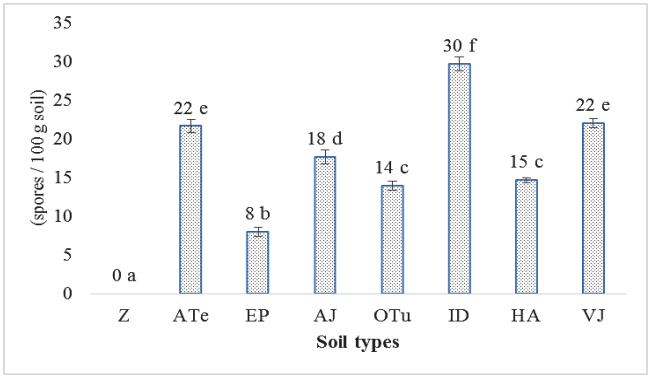


Fig. 4. The effect of inoculum sources of arbuscular mycorrhiza on spore density. Means followed by the same letter (s) are not significantly different at 5% level by DMRT

The findings that inoculum source from ID had the highest spore productivity in the present pot cultural experiment using zeolite was in contrast with the result of exploration mycorrhizal density in situ habitat or in rhizosphere of elephant grass in natural conditions that showed the lowest spore density (Table 2). These phenomena might be related with that mycorrhiza have some criteria to adapt to environmental conditions for germination (Brundrett et al., 1996).

Inoculum source from AJ showed the highest plant height, plant dry weight, P concentration in plant tissue and P uptake. The inoculum EP showed the highest

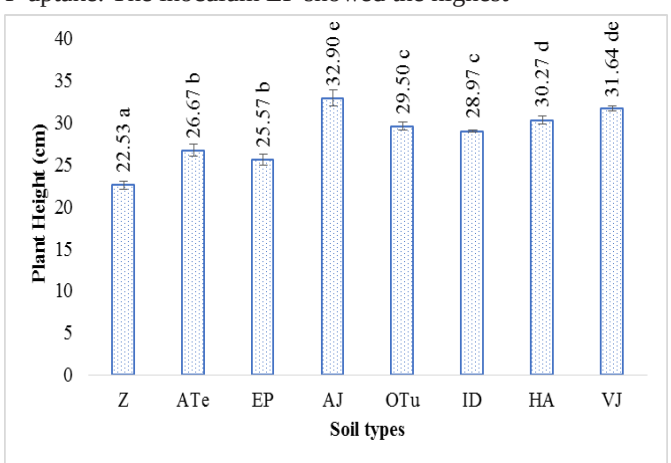


Fig. 5. The effect of inoculum sources of arbuscular mycorrhiza on plant height. Means followed by the same letter (s) are not significantly different at 5% level by DMRT

fresh weight of the plant compared with other treatments. The high plant fresh weight by inoculum EP might indicate the role of mycorrhiza in increasing water uptake for plants, resulting in high water content in plants. AM colonization on plant roots can extend the range of nutrient uptake of P, water, and other nutrients due to the external hyphae that grows and develops through the hair of the roots (Jarstfer & Sylvia, 1993).

As shown from Fig. 2, Fig. 5, Fig. 6, Fig 7 and Fig. 8 the inoculum AJ showed the lowest infectivity but indicated the highest level of plant height, plant dry weight, and P uptake. The inoculum EP showed the lowest spore density but indicated the highest level of plant fresh weight and plant dry weight. Thus, the lower AM infectivity and spore productivity do not mean resulting in lower plant biomass.

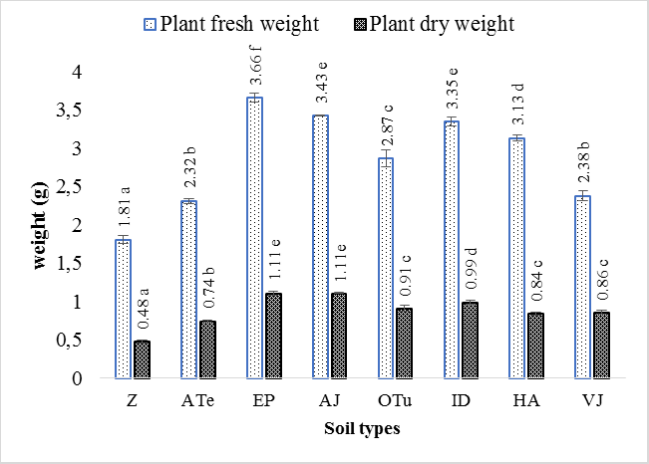


Fig. 6. The effect of inoculum sources of arbuscular mycorrhiza on plant fresh weight and plant dry weight. Means followed by the same letter (s) are not significantly different at 5% level by DMRT

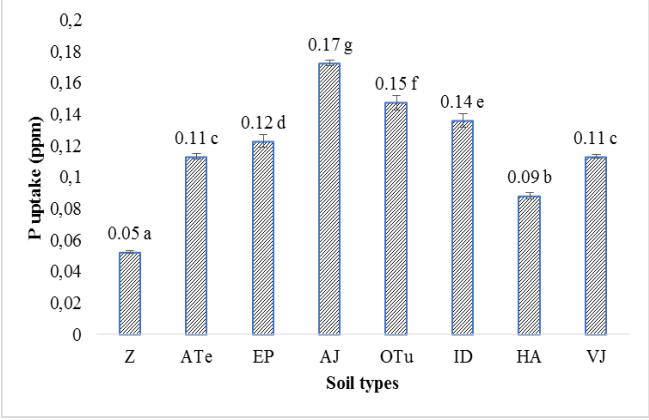


Fig. 7. The effect of inoculum sources of arbuscular mycorrhiza on P uptake. Means followed by the same letter (s) are not significantly different at 5% level by DMRT

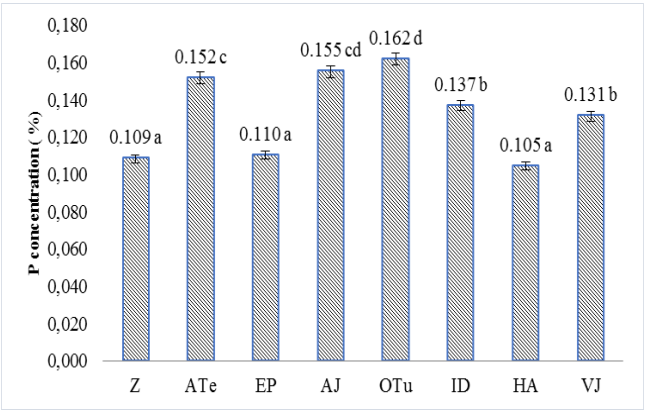


Fig. 8. The effect of inoculum sources of arbuscular mycorrhiza on P concentration in plant tissue. Means followed by the same letter (s) are not significantly different at 5% level by DMRT

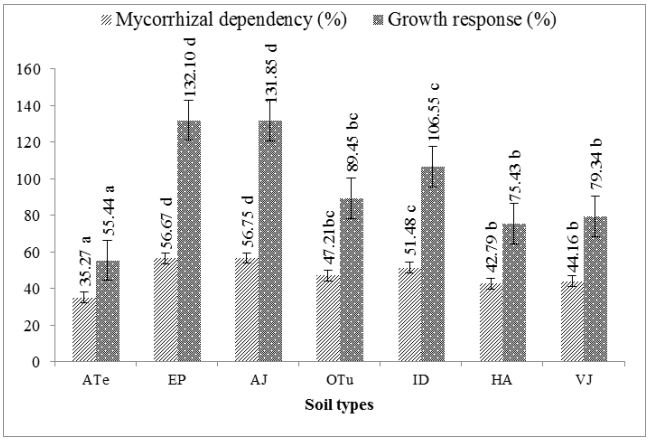


Fig. 9. The effect of inoculum sources of arbuscular mycorrhiza on mycorrhizal dependency (MD) and growth response (GR). Means followed by the same letter (s) are not significantly different at 5% level by DMRT

Mycorrhizal dependency (MD) was defined as the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield, at a given level of soil fertility (Gerdemann, 1975). MD was determined by expressing the difference between the dry weight of the mycorrhizal plant and the dry weight of the non-mycorrhizal plant as a percentage of the dry weight of the mycorrhizal plant (Plenchette et al., 1983). The other measurement to evaluate the response of plant, it can be expressed by growth response (GR) which calculated by the difference between dry weight of mycorrhizal plant and the dry weight of the non-mycorrhizal plant as a percentage of the dry weight of the non-mycorrhizal plant (Hetrick et al., 1992).

Fig. 9 shows that the treatments of EP and AJ indicated the highest MD and GR on zeolite, whereas ATe showed

the lowest MD and GR on zeolite. Positive correlation was found between MD ($r= 0.829$, $p<0.05$) and GR ($r= 0.819$, $p<0.05$) with P uptake. This phenomenon suggested that the increase of P uptake was caused by the role of mycorrhiza that resulted in the high of MD and GR for the treatment of EP and AJ. MD is closely related with the level of soil fertility and availability of nutrient, especially phosphorus. The status of phosphorus in soil determines the development and efficiency of mycorrhiza (Menge et al., 1978; Ortas et al., 2002). The highest level of MD should not exceed 100% and that the lowest should be 0%. Plant having MD value of 100% indicates that the plant will fail to grow without mycorrhiza. On the other hand, plant with MD value of 0% if the difference between the dry weight of the mycorrhizal plant and the dry weight of the non-mycorrhizal one is zero or not statistically significant (Plenchette et al., 1983).

Conclusions

Based on the exploration results:

• The highest spore density was found from the rhizospheric soil of elephant grass of ATe, then the second level from HA, the third level from by AJ and VJ, and the lowest level from EP and ID.

• Soil pH H_20 and soil available P were significantly negatively correlated with AM spore density with the correlation coefficient of $r= -0.495$; $p<0.05$ and $r= -0.550$, $p<0.05$, respectively, indicating that the increase of soil pH H_20 and available P followed by the decrease of AM spore density.

Based on the zeolite pot cultural experiment:

• The inoculum source of AJ showed the lowest AM root infectivity but indicated the highest level of plant height, plant dry weight, and P uptake.

• The inoculum source of EP showed the lowest spore density but indicated the highest level of plant fresh weight and plant dry weight.

• The inoculum source of ID showed the highest AM root infectivity and spore density however its mycorrhizal effectivity on plant growth was lower than AJ and EP, but higher than the other inoculum sources.

Based on the results of exploration in natural conditions, the rhizospheric soil of elephant grass from AJ and EP were categorized having low spore density, and based on the pot cultural experiment using zeolite, these two inoculums of AJ and EP also showed low AM infectivity, but resulted in the highest AM effectivity on plant growth.

Based on the results of exploration, the rhizospheric soil from ATe indicated the highest spore density, however based on the zeolite pot cultural experiment the inoculum ATe showed the lowest AM effectivity on plant dry weight.

Based on zeolite pot cultural experiment, it was found that the inoculum sources of EP and AJ showed the highest value of mycorrhizal dependency (MD) and growth response (GR), whereas the inoculum of ATe showed the lowest MD and GR. To obtain effective biofertilizer, it is needed continued examinations for the functional capability of AM in target conditions.

Acknowledgements

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