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## Diversity of arbuscular mycorrhiza in the rhizosphere of Cajeput in agroforestry system with different fertilizer management of maize

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### Diversity of arbuscular mycorrhiza in the rhizosphere of Cajeput in agroforestry system with different fertilizer management of maize.

#### Parwi, B Pudjiasmanto, D Purnomo and VR Cahyani

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Abstract. This study investigated the diversity of arbuscular mycorrhiza in rhizosphere of cajeput with different fertilizer management of maize. This research was conducted by observation on cajeput agroforestry system in Ponorogo that have different fertilizer management of maize: conventional management (CM), universal management (UM) and alternative management (AM1, AM2, and AM3). The result showed that the highest infection of arbuscular mycorrhiza was observed in the plot of AM3, while the lowest colonization was observed in the plot of CM. Infection of arbuscular mycorrhiza in roots cajeput from five fertilizer management, ranging from 32.64% - 63.33%. In all fertilizer management, there were eight species of arbuscular mycorrhiza which five species were Glomus genus, one species was Acaulospora genus and two species were Gigaspora genus. Glomus constrictum was the dominant species in all fertilizer management. Acaulospora favoeta was found only in the plot of AM3. Spore density varies between 150-594 / 100g of soil. The highest spore density was observed in the plot of AM3, while the lowest spore density was observed in the plot of AM1. The highest diversity index value of arbuscular mycorrhiza (Species richness and Shannon-Wiener) was observed in the plot of AM3.

#### 1. Introduction

Arbuscular mycorrhiza is favorable soil microorganisms to host plants and ecosystem. Arbuscular mycorrhiza obtains carbon from the host plant. On the other hand, arbuscular mycorrhiza has a role for growth and yield crop, namely: increase adsorption nutrient and water [1], can protect the plant from root pathogens [2], preventing stress plants from unfavorable environments [3]. Furthermore, arbuscular mycorrhiza can improve soil fertility on degraded soils [4].

The diversity of arbuscular mycorrhiza depends on soil type, plant type and climate condition. The diversity of arbuscular mycorrhiza is also dependent on fertilization management. High N fertilization has a negative impact on the diversity of arbuscular mycorrhiza [5]. On the other hand, fertilization of manure and straw have a positive effect on the diversity of arbuscular mycorrhiza [6].

Arbuscular mycorrhiza exist in rhizosphere Cajeput in an agroforestry system in Sukun Village, district of Ponorogo has an intercropping system with a permanent cycle of maize as the main plant, soybeans and fallow. Each farmer has differences in fertilizer management for maize cultivation. Suspected that different fertilizer management will affect the diversity of arbuscular mycorrhiza in the rhizosphere of cajeput. This study investigated the diversity of arbuscular mycorrhiza in rhizosphere of cajeput (*Melaleuca leucadendron* LINN) with different fertilizer management of maize (*Zea mays*)

L). The information diversity of arbuscular mycorrhiza in cajeput rhizosphere can be used as a consideration in fertilizer management to create a sustainable agroforestry system

#### 2. Materials And Methods

#### 2.1. Location of sampling sites

This research was conducted by observation on cajeput agroforestry system in Ponorogo (7°52' S, 111°35' E, altitude 265 m) that have different fertilizer management of maize: conventional management (CM), universal management (UM) and alternative management (AM1, AM2, and AM3). Conventional management (CM) used 360 kg Urea / ha + 200 kg NPK / ha +2 t of chicken manure / ha. Universal management (UM) used 650 kg Urea / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha. Alternative management of AM1 (650 kg Urea / ha + 330 kg NPK / ha + 4 t of chicken manure / ha), AM2 (730 kg Urea / ha + 430 kg NPK / ha + Maize straw) and AM3 (650 kg Urea / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 330 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of chicken manure / ha + 430 kg NPK / ha + 3.3 t of

#### 2.2. Collection of root and soil samples

Soil samples were taken randomly from the rhizosphere of cajeput on an agroforestry systems. Each treatment was done randomly by three replications. In each replication, the soil sample was taken randomly for about five sub samples, so that total samples were  $5 \times 3 \times 5 = 75$  sub samples. Each sub sample comprised of 2 kg soil, which was put into a polybag and kept in room temperature before having soil analysis. Before the implementation of soil analysis, the same replication was mixed to obtain soil samples as a composite of sub samples.

Root samples were cleaned with running water to remove soil particles, then inserted into bottles containing 60% alcohol.

#### 2.3. Estimation infection of arbuscular mycorrhiza

The technique of Phillips and Hayman [7] was used to detect infection of arbuscular mycorrhiza in cajeput root. Infection of Arbuscular mycorrhiza was determined from fress root. Root samples were cut into 1 cm and inserted into a glass beaker containing 10% KOH at 70-80 °C for 10 minutes. The KOH solution was removed, and the sample was washed with water. Root samples were treated using 1% HCl and stained with with Staining solutions (glycerol : lactic acid : aquadest = 2 : 2 : 1 + trypan blue 0.05%). Infection of arbuscular mycorrhiza was observed under the microscope. Infection of arbuscular mycorrhiza percentage was calculated from the number root infection segment out of total root segment

#### 2.4. Isolation of arbuscular mycorrhiza

Mycorrhiza spores were isolated by wet sieving [8] and decanting by sucrose centrifugation [9] procedures. 100 g soil was dissolved into 500 ml of water. The suspension was filtered with 250  $\mu$ m, 90  $\mu$ m, and 45  $\mu$ m sieves filters. Filtrate on sieve 45  $\mu$ m was added 60% sugar and stirred for 10 minutes. The spores were filtered with a 45 $\mu$ m sieve and placed in a petridish to be observed under a binocular stereomicroscope. Spores were sorted into groups and counted.

#### 2.5. Identification of arbuscular mycorrhiza

Isolation of mycorrhiza spores was based on spore morphology including spore color, spore size, the number of spore walls, bolbours, hyphae and Melzer reagent. The identification is based on the morphological description provided by The international collection of vesicular arbuscular mycorrhizal fungi (*http://invam.caf.wvu.edu*) and originally published species descriptions.

#### 2.6. Statistical analysis

The diversity of arbuscular mycorrhiza was measured by spore density, Relative abundance, Species richness and Shanon Wiener index [10]. The formula used to calculate these parameters is given in Table 1. Data analysis was done using one-way analysis of variance (ANOVA) by SPSS program. LSD analysis (Students t least significant difference) was used to compare the treatments. The Pearson correlation coefficient was used to determine the relationship between spore density with Species Richness. Regression analysis was calculated to assess the significance of the relationship between spore density and species richness

Table 1. Diversity measures used to describe AM communities				
Spore density	The number spores in 100 g soil			
Relative abundance (RA)	(Spore number of species / total number of identified spore samples) x 100			
Species Richness (SR) was estimated from Margalef Indeks (D <sub>Mg</sub> )	$D_{Mg} = S - 1/ln N$			
Shannon-Wiener index of diversity (H')	H' = - pi (ln pi)			
Pi is the relative abundance of each ide	entified species per sampling site and calculated by the			

Pi is the relative abundance of each identified species per sampling site and calculated by the following formula,

Pi = ni/Ni where n is the spore numbers of a species and N is the total number of identified species per sampling sites

#### 3. Results And Discussion

#### 3.1. Diversity of arbuscular mycorrhiza

Soil samples of cajeput rhizosphere were collected from five location that different of fertilization management revealed the presence of several arbuscular mycorrhiza species. A total of eight species of arbuscular mycorrhizas were wet sieved from the soil sample collected from five different of fertilization management. The identified species were *Glomus claroideum, Glamus lamellosum, Glomus coronatus, Glomus ambisporum, Glomus constrictum, Gigaspora rosea, Gigaspora margarita* and *Acaulospora favoeta* (Table 2). Similarly in this study also eight arbuscular mycorrhiza species were isolated by Pagano and Scotti [11] from two species of *Eucalyptus* plants in semiarid Brazilian. A number of arbuscular mycorrhiza species in this research was highest than in rhizosphere of *Eucalyptus globules* in Portugal (five species) was reported by Silvia *et al*, [12].

Spore abundance arbuscular mycorrhiza was highest *Glomus constrictum* (60.87% - 73.56%), followed by *Glomus claroideum* (13.61% - 26.81%), and lowest *Gigaspora rosea* (2.49% - 5.66%). Spore abundance arbuscular mycorrhiza was dominated genera *Glomus*. A similar observation of spore abundance arbuscular mycorrhiza was reported Pagano and Scotti [11] that the dominant arbuscular mycorrhiza on Eucalyptus is *Glomus sp*. Furthermore, Chen and Dell [13] proposed that spore abundance arbuscular mycorrhiza in Eucalyptus rhizosphere in China was highest *Glomus mossae* (67%), followed by *Glomus geosporum* (15.4%) and lowest *Acaulospora scrobicullata* (2.6%). On the other hand, Silvia *et al.* [12] which states that arbuscular mycorrhiza colonizing in the Eucalyptus rhizosphere was dominated by *Gigaspora margarita* (40%). Spore density in the agroforestry system of Cajeput ranges from 150 - 594/ 100g of soil. Spore density was highest in e plot of AM3 and lowest in the plot of CM (Figure 1).

Diversity indices (H' and SR) of arbuscular mycorrhiza was observed different on five kind fertilizer management. Shannon-wiener index (H') in the plot of CM ( $1.06 \pm 0.11$ ) was higher than in the plot of UM ( $0.94 \pm 0.05$ ), although the number spores in the plot of CM was lower than in the plot of UM. Shannon Wiener index was determined number spores and species distribution [14]. Shannon-Wiener index (H') was lowest ( $0.90 \pm 0.07$ ) in the plot of AM1 and highest ( $1.07 \pm 0.03$ ) in

the plot of AM3. Richness species (SR) was highest  $(7.17 \pm 0.57)$  in the plot of AM3 and lowest  $(5.46 \pm 0.58)$  in the plot of AM1 (Table 3). The correlation analysis found that the spore density was positively correlated with Richness species (r = 0.848) (Figure 2). A similar observation was reported by Kavitha and Nelson [15] that spore density is positively correlated with Richness species (r=0.618)

Table 2. Relative abundance arbuscular mycorrhiza						
Species	CM	UM	AM1	AM2	AM3	
Glomus Claroideum	23.34±6.92	13.61±4.93	17.41±5.23	23.00±1.43	26.81±2.21	
Glomus lamellosum	$0.41 \pm 0.71$	1.93±0.76	$0.42 \pm 0.72$	$1.06\pm0.64$	$0.46 \pm 0.42$	
Glomus coronatus	$3.70{\pm}1.45$	1.23±0.76	2.73±3.23	$2.19\pm0.82$	$2.68{\pm}1.64$	
Glomus ambisporum	2.31±2.46	$2.53 \pm 0.80$	$2.78{\pm}1.70$	$3.06 \pm 2.05$	$1.27 \pm 0.25$	
Glomus Constrictum	61.87±9.37	73.56±3.17	71.63±4.14	64.73±2.87	$60.87 \pm 2.35$	
Gigaspora Rosea	$3.03 \pm 2.76$	4.35±1.79	3.20±2.29	$2.49 \pm 2.14$	5.66±1.63	
Gigaspora Margarita	5.30±6.39	$2.79 \pm 0.37$	1.83±1.63	$3.46{\pm}1.70$	2.13±0.91	
Acaulospora favoeta	0	0	0	0	$0.02 \pm 0.006$	

CM: Conventional management, UM: Universal management, AM: Alternative management 1, AM2: Alternative management 2, AM3: Alternative management 3.



**Figure1**. Spore density (CM: Conventional management, UM: Universal management, AM: Alternative management 1, AM2: Alternative management 2, AM3: Alternative management 3).

Table 3.	Diversity	of	arbuscular	mycorrhiza
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Fertilizer Mangement	Shannon – Wiener(H')	Species Richness (SR)
СМ	1.06±0.11 c	5.47±0.58 a
UM	0.94±0.05 ab	6.50±0.58 b
AM1	0.90±0.07 a	5.46±0.58 a
AM2	1.05±0.03 bc	6.84±0.00 c
AM3	1.07±0.03 c	7.17±0.57 c

CM: Conventional management, UM: Universal management, AM1: Alternative management 1, AM2: Alternative management 2, AM3: Alternative management 3.

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Figure 2. Linear regression between spore density with Species Richness

#### 3.2. Infection of arbuscular mycorrhiza

Infection of arbuscular mycorrhiza was observed in the five plots studied with different infection of arbuscular mycorrhiza. Infection of mycorrhiza was highest in the plot of AM3(63%) and lowest in the plot of CM (36%) (Figure 3). A similar observation infection of arbuscular mycorrhiza (35-55%) in rhizosphere *Eucalyptus camaldulensis* and *E.grandis* in semiarid Brazil was reported Pagano and Scotti [11]. However, infection of arbuscular mycorrhiza only of 16-39% in rhizosphere of *Eucalyptus globules, E. maideni* and *E. sideroxylon* rhizosphere was reported from Northern Algeria [16] and only of 1 - 26% in rhizosphere of *Eucalyptus grandis* in native forest Northern Queensland Australia was reported Adams *et al*, [17]. Infection of arbuscular mycorrhiza in plant roots depends on host plants, soil, climate and land history [13].



**Figure 3**. Infection of arbuscular mycorrhiza. (CM: Conventional management, UM: Universal management, AM1: Alternative management 1, AM2: Alternative management 2, AM3: Alternative management 3).

#### 4. Conclusion

Communities of arbuscular mycorrhiza in the rhizosphere of cajeput in agroforestry system showed varies in diversity on different fertilizer management of maize. The highest infection of arbuscular

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mycorrhiza, highest spore density and highest diversity index value of arbuscular mycorrhiza were found in the plot of AM3. The lowest colonization was observed in plot of CM, and lowest spore density was observed in the plot of AM1. In all fertilizer management, there were eight species of arbuscular mycorrhiza which five species were *Glomus* genus, one species was *Acaulospora* genus and two species were *Gigaspora* genus. Arbuscular mycorrhiza species were dominated by *Glomus* constrictum in all fertilizer management. Especially in the plot of AM3 was found *Acaulospora* favoeta.

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