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Coal Waste Powder Amendment and Arbuscular Mycorrhizal Fungi Enhance the Growth of Jabon (*Anthocephalus cadamba* Miq) Seedling in Ultisol Soil Medium

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ABSTRACT

Coal powder waste application on low nutrient media is expected to be able to increase plant growth and to improve Arbuscular mycorrhizal fungi (AMF) development. The objective of this research was to determine the effect of coal waste powder on the growth of *Anthocephallus cadamba* Jack and AMF development grown on ultisol soil. Two factors in a completely randomised experimental design was conducted under greenhouse conditions and Duncan Multiple Range Test was used to analyse of the effect the treatment. The first factor was ultisol soil ammended with coal waste powder (control, soil amanded with coal waste 5%, soil amanded with coal waste 10% and soil amanded with coal waste 15%) and the second factor was AMF inoculation (uninoculated control, inoculated with *Gigaspora margarita*). Plant height, diameter, shoot dry weight, percentage of AMF colonization and nutrient uptake were measured in this experiment. Results of this study showed that coal amendment and AMF when applied separately significantly increased height, diameter, shoot dry weight, root dry weight and nutrient uptake of 12 weeks *A. cadamba* seedling, but when the coal waste powder and AMF were combined the plant growth parameters were lower than those applied separately but significantly higher than control. The application of coal waste powder or AMF in ultisol soil could increase *A. cadamba* growth and development.

Keywords: AMF development, coal waste, nutrient uptake, plant growth

INTRODUCTION

The majority of soils in the tropical region are acid soil (Ultisol, Oxisol). At least 500 million hectares (16.2%) of the African continent (Bationo *et al*. 2006) and 38 % of the land area in Southeast Asia has acidic upland soil (van Uexkull and Bosshart 1989). In Indonesia, Ultisols soils cover 45.794.000 ha, almost 25% of total land surface (Subagyo *et al.* 2004) which are wide spread in Kalimantan (21.938.000 ha), Sumatera (9.469.000 ha), Maluku and Papua (8.859.000 ha), Sulawesi (4.303.000 ha), Java (1.172.000 ha), and Nusa Tenggara (53.000 ha) (Subagyo *et al.* 2004). The characteristics of these soils are low nutrient content, high acidity with aluminium and Mn toxicity, low organic matter and biodiversity and very low mineralization and nitrification (Kochian *et al.* 2004). Such conditions are major constraints to agricultural and forestry plant growth and productivity.

Many efforts have been applied to overcome the problems of tropical acid soil and infertility. Commonly the application of lime in the form of calcium and/or magnesium carbonates, in order to increase soil pH, and modifying its chemical properties are widely used in the field (Anetor and Akinrinde 2007). The addition of organic materials to acid soils can have a direct effect on soil organic matter content, reduce Al toxicity, increase of soil pH and soil biota, aggregate stability, soil enzymatic activities, water soluble C and water soluble carbohydrates as well as nutrient availability, especially P (Escobar and Hue 2008; Bougnom *et al.* 2010; Medina and Azcon 2010). The application of charcoal to acid soils significantly increase their pH, soil organic C, N, P, K, Ca, Mg, decrease CEC, Al^{3+} and increase diameter and height of *Accacia mangium* growth (Siregar 2007). The used of biochar (Matsubara *et al.* 2002); wood, leaf and rice husk ash (Insam *et al.* 2009; Nwite *et al.* 2011); coal combustion fly ash (Karmakar *et al.* 2010) for improving acid soil properties and plant growth have been well documented. However, no data on the effects of soil amendment coal waste powder on the growth of forest trees plant are available. Coal waste powder are available in a large amount around coal mine company and has similar properties as coal combustion ash, therefore it will have a potential as acid soil amandment.

Arbuscular mycorrhizal fungi (AMF) is one of the soil microorganism that forms essential components of sustainable soil-plant system (Hause and Fester 2005). These fungi provide numerous benefits to their host, including better phosphours nutrition in acidic soil (Gossous and Mohammad 2009, Guissou 2009), increasing absorption of Nitrogen (He *et al.* 2009, Rotor and Delima 2010), producing plant growth hormones (Herrera-Medina *et al.* 2007), defending root against soil borne diseases (Bakhtiar *et al.* 2010) and increasing plant growth and productivity (Wu *et al.* 2010; Duponnoisa *et al.* 2005).

Jabon (*Anthocepalus cadamba* Miq.) known as 'kadam' is one of potential tropical forest tree species and has been used for industrial plantations. The kadam wood is suitable for plywood, flooring, toys, boxes and other end use product. The kadam root and fruits are also suitable for antifungal and antimicrobial (Mishra and Sidique 2011; Acharrya *et al.* 2011; Umachigi *et al.* 2007). Kadam is one of pioner tree species that very prospective for reforestation and afforestation on land post mining area by using coal waste and arbuscular mycorrhizal fungi. However, there is no data available yet on the interaction effect of AMF and soil amendment coal powder residu on kadam growth.

The aims of this study were to determine (i) effect of residue coal powder soil amendment on kadam growth (ii) effect of AMF on kadam growth (iii) effect of interaction coal powder residu and AMF on kadam growth which were grown in ultisol soil.

MATERIALS AND METHODS

Soil and Coal Powder

Surface soil (0-20 cm) was collected from Jasinga Forest Reseach Station, Bogor, air dried, sieved (2 mm) and stored in the bag until used. The physicochemical properties of soil were determined following standard method in Soil Laboratory, Faculty of Agriculture Bogor Agriculture University, Bogor (Table 1).

Coal residu was obtained from PT. Marunda Graha Mineral, a mine company located in Murung Raya District, Central Kalimantan. Coal were broken and sieved (2 mm) than it was stored until used. The most important properties of coal powder used are shown in Table 1. It was determined by standard procedure in Soil Laboratory, Faculty of Agriculture Bogor Agriculture University. The soil and coal powder were mixed in a proportion of 100:0; 95:5; 90:10 and 85:15 (v/v), respectively. Mixture media were autoclaved at 121 ^{o}C for 1 hour.

Table 1. Chemical characteristics of soil medium and coal powder used.

Chemical characteristic	Soil medium	Coal powder
pH H_2O	4.5	6.5
pH KCl	3.7	5.3
Organic-C (%, Walkley & Black) %	2.64	15.6
Total-N (%, Kjeldhal)	0.24	0.95
Ratio C/N	11	15.96
Available-P (mg kg^{-1}, Bray I)	8.6	71
Total-P (mg $kg^{-1,}$ HCl 25 %)	206.6	568.5
Ca (me 100 g^{-1})	0.47	7.32
Mg (me 100 g^{-1})	0.28	1.29
K (me 100 g^{-1})	0.17	1.14
Na (me 100 g^{-1})	0.13	2.8
CEC (me 100 g^{-1})	23.38	19.46
Base saturation (%)	4.49	64.49
Exch-Al (me 100 g^{-1})	1132	Not detected
Exch-H (me 100 g^{-1})	0.51	0.04
Fe (me 100 g^{-1})	4.08	26.8
Cu (me 100 g^{-1})	0.08	0.4
Zn (me/100 gram)	0.68	1.88
Mn (me 100 g^{-1})	7.56	8.92

Seedling and Fungal Materials

Seeds of *A. cadamba* were obtained from Silviculture Laboratory, Faculty of Forestry, Bogor Agriculture University. Seeds were shown in plastict box containing sterilized soil and placed in green house for two month. Plants were watered as needed. Mycorrhizal spores of *Ggaspora margarita* were obtained from Silviculture Laboratory, Faculty of Forestry, Bogor Agriculture University. Spores were surface sterilized following the method of Budi *et al.* (1999) and stored in refrigerator until used.

Mycorrhizal Spores Inoculation

Two month old uniform *A. cadamba* seedlings with four leaves were transplanted into 500 ml polybag containing sterilized mixed ultisol soil and coal powder. Plants were wether inoculated with surface sterilized arbuscula mycorrhizal spores *G. margarita* or not as a control at transplanting. Each inoculated plant received 50 surface sterilized arbuscula mycorrhizal spores. Plants were grown for twelve weeks in the green house and watered as needed.

Harvesting and Parameter Measurement

Plants were harvested twelve weeks after transplanting and evaluated for height, diameters, shoot and root dry weight. Shoot and root weights were recorded after drying at 70 °C to constant weight. N, P and K concentrations were measured following standard procedure in Soil Laboratory, Faculty of Agriculture, Bogor Agriculture University. Mycorrhizal colonisation were evaluated after cleaned with 2.5% KOH , and stained with 0.05% trypan blue in acidic glycerol (Koske and Gemma 1989). Percentage of mycorrhizal colonisation were determined according to the method of Biermann and Linderman (1981).

Experimental Design and Data Analysis

The experiments were done with a factorial design with 2 levels of arbuscula mycorrhizal inoculation (inoculated and uninoculated), and 4 levels of coal waste powder as soil amendment (control, and ultisol soil amanded with 5%, 10% and 15% of coal waste powder). The experiment was arranged in a completely randomized design in a polybag culture with 5 replications.

All data were analysed by analysis of variance procedure (ANOVA) and followed by further tests using Duncan Multiple Range Test (DMRT) at 5% level by using the MStat program.

RESULTS AND DISCUSSION

Coal amendment significantly increased height, diameter, shoot dry weight and root dry weight of 12 weeks *A. cadamba* seedling in all levels of coal added (Table 2). The plant height, diameter, shoot dry weight and root dry weight increased with increasing amount of coal added regardless AMF inoculation (Table 2). The plant height increased by 235.8%, 252.1% and 487.2% when soil amanded by coal 5%, 10% and 15%, respectively (Figure 1), while the plant diameter with the same treatment increased by 163.5%, 155.4% and 187.84%, respectively as compared to control. The greatest shoot and root plant dry weights were occured at highest level of coal added, which increased by 1221.6% and 753.9%, respectively as compared to control (Figure 1).

Arbuscula mycorrhizal fungi *G. margarita* inoculation significantly increased height, diameter, shoot and root dry weight of A. cadamba seedlings (Table 2). Mycorrhizal plant height, diameter, shoot and root dry weights were comparabel to coal treatment plants (Table 2), and their height had much greater 54.5% than those coal treatment plants and 541.7% much higher than control plants (Figure 1). Mycorrhizal root colonization decreased with increasing amount of coal added to the soil (Table 3). There was no evidence of AMF colonization in uninoculated plant. Interaction between coal and arbuscular mycorrhizal fungi significantly increased plant height, diameter, shoot dry weight and root dry weight and was much greater than control plant (Table 2). Interaction between arbuscular mycorrhizal fungi with coal at all level doses decreased plant height, diameter, coal

Table 2. The effect of coal powder on arbuscular mycorrhizal root colonization.

Mycorrhizae	Coal powder % (v/v)	Mycorrhizal roots colonization (%)
Control	0	0
G. margarita	0	90.00 a
	5	84.67 ab
	10	81.33 ab
	15	60.00 c
Significancy		
Coal powder		**
Mycorrhizae		ns
Interaction		**

**= significantly different at confidence level of 0.01%. Numbers followed by same letter within coloum are not significant different (P 0.05) by DMRT test.

Table 3. The effect of coal powder and arbuscular mycorrhizae on the growth of kadam seedling at 12 weeks after planting.

Mycorrhizae	Coal powder % (v/v)	Height (cm)	Diameter (cm)	Shoot dry weight (g)	Roots dry weight (g)
Control	0	7.2 e	1.48 b	0.37 c	0.13 d
	5	24.18 d	3.9 a	2.91 abc	0.88 abc
	10	25.35 cd	3.78 a	2.88 abc	0.84 abc
	15	42.28 abc	4.26 a	4.89 ab	1.11 abc
G. margarita	0	46.2 ab	4.04 a	3.53 ab	0.76 abcd
	5	32.46 bcd	4.12 a	3.67 ab	0.66 bcd
	10	41.3 abcd	3.74 a	3.25 abc	0.48 bcd
	15	39.46 abcd	3.96 a	3.72 ab	0.71 abcd
Significancy					
Coal powder		**	**	**	**
Mycorrhizae		**	**	*	*
Interaction		**	**	**	*

**= significantly different at confidence level of 0.01% and *= significantly different at confidence level of 0.05%. Numbers followed by same letter within coloum are not significant different (P 0.05) by DMRT test.

shoot dry weight and root dry weight as compared to plants wether inoculated by AMF or treated by coal powder separately (Figure 1).

Interaction of coal amendment and AMF inoculation significantly increased leaf tissue N, P and K, which was greater than control plant (Table 4). Coal soil amendment had much greater leaf tissue N, P and K than those plants tissue inoculated by AMF alone. Leaf tissue N, P and K increased with increasing coal amount added to the soil in uninoculated mycorrhizal plant, while in inoculated mycorrhizal plant, leaf tisue N, P and K decreased at 10% and 15% coal added level (Table 4).

Discussions

Previous study demonstrated that coal combustion fly ash could improve acid soil properties and plant growth (Karmakar *et al.* 2010). In this study coal waste soil amendment increased kadam seedling growth and development. Coal is derived from organic matter and as geological processes apply presure to organic matter over time and is transformed to different product based on their organic maturity which are; lignite coal, bituminous coal and atracite (WCA 2009). Coal provides the primary source of energy for many countries and large quantities of waste products are produced during the combustion of coal in coal-fired power stations (Seshadri *et al.* 2010). The combustion of

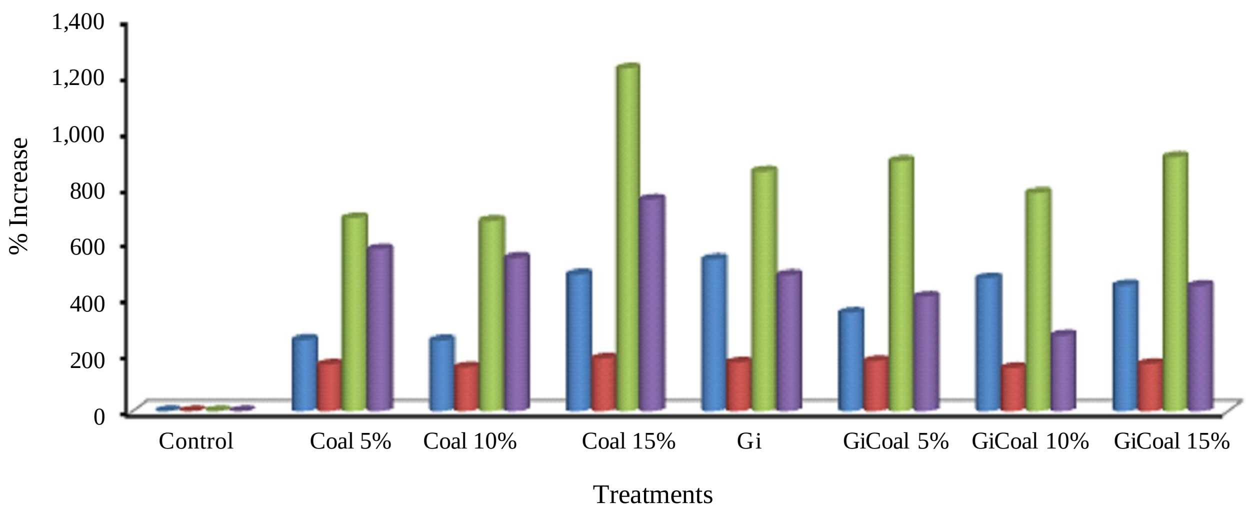

Figure 1. Percentage of increased parameters growth as compared to control. ■ = height (cm), ■ = diameter (mm), ■ = shoots dry weight, and ■ = roots dry weight.

Table 4. Nutrient (N, P, and K) uptake by kadam seedling as affected by mycorrhizae and coal powder applications.

Mycorrhizae	Coal powder % (v/v)	N (g plant^{-1})	P (g plant^{-1})	K (g plant^{-1})
Control	0	10.71	1.52	12.23
	5	54.93	5.39	70.59
	10	67.44	5.90	40.18
	15	104.34	11.83	76.52
G. margarita	0	52.42	10.91	76.36
	5	96.06	12.67	105.56
	10	75.18	6.80	43.32
	15	77.06	8.67	65.02

to generate electricity in Termal Tower Plant produces solid wastes like fly ash and bottom ash (Seshadri *et al.* 2010). Several studies demonstrated that coal combustion residues (fly ash) improved soil properties and increased plant growth and development when it were used as soil amendment (Ram *et al.* 2006; Ram *et al.* 2007; Karmakar *et al.* 2010). This study demonstrated that coal waste powder with its chemical characteristict showed high pH, P, C-organic, Ca and Mg contents and cantained a trace element (Table 1). It also could increase height, diameter, shoot dry weight and root dry weight of Kadam seedling. Although the pH of tretment soil was not recorded in this study, numerous study demonstrated that the application of fly ash combustion as soil amandement could increase the soil pH (Seshadri *et al.* 2009), and in turn influencing the reaction and transformation of nutrient avaibility particularly P for plant growth and development. Phosporous is one of the mineral nutrients essential for plant growth (constituting up to 0.2% of the dry weight of the plant cell) and development (Shtark *et al.* 2010). The application of fly ash in soils have been reported to improve growth and yield and mineral composition of the rice plants, and reduced the N fertilizer when applied with bluegreen biological fertilizer and N fertilizer (Tripathi *et al.* 2008). Other study by Hill and Lamp (1980) demonstrated that the used of fly ash could realease Mg at rates comparable to most Mg fertilizer. Seoane and Leiros (2001) showed that the application of fly ash increased gradually soluble Ca over time in field condition. The used of coal waste powder in this study also contributed Ca and Mg to the soil and plant growth and development, gave similar results of those used fly ash as soil amandement. The evidence of soil nutrient improvement by coal waste powder in this study was also indicated by leaf N, P and K content which increased by increasing coal added (Table 4).

The results of this study demonstrated that the growth and development of kadam seddling improved by Arbuscular mycorrhizal fungi *G. margarita* inoculation, in particularly in soil without coal amendement (Table 2). The improvment of non tropical plant growth and development by AMF in acidic soil have been reported (Marco *et al.* 2006; Nagarathna *et al.* 2007; Ortas 2010; Duponnoisa 2005; Arpana and Bagyaraj 2007), due to the improvement of soil P absorption. As shown in Table 1. the soil chemical characteristic used in this study had low pH, low P avaibility, high P total and high aluminium (Al), indicating P immobilization in the soils were occured. In such soil conditions, the absorbtion of P by plant root is limited. Extramatrical hyphae of mycorrhizal fungi including *G. margarita*, by growing into soil not colonized by root, can absorb P from the soil solution and translocate it to the root (Smith and Read 2007). Mycorrhizal plant could have dense extramatrical hyphae extend up to 25 cm from the root, that facilitate increasing the volume of soil explored for nutrient that are not accessed by root (Smith and Read 2007; El Seoud 2008). In this study, leaf N, P and K of arbuscular mycorrhizal plants were greater than control (Table 4) indicating the mycorrhizal fungi *G. margarita* has been functioned. In addition, the production of phosphatase enzyme by mycorrhizal fungi have been reported by several researcher (Wang *et al.* 2011). This enzyme can facilitate the solubilization of Al/Fe-P bound in acid soil to be released to the soil and absorbed by mycorrhizal hyphae or root plant.

The application of coal waste powder in this study decreased arbuscular mycorrhizal root colonization (Table 2). and in turn decreased also plants height, diameter, shoot dry weight and root dry weight (Table 4) with increasing coal added. Previous study demonstrated that the AM root colonizations were affected by soil P avaibility, in plants with a high P status root colonization were reduced or absent, whereas low P levels enhanced root colonization (Vierheirlig 2004; Karandashov and Bucher 2005; Bucher 2007; Sabannavar and Lakshman 2009). Smith and Read (2007) reported that a decreasing of the cell membran permeability in plants grown at hight P condition, resulting in a lower leakage of amino acids and sugar, has been linked with a lower root colonization. Mycorrhizae is a mutual symbiotic organism that their life cycle are depend on the sugar and amino acid from the host plant. As shown in Table 1 that coal waste powder contains high available P that can contribute to soil P avaibility and in turn decrease mycorrhizal root colonization and plant growth. This finding reveals that there was no synergetic effect between coal waste powder and mycorrhizal arbuscular fungi *G. margarita* for improving Kadam growth in ultisol soil medium so that the application should be separately.

CONCLUSIONS

In overall, the important findings of this research that ultisol soil for Kadam plant growth medium could be improved by application of arbuscular mycorrhizal fungi as well as coal waste powder amandments. This findings implie the prospective and potential use of arbuscular mycorrhizal fungi and coal waste for the succesful of post mine land rehabilitation as well as forest land rehabilitation which dominated by Ultisol soil.

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