Effects of Mycorrhiza Doses and Manure Types on Growth and Yield of Cassava in Gunungkidul

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ABSTRACT

Gunungkidul is a production center of cassava (***Manihot esculenta*** Crantz), a carbohydrate source and raw material for food industry. AMF inoculation in cassava plants is known to increase biomass production. However, little studies have been conducted on the response of cassava to mycorrhizal inoculation and organic fertilizer. Therefore, this study was aimed at examining the effects of AMF inoculation and types of manure on the AMF colonization and yield of cassava in Gunungkidul. The research was carried out by planting cassava in Alfisol Gunungkidul arranged in a randomized complete block design with two factors, AMF doses of 25g; 50; and 75g/plant; and types of manure i.e. cow, goat, and poultry manure, for five months period. Rhizosphere soil and root samples were analyzed for AMF colonization and the spores number. The results showed that AMF-infected cassava roots combined with cow or goat manure application produced more spores than poultry manure. AMF infection and manure, thus, significantly resulted in better root proliferation, root forehead weight, tuber diameter, and cassava products, than the absence of both treatments. Cow manure combined with AMF at a dose of 25 g/plant significantly affected the dry weight of cassava roots. This study implies that applying AMF and manure provide a substantial contribution on the growth and production of cassava.

Keywords: AMF, Cassava, Gunungkidul, Manure

ABSTRAK

Gunungkidul merupakan sentra singkong (Manihot esculenta Crantz), sebagai salah satu sumber kabohidrat dan bahan baku industri di Indonesia. Penelitian ini bertujuan untuk mengkaji pengaruh dosis inokulasi Mikoriza (Arbuscular Mycorrhizal Fungi-AMF) dan jenis pupuk kandang terhadap kolonisasi pada akar, pertumbuhan dan hasil singkong di Gunungkidul. Metode penelitian yaitu singkong ditanam di lahan Alfisol Gunungkidul dengan rancangan acak kelompok lengkap dan diberi perlakuan faktorial dosis AMF (25g, 50, 75g/tanaman) dengan jenis pupuk kandang (sapi, kambing, ayam). Tanah rhizosfer tanaman singkong dan sampel akar dianalisis kolonisasi Mikoriza dan jumlah sporanya. Parameter pertumbuhan tanaman dan hasil singkong selama 5 bulan dilakukan dianalisis. Hasil menunjukkan bahwa AMF menginfeksi akar singkong 100% dan aplikasi pupuk kandang sapi atau kambing menghasilkan spora lebih banyak dari pupuk kandang ayam dan nyata lebih baik terhadap proliferasi akar, berat kering akar, diameter ubi dan hasil ubi singkong. Pupuk kandang sapi dengan dosis AMF 25g/tanaman nyata saling berpengaruh terhadap berat kering akar tanaman singkong, sehingga disarankan penggunaan pupuk kandang sapi dengan mikoriza ini pada budidaya singkong karena dapat meningkatkan pertumbuhan dan hasil.

Kata kunci: *Mikoriza, Singkong, Gunungkidul, Pupuk Kandang*

INTRODUCTION

Symbiotic associations between Arbuscular Mycorrhizal Fungi (AMF) with plant roots often occur in almost 80% of terrestrial plants (Brundrett & Tedersoo, 2018; Zhang et al., 2019). AMF symbiosis with plants plays an essential role in the absorption of minerals, especially phosphorus ions exposed to soil and micronutrients, and increases the plant's resistance to pathogens, drought stress, and heavy metals so that it is potentially used as an environmentally friendly biological fertilizer (Jiang et al., 2017; Ryan & Graham, 2018). AMF inoculation in cassava plants can increase biomass production (De Bauw et al., 2021). Still, the variety strongly influences the association, species, and number of AMFs and their cultivation techniques (Ryan & Graham, 2018). The research of (Saputro

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et al., 2016) showed that the provision of a 75-gram crude AMF/plant was the most effective dose for the growth of *Albizia chinensis* plant. Some AMF genera associated with cassava are the genus *Glomus* sp., *Gigaspora*, sp., and *Acaulospora* sp. According to (Lone et al., 2017), the crude inoculum dose is AMF 20 grams/plant for agriculture.

Gunungkidul is a production center of cassava, a food source of carbohydrates in Indonesia used as raw material for the food processing industry, animal feed ingredients, and bioethanol (Hidayat et al., 2016; Ogundare, 2017). Cassava plants are easy to grow. However, fertilization is needed in its cultivation to get the optimal yield, and synthetic fertilizers are usually widely used. According to (Biratu et al., 2018b), the effect of synthetic fertilization on cassava depends on previous cropping patterns, soil type, and season. The application of 2.8 tons/ha of manure and *NPK* fertilizer (100:22:83) at the beginning of the wet season increased a higher yield compared to the application at the end of the dry season. The soil in Gunungkidul is weathering limestone with low organic matter content. The soil is infertile, dry, and fragile during the dry season. For this reason, it is necessary to study the proper organic matter to be applied for the sustainability of cassava cultivation in Gunungkidul by AMF inoculation.

Environmental factors, such as temperature, humidity, pH, and organic matter, affect the development of AMF (Ryan & Graham, 2018; Valverde Barrantes et al., 2017). Cow manure improves soil fertility and cassava production (Ognalaga et al., 2017). In intensive agriculture, the AMF population is lower than in low-input systems. In contrast, according to (Chandhana & Kerketta, 2021), goat manure can increase cassava weight and protein content. The advantages of chicken manure applications are improving soil physical properties, water binding capacity, organic matter, and soil nutrient content (Biratu et al., 2018a; Biratu et al., 2018b). For this reason, this study aimed to examine the effects of mycorrhizal fungi inoculation dose and type of manure on the colonization of roots, growth, and yield of cassava in Gunungkidul.

MATERIALS AND METHOD

The research was conducted in Alfisol soil in Gunungkidul, arranged in a randomized complete block design consisting of two factors. The first factor was AMF dose (25g, 50, and 75g/plant), and the second was the type of manure (cow, goat, and poultry). Each treatment combination was replicated three times, each consisting of eight plants.

Gunungkidul indigenous AMF inoculum was obtained by multiplication of the trapping method for three months, then applied in the planting hole before planting cassava seeds with a spacing of 1x1 m. The type of manure treatment was given a week before planting (Selvakumar et al., 2016)

The number of infections was observed using microscopic analysis, according to the method of Kormanik &McGraw, and calculated based on the AMF colonization in the roots of cassava plants. The amount of AMF spores was calculated by extracting 100 g of rhizosphere soil using the wet sieving and decanting technique (Selvakumar et al., 2016). Dry root weight, the number of primary and secondary roots, plant height, and the number of leaves were determined when the plants aged 1, 2, and 3 months. Meanwhile, the length, diameter, number, and weight of the tubers were determined by harvesting 5-month-old plants.

Statistical analysis

The data of AMF colonization and the number of spores, root dry weight, number of primary and secondary roots, plant height, number of leaves, tuber's length, diameter, number, and weight were analyzed using analysis of variance. If there was a significant difference between treatments, the data were subjected to Duncan Multiple Range Test at a significance level of 5%.

RESULTS AND DISCUSSION

AMF colonization of cassava roots

Mycorrhizae colonize cassava plants by infecting roots (Straker et al., 2010). The percentage of internal hyphae formation, external hyphae, arbuscular, or vesicles on the roots indicate mycorrhizal colonization at the roots of cassava plants (Ryan & Graham, 2018). Based on microscopic analysis of roots colonized by AMF, this study reported compatibility between mycorrhizae and cassava plant roots, as indicated by mycorrhizal colonization of cassava roots by 100%. However, there was no interaction between AMF doses and types of manure. The AMF dose or types of manure did not affect anything. The development of AMF colonization is presented in Figure 1.

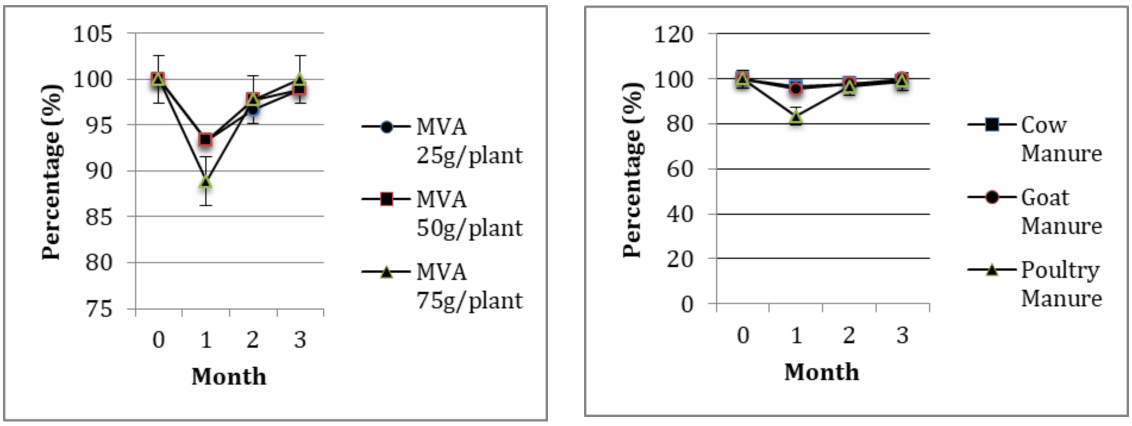
This result showed that the compatibility of Gunungkidul indigenous mycorrhizae with cassava roots was excellent, as indicated by the percentage of AMF colonization at a dose of 25 g/plant, which was not significantly different from that at doses of 50 g/plant or 75 g/plant (Table 1). The colonization percentage was slower with the higher AMF dose due to the competition between spores.

From trapping results, AMF first infected 100% of corn plants. However, after the inoculation of cassava plants, the percentage of AMF colonization decreased in the first month. It is because the

Table 1. The percentage of mycorrhizal colonization and spores at the roots of cassava plants in the 3rd month

Treatments	Mycorrhizal Colonization (%)	Number of spores (spores / 100g of soil)
Mycorrhizal Dosage:		
25 g/plant	100 a	62.3 a
50 g/plant	100 a	67.2 a
75 g/plant	100 a	62.2 a
Manure types:		
Cow manure	100 p	66.6 p
Goat manure	100 p	69.6 p
Poultry manure	100 p	52.8 q
Interaction	(-)	(-)

Remarks: Means followed by different letters are significantly different based on the F test at a significance level of 5%; (-) indicates no interaction between treatments



(a) (b)

Figure 1. Development of AMF colonization as affected by (a) AMF dose and (b) Type of manure

AMF infection process was taking place at the root of the plant, and it turned out that the 75 g dose resulted in the lowest colonization (88%). Likewise, AMF colonization in plants treated with poultry manure was the lowest (83%) compared to those treated with cow manure (96%) and goat manure (95%). Later in the next 2^{nd} and 3^{rd} months, the overall colonization reached 100%, and there was no mutual influence and a significant difference between treatments (Table 1).

The number of AMF spores and their diversity

AMF symbiosis with plant roots gets energy from the host and develops to produce spores. In the third month, AMF dose and type of manure did not influence the number of spores (Table 1). Still, the highest number was in cow manure (66.6 spores/100 g of soil) and goat manure (69.6 spores/100 g of soil), which was significantly different from that in poultry manure (52.8 spores/100 g of soil). While the development of the number of spores in the rhizosphere of cassava plants over three months showed an increasing number of spores, there was no significant interaction effect between AMF doses and types of manure (Figure 2).

AMF spore production increased rapidly after the 2^{nd} month, reaching 66.78 spores/100g of rhizosphere soil, but it was not affected by the AMF dose. In contrast, cow manure (67 spores/100g of rhizosphere soil) and goat manure (68 spores/100g of rhizosphere soil) were the best organic matter to increase the number of AMF spores compared to poultry manure (53 spores/100g of rhizosphere soil). Based on the identification of spore types, it was dominated by *Glomus* sp., although some *Gigaspora* sp. and *Acaulospora* sp. also existed.

The AMF in symbiosis with plant roots obtains energy from the host and develops to produce spores (Zhang et al., 2019). The three-month observations showed that the number of spores in the cassava rhizosphere increased, along with the increase in the percentage of AMF colonization, which was not affected by various AMF doses (Figure 2). However, the number of spores in plants fertilized with cow or goat manure was significantly higher ($p < 0.05$) than those of poultry manure. Cow manure and goat manure were the best organic materials to increase the number of mycorrhizal spores and could replace one another. According to (Begoude et al., 2016), the type of fertilization in cassava cultivation affects the indigenous AMF population. (Biratu et al., 2018b) support the statement by stating that chicken manure weakens the appearance and composition of cassava nutrients.

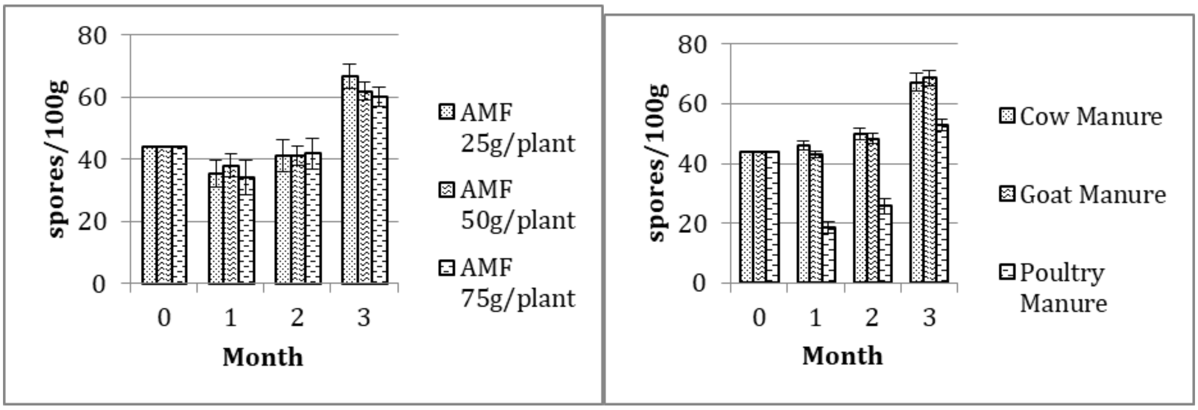
Identification of AMF

The type of spores identified was dominated by *Glomus* sp., although several Gigaspora sp. and Acaulospora sp. were observed. According to (Begoude et al., 2016), *Glomus* sp. dominates the tropics and is usually present in soils. The previous study by (Astuti et al., 2020) showed that the genus *Glomus* sp., *Gigaspora* sp., and *Acaulospora* sp. identified the indigenous AMF spores of Gunungkidul. (Lopes et al., 2019) supported the result by showing that AMF colonization in cassava plants could reach 93%, usually from the genus *Glomus*, *Gigaspora*, and *Acaulospora*.

AMF association in cassava roots

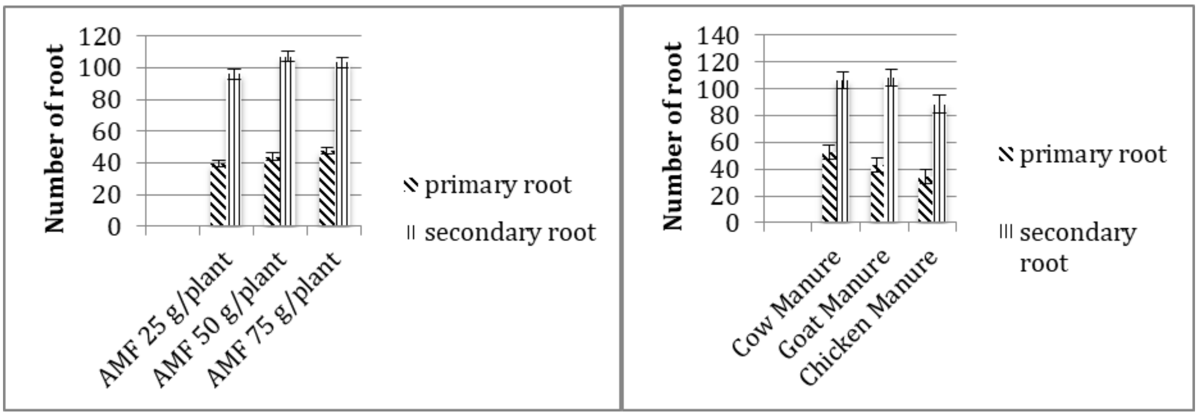
Mycorrhizal spore infection into the roots of cassava plants will stimulate root branching (Zhang et al., 2019). AMF infection affects the root in terms of length, dry weight, and proliferation, as indicated by the number of primary and secondary roots (Figure 3).

Mycorrhizal spore infection into the roots of cassava plants will stimulate root branching. Various AMF doses showed the same effect on the number of spores, so the number of primary and secondary



(a) (b)

Figure 2. Number of spores as affected by (a) AMF dose and (b) Type of manure



(a) (b)

Figure 3. The average number of primary and secondary roots at the 12th week as affected by (a) AMF dose and (b) Type of Manure

roots was also not affected by AMF doses. However, in the treatment of manure types, the number of spores was high, so the number of primary and secondary roots was also high. The primary and secondary roots of plants fertilized with cow and goat manure were significantly higher than those fertilized with poultry (Figure 3).

The results showed a correlation between the number of spores with the number of primary and secondary roots. However, the treatment of AMF doses did not affect the number of spores and the number of primary and secondary roots. Meanwhile, the type of manure significantly affected the number of spores and the number of primary and secondary roots. The high number of spores in the treatment of cow and goat manure stimulated the number of primary roots (52.71 and 43.28, respectively) and secondary roots (108.43 and 108.57, respectively). This result was significantly higher than in poultry manure, which produced primary and secondary roots of 34.33 and 88.50, respectively. Another effect observed was on the root dry weight of cassava plants, which was the highest in the treatment of cow manure with an AMF dose of 25 g (2.96 g) (Table 2).

AMF infection affected root proliferation, so the root dry weight increased. There was an interaction effect of AMF dose and type of manure on the root dry weight of cassava plants, which was the highest (2.96g) at AMF dose of 25 g/plant combined with

Table 2. Average root dry weight at week 12 (gram)

AMF Dose	Types of Manure			Average AMF Dose
	Cow	Goat	Poultry	
25 g/plant	2.96 a	2.14 bc	1.30 bc	2.13
50 g/plant	1.28 bc	1.27 bc	1.99 ab	1.51
75 g/plant	0.76 c	0.85 c	1.43 bc	1.01
Average Types of Manure	1.66	1.42	1.57	(+)

Remarks: Means followed by different letters are significantly different based on the DMRT test at a significance level of 5%; (+) indicates an interaction between treatments

Table 3. Average growth and products of cassava

Treatments	Height (cm)	Number of leaves (strands)	Number of tubers /plant	Diameter of tuber (cm)	The length of tuber (cm)	Weight of tuber/ plant (kg)	Cassava yield (ton / Ha)
AMF Dose:							
25 g/plant	237.44 a	224.67 a	11.22 a	**32.20 a**	22.52 a	**3.87 a**	**38.76 a**
50 g/plant	234.67 a	224.89 a	11.78 a	31.18 ab	22.50 a	3.79 ab	37.92 ab
75 g/plant	236.45 a	216.45 a	11.56 a	29.71 b	21.54 a	3.65 b	36.53 b
Types of Manure:							
Cow	242.45 p	218.00 p	13.78 p	31.15 p	22.44 p	3.86 p	38.63 p
Goat	225.22 p	224.33 p	10.33 q	30.47 p	21.34 p	3.34 q	33.40 q
Poultry	240.44 p	219.67 p	10.44 q	29.46 p	22.79 p	3.41 q	34.13 q
Interaction	(-)	(-)	(-)	(-)	(-)	(-)	(-)

Remarks: Means followed by different letters are significantly different based on the DMRT test at a significance level of 5%; (-) indicates no interaction between treatments

cow manure, while the lowest was 0.76-0.85 g at AMF dose of 75g / plants combined with cow or goat manure (Table 2).

Cassava growth and yield

The analysis of variance showed no interaction effect of AMF dose and the type of manure (cow, goat, and poultry) on all growth variables and cassava yields. Still, each factor affected the yield of cassava independently (Table 3).

The AMF dose of 25-50 g per plant resulted in the highest value of tuber diameter and tuber weight per plant, reaching 37.92-38.76 tons per hectare, compared to 75g/plant (36.53 tons). Meanwhile, the application of cow manure significantly increased the number of tubers and the weight of tubers per plant, resulting in the highest values per hectare (38.63 tons) compared to goat or poultry manure (34.13 tons).

The results of this study indicated that the treatment of Gunungkidul indigenous AMF doses and types of manure on cassava plants could increase root proliferation and dry weight so that it had a significant effect on the tuber. However, the effects on the plant growth, height, and number of leaves were not significant (Table 3). The application of AMF at a dose of 25 g/plant had the most significant effect on the diameter of the tuber (32.20 cm) and the most substantial cassava yield (3.87 kg/ plant). At the same time, cow manure affected the highest number of tuber (13.78 tuber/plant) and the highest cassava yield (3.86 kg per plant). This result is in line with the opinion of (Lehmann et al., 2017) that AMF symbiosis in plants can increase nutrient absorption and resist drought stress, thereby increasing plant growth and yield.

CONCLUSIONS

The results showed that AMF-infected cassava roots and cow or goat manure application produced more spores than poultry manure. AMF infection and manure, thus, significantly resulted in better root proliferation, root forehead weight, tuber diameter, and cassava products, than the absence of both treatments. Cow manure combined with AMF at a dose of 25 g/plant significantly affected the dry weight of cassava roots. This study implies that applying AMF and manure provide a substantial contribution on the growth and production of cassava.

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