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Integration of mycorrhiza hyphae into organic fertilizer granules on the growth of sugarcane crops

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Abstract

Arbuscular mycorrhizae fungi (AMF) are important for tropical soils where soil phosphorus (P) availability is low so that plants can use P with higher efficiency. AMF can increase the P concentration in the plant root area, by releasing the bonds of fixed P into available P. One of the obstacles in utilizing AMF fertilizer is the limited availability of AMF inoculant in the form of AMF spores. In addition, AMF spore production takes a long time. AMF in the form of hyphae is an alternative as an inoculant because it has a faster infection capacity than AMF spores and does not break apart like AMF spores when made in the form of granular organic biofertilizer. The aim of this research was to analyze the effectiveness of various doses of AMF hyphae granule organic fertilizer on sugarcane plants. This research was conducted using a randomized block design. The treatment doses used were control (without AMF), 750 kg/ha, 1000 kg/ha, 1500 kg/ha, and 2000 kg/ha AMF hyphae granule organic fertilizer which was repeated five times. The results of the research showed that the application of AMF hyphae granule organic fertilizer at a dose of 1500 kg/ha could increase plant height, stem diameter, number of tillers and root infections of sugarcane plants. The P status of sugarcane plants increased from medium to high with a plant P content of 0.442% P_2O_5. This effect was seen more clearly in sugarcane plants grown in the field than grown in the greenhouse.

Keywords: Arbuscular mycorrhizae fungi; Hyphae; Granule; Organic fertilizer; Sugarcane

1 Introduction

Mycorrhiza is a form of association between certain soil fungi and the roots of taller plants. This mycorrhizal fungus can supply nutrients regularly to plants. This fungus develops in roots and in root cortex cells [1]. Vesicular Arbuscular Mycorrhiza is included in the endomycorrhiza group where this type has a very wide distribution area, while the other types have a limited distribution area. According to Mosse [2], plants associated with mycorrhizal fungi will be more efficient in absorbing nutrients, assimilating phosphorus quickly, increasing the absorption of nitrogen, sulphur, Zn, and other essential elements. The rate of nutrient absorption is almost four times compared to normal roots, while the area of root absorption increases up to 80 times. Besides that, mycorrhizal fungi can protect infected roots against various pathogens carried through the soil.

One of the obstacles in popularizing biofertilizers, especially AMF, is the limited availability of quality AMF inoculants. Good inoculant quality is characterized by a high number of AMF spores and AMF colonization on the roots. The higher the number of mycorrhizal spores in the inoculant carrier medium, the better the quality of the inoculant. On the other hand, AMF spore production takes a long time, around 3 to 4 months, while the need for AMF biofertilizer is increasing.

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Some types of AMF species require certain host plants to produce high numbers of spores. The host plants required for inoculant propagation must be able to adapt to local climate conditions, grow normally in the growing medium, be easy to maintain and be resistant to root pathogens and insects [3]. Mycorrhizal infected plants can produce external hyphae several weeks after AMF colonizes the host plant. The presence of hyphae structures since the age of 10 days after inoculation [4]. AMF external hyphae have a rapid infection capability compared to AMF spores as an inoculant when applied in pot culture and in the field. Likewise, hyphae production will be shorter than AMF spore production. However, AMF hyphae have problems developing and will die if they do not find suitable roots within a week or more. In the Scutellospora type, hyphae grow from the germination site in the spore [5].

Obstacles to the use of beneficial microbes as inoculant products are still limited by the low viability of functional microbes and the form of powdered biofertilizer which is easily scattered during application. Granular organic fertilizer is a type of organic fertilizer which is produce from composting agricultural waste bagasse, livestock manure, and sugar waste cake filtered. The benefits of this organic fertilizer on the soil can increase the soil's ability to retain water and nutrients by increasing the cation exchange capacity of the soil so that nutrients are not easily lost or leached [6]. Organic fertilizer can also increase soil macro and micronutrients. The physical and biological properties of soil can be improved by the presence of organic fertilizer, by increasing the stability of soil aggregates and increasing the community and activity of soil microbes.

One of the obstacles to the use of mycorrhizal spores in making granulated organic fertilizer is that the spores are damaged during the mixing process with the carrier medium. The rupture of spores results in the loss of the opportunity for the spores to germinate and infect the host plant. The use of AMF hyphae in the form of fresh hyphae is more resistant to the inoculant mixing process. The obstacle to using mycorrhizal hyphae is that their viability is relatively short compared to spores. Therefore, it is necessary to analyze the ability of granular organic fertilizer containing AMF hyphae by applying to sugarcane plants in the greenhouse and in the field. The aim of this research was to analyze the effectiveness of various doses of AMF hyphae granule organic fertilizer on sugarcane plants.

2 Materials and Methods

The research was carried out in the greenhouse and experimental garden of the Faculty of Agriculture, Padjadjaran University, West Java, Indonesia.

2.1 Preparation planting media of sugarcane stalk seedling

The sugarcane stalk seedling used were of Plant Cane (PC) which is a sugarcane plant seedling that was first planted that had never been planted before on the field. The Inceptisols topsoil is taken from a depth of 20 cm and air-dried for two weeks. The soil is crushed and sifted to a size of 2 mm and put into 20 kg capacity pot containing non-sterile Inceptisols soil. PC sugarcane stalk seedling plants were planted in a greenhouse and field without shade. This research was conducted for 4 months.

2.2 AMF hyphae granule organic fertilizer

The materials used for organic fertilizer granule were bagasse, livestock manure, and sugar waste cake filtered (”blotong”) which composted and mixed with AMF hyphae *Glomus* sp. and *Gigaspora* sp. That mixed organic fertilizer made granule at Jatitujuh Sugar Factory plantation, West Java.

2.3 Experimental design

This research was conducted using an experimental method using a Randomized Block Design. The treatment used was a dose of AMF hyphae granule organic fertilizer (D), the treatment used consisted of:

D1: control (without AMF hyphae granule organic fertilizer)
D2: 750 kg/ha is equivalent to 9.375 g/pot of AMF hyphae granule organic fertilizer
D3: 1000 kg/ha is equivalent to 12.5 g/pot of AMF hyphae granule organic fertilizer
D4: 1500 kg/ha is equivalent to 18.75 g/pot of AMF hyphae granule organic fertilizer
D5 : 2000 kg/ha equivalent to 25.0 g/pot of AMF hyphae granule organic fertilizer

All treatments were repeated five times and given inorganic fertilizer according to recommendations for planting sugarcane seedlings. The parameters observed were the root AMF infection, sugarcane plant growth (plant height, stem diameter, number of tillers), and sugarcane plant P uptake (wet digestion method).

2.4 Statistical analysis

Variance analysis (ANOVA) was conducted for all parameters using Statistical Product and Service Solutions (SPSS) version 15.0. The differences between treatments were analyzed using Least Significant Difference at $p < 0.05$.

3 Results and Discussion

3.1 Sugarcane plant height

The growth of sugarcane until 16 WAP planted in the field is different from that grown in the greenhouse. Providing AMF hyphae granule organic fertilizer dose of 1000 kg/ha at 16 weeks after planting (WAP) had the effect of increasing the height of sugarcane plants in the field, as well as the height of sugarcane plants in the greenhouse. In field experiments, increasing the dose of AMF hyphae granule organic fertilizer above 1000 kg/ha did not increase plant height.

Table 1 Sugarcane plant height after application of various doses of AMF hyphae granule organic fertilizer in the field

Treatments	Plant height in the field (cm)							
	4 WAP		8 WAP		12 WAP		16 WAP	
Control (D_1)	11.03	a	17.50	a	25.10	a	37.70	a
500 kg/ha (D_2)	11.77	a	22.65	bc	32.83	c	40.90	ab
1000 kg/ha (D_3)	12.57	a	23.03	bc	32.98	bc	46.80	b
1500 kg/ha (D_4)	10.74	a	21.68	b	28.80	ab	39.70	ab
2000 kg/ha (D_5)	11.96	a	25.50	c	34.68	c	40.75	ab

Note: The average values in the same column marked with the same letter are not significantly different according to the Least Significant Difference Test at the 5% level

In the greenhouse. the application of AMF hyphae granule organic fertilizer has the effect of increasing plant height starting from 4 WAP. At the end of the observation (16 WAP), sugarcane plants given AMF hyphae granule organic fertilizer at a dose of 1000 to 2000 kg/ha showed higher plant height compare the control. This is related to greenhouse conditions where the temperature difference between morning, afternoon, and evening is not too high compared to in the field so that more volatile nutrients such as N are not lost and are used to increase plant height.

Table 2 Sugarcane plant height after application of various doses of AMF hyphae granule organic fertilizer in the greenhouse

Treatments	Plant height in greenhouse (cm)							
	4 WAP		8 WAP		12 WAP		16 WAP	
Control (D_1)	17.38	a	17.50	a	33.80	ab	37.40	a
500 kg/ha (D_2)	20.90	ab	22.65	bc	34.95	ab	40.90	ab
1000 kg/ha (D_3)	18.97	ab	23.03	bc	33.15	ab	43.50	b
1500 kg/ha (D_4)	21.95	b	21.68	b	36.20	b	42.40	b
2000 kg/ha (D_5)	17.36	a	25.50	c	30.45	a	45.10	b

Note: The average values in the same column marked with the same letter are not significantly different according to the Least Significant Difference Test at the 5% level

3.2 Sugarcane Stem Diameter

In the field and greenhouse. the application of AMF hyphae granule organic fertilizer began to have an effect on increasing the diameter of sugarcane plants at the age of 8 WAP. At the end of the observation (16 WAP), application of AMF hyphal granule organic fertilizer at a dose of 500 kg/ha in the field increased stem diameter, the effect was different from the highest dose (2000 kg/ha). In the greenhouse experiment. the application of AMF hyphal granule organic

fertilizer up to a dose of 2000 kg/ha did not increase the diameter of sugarcane stems compared to without AMF inoculant application. Sugarcane stem diameter is one part of the plant that influences sugarcane yield [7].

Table 3 Diameter of sugarcane plant stems after application of various doses of AMF hyphae granule organic fertilizer in the field

Treatments	Stem diameter in the field (mm)							
	4 WAP		8 WAP		12 WAP		16 WAP	
Control (D_1)	6.27	a	10.05	a	14.25	a	18.07	a
500 kg/ha (D_2)	6.61	a	12.14	b	17.59	bc	21.03	b
1000 kg/ha (D_3)	7.05	a	12.57	b	18.41	bc	21.93	b
1500 kg/ha (D_4)	6.46	a	11.58	ab	16.95	b	21.66	b
2000 kg/ha (D_5)	6.93	a	12.98	b	19.33	c	22.65	b

Note: The average values in the same column marked with the same letter are not significantly different according to the Least Significant Difference Test at the 5% level

The stem diameter of sugarcane plants in the greenhouse is smaller than that of sugarcane plants planted in the field with the same fertilizer dose. It seems that the nutrients absorbed by sugarcane plants in the field are used to increase the diameter of the stem rather than being used to increase plant height.

Table 4 Diameter of sugarcane plant stems after application of various doses of AMF hyphae granule organic fertilizer in the greenhouse

Treatments	Stem diameter in Greenhouse (mm)							
	4 WAP		8 WAP		12 WAP		16 WAP	
Control (D_1)	6.42	a	10.58	a	12.99	a	14.10	a
500 kg/ha (D_2)	7.28	a	11.84	ab	14.38	ab	14.70	a
1000 kg/ha (D_3)	6.49	a	11.90	ab	14.59	ab	15.70	a
1500 kg/ha (D_4)	7.08	a	12.88	b	14.64	ab	14.90	a
2000 kg/ha (D_5)	6.13	a	10.96	a	15.56	b	16.00	a

Note: The average values in the same column marked with the same letter are not significantly different according to the Least Significant Difference Test at the 5% level

3.3 Number of sugarcane tillers

Table 5 Number of sugarcane tillers after application of various doses of AMF hyphae granule organic fertilizer in the field

Treatments	Tillers number in the field							
	4 WAP		8 WAP		12 WAP		16 WAP	
Control (D_1)	0.20	a	0.90	a	4.80	a	8.50	a
500 kg/ha (D_2)	1.20	a	5.30	b	8.10	b	13.50	bc
1000 kg/ha (D_3)	1.30	a	5.10	b	8.22	b	11.60	b
1500 kg/ha (D_4)	1.20	a	6.90	b	10.10	b	15.10	c
2000 kg/ha (D_5)	0.70	a	5.50	b	10.30	b	13.10	bc

Note: The average values in the same column marked with the same letter are not significantly different according to the Least Significant Difference Test at the 5% level

The most striking difference is mainly in the number of sugarcane tillers. Sugarcane plant was planted in the field tillers was begin to grow at 4 WAP, while in the greenhouse until 8 WAP the sugarcane tillers have not yet grown. This is related to the microenvironment in which sugarcane plants grow. The high temperature in the greenhouse and less than optimal air flow causes the evapotranspiration process to be faster than in the field. The water that has been absorbed by the sugarcane roots, which should be used to form carbohydrates, evaporates more quickly so that the formation of plant biomass (tillers) is hampered. With a dose of AMF hyphae granule organic fertilizer of 500 kg/ha, the number of sugarcane tillers can be increased.

Table 6 Number of sugarcane tillers after application of various doses of AMF hyphae granule organic fertilizer in the greenhouse

Treatments	Tillers number in Greenhouse							
	4 WAP		8 WAP		12 WAP		16 WAP	
Control (D_1)	0.00	a	0.00	a	0.00	a	0.00	a
500 kg/ha (D_2)	0.00	a	0.00	a	0.00	a	0.30	a
1000 kg/ha (D_3)	0.00	a	0.20	a	0.22	a	0.60	a
1500 kg/ha (D_4)	0.00	a	0.00	a	0.00	a	0.00	a
2000 kg/ha (D_5)	0.00	a	0.20	a	0.20	a	0.40	a

Note: The average values in the same column marked with the same letter are not significantly different according to the Least Significant Difference Test at the 5% level

3.4 AMF root infection of sugarcane

AMF root infection of sugarcane plants root at the age of 8 WAP in the field increased according to the increase in the dose of AMF hyphal granule organic fertilizer given. In contrast. in the greenhouse. application of AMF hyphae granule organic fertilizer did not increase root infection of sugarcane plants. At the end of the observations (16 WAP) in both field and greenhouse experiments. the application of AMF hyphae granule organic fertilizer at a dose of 1000kg/ha increased AMF root infection of sugarcane plants.

At the beginning of planting sugarcane seedlings in a greenhouse or in the field. AMF inoculation is a beneficial treatment to provide the opportunity for AMF hyphae to immediately contact plant roots and infection the roots. At 7 days after transplanting application of AMF in the nursery increased the colonization by 41.3% and had the effect of increasing stem biomass from 11 to 61% depending on sugarcane variety [8].

Table 7 Root infection of sugarcane plants after application of various doses of AMF hyphae granule organic fertilizer in the field and greenhouse

Treatments	Root infection in the field (%)								In greenhouse (%)			
	4 WAP		8 WAP		12 WAP		16 WAP		8 WAP		16 WAP	
Control (D_1)	16.67	a	36.67	a	51.33	a	48.67	a	39.33	a	56.67	a
500 kg/ha (D_2)	44.00	b	50.00	b	66.67	b	59.33	b	48.67	a	58.67	ab
1000 kg/ha (D_3)	46.67	b	57.33	b	71.33	bc	68.00	bc	47.33	a	59.33	ab
1500 kg/ha (D_4)	59.33	c	59.33	b	76.00	bc	73.33	c	50.67	a	66.67	b
2000 kg/ha (D_5)	60.67	c	70.00	c	81.33	c	74.67	c	46.67	a	62.00	ab

Note: The average values in the same column marked with the same letter are not significantly different according to the Least Significant Difference Test at the 5% level

3.5 P concentration in sugarcane leaves

In field experiments. the P concentration of sugarcane plants increased due to the application of AMF hyphae granule organic fertilizer starting at a dose of 1500 kg/ha. whereas in the greenhouse the increase in this dose was no different from other treatments. An increase in plant P concentration in the greenhouse was only achieved when AMF hyphae granule organic fertilizer was applied at a dose of 2000 kg/ha. Application of AMF inoculum significantly increased P

concentration on the leaves of all sugarcane clones but varied among clones [4]. The increasing P uptake was related to the ability of root colonization [9].

P concentrations in sugarcane plants above 0.3% indicate that the P content of sugarcane plants is in high nutrient status. whereas below 0.3% the P status of sugarcane plants is in moderate or sufficient nutrient status [10]. The role of AMF in AMF hyphae granule organic fertilizer is clearly visible when applied in the field by changing the P status of plants from sufficient to high. Besides that. plants in symbiosis with mycorrhizae also increase ecosystem repair by reducing the leaching of elements in the soil. Mycorrhizae will suppress the loss of N and P elements by 40% and 50%. respectively in soil [11].

Table 8 P concentration of sugarcane plants after application of various doses of AMF hyphae granule organic fertilizer in the field and greenhouse

Treatments	P (% P_2O_5) concentration			
	In the field		In greenhouse	
Control (D_1)	0.298	a	0.360	a
500 kg/ha (D_2)	0.330	a	0.400	ab
1000 kg/ha (D_3)	0.302	a	0.402	ab
1500 kg/ha (D_4)	0.442	b	0.406	ab
2000 kg/ha (D_5)	0.454	b	0.422	b

Note: The average values in the same column marked with the same letter are not significantly different according to the Least Significant Difference Test at the 5% level

From the experiment above. the application of AMF hyphae granule organic fertilizer at a dose of 1500 kg/ha has advantages compared to other treatments. especially in field experiments. A very clear effect can be seen from the increase in the number of tillers. infection AMF of sugarcane plant roots. and plant P concentration in sugarcane plants. This shows that the dose of AMF hyphae granule organic fertilizer can play a role in increasing the P status of plants through the absorption of P in the soil through its external hyphae. This is in accordance with the opinion of Wang et al. [12] mycorrhizae also appear to have a significant role in increasing the growth of forest plant seedlings in the nursery, the increase in the growth, and nutritional content in plants compared to plants without mycorrhizal inoculation.

4 Conclusion

Providing AMF hyphae granule organic fertilizer at a dose of 1500 kg/ha increased plant height, stem diameter, number of tillers, and AMF root infection of sugarcane plants. The P status of sugarcane plants increased from medium to high, namely 0.442% P_2O_5. This effect was seen more clearly in sugarcane plants grown in the field than in those grown in the greenhouse.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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