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Cocoa Seedlings Growth on Marginal Soil as Affected by Application of Dry Walnut Leaf Compost and Arbuscular Mycorrhiza Fungi

Ferdi Zul Fandri[1)], Setiyono[2*)], Ayu Puspita Arum[3)], Ahmad Ilham Tanzil[4)], Tri Wahyu Saputra[5)], Dyah Ayu Savitri[6)], and Susan Barbara Patricia[7)]

[1)4)5)]Agrotechnology Study Program, Faculty of Agriculture, Universitas Jember, Jln Kalimantan, Jember, Indonesia
[2)3)6)7)]Agricultural Science Study Program, Faculty of Agriculture, Universitas Jember, Jln Kalimantan, Jember, Indonesia
[*)]Corresponding Author: setiyono.faperta@unej.ac.id
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Abstract

Some cocoa (*Theobroma cacao* L.) plantations in Jember are found on marginal land based on their chemical fertility. For optimal use, the land's fertility needs to be increased, including its biological fertility. This study aims to investigate the effect of providing compost made from dry walnut leaves and arbuscular mycorrhizal fungi on the growth of cocoa seedlings. This research used a completely randomized design consisting of 2 factors and 3 replications: compost dose factor consisting of three levels: 0 g polybag^{-1}, 60 g polybag^{-1}, and 120 g polybag^{-1}. The mycorrhizal dose factor consists of three levels: 0 g polybag^{-1}, 15 g polybag^{-1}, and 30 g polybag^{-1}. The parameters observed included plant height, number of leaves, stem diameter, root volume, plant fresh weight, plant dry weight, and mycorrhizal infection. The results of the research showed a significant interaction between the treatment dose of dry walnut leaf compost and the mycorrhizal dose, where the combination of treatment with a compost dose of 120 g plant^{-1} and mycorrhizal dose of 30 g plant^{-1} was the best treatment combination. The compost dose treatment significantly affects plant height, number of leaves, stem diameter, and plant dry weight, with the best treatment being a compost dose of 120 g plant^{-1}. The mycorrhizal dose treatment shows a highly significant effect on plant height, number of leaves, stem diameter, and plant dry weight, with the best treatment being a dose of 30 g pla-nt^{-1}. Mycorrhiza gave a positive correlation to the dry weight of cocoa seedlings.

Keywords: Cocoa seedlings, compost, mycorrhiza, marginal soil

INTRODUCTION

Cocoa (*Theobroma cacao L.*) is a plantation commodity widely cultivated in Indonesia and is vital to the Indonesian economy. Indonesia is ranked seventh as the world's largest cocoa bean exporting country (ICCO, 2022).

Cocoa production in East Java, including Jember, Indonesia experienced a decline of 1.2% (BPS, 2022) caused by old plants, marginal land, conversion of cocoa plantations, and the government's focus on food crops (Rusli *et al.*, 2022). However, there is still a potential to increase the productivity of cocoa plants, including by improving the quality of planting material during the nursery period and improving cultivation techniques. The obstacles in the nursery are the limited nutrients and the lack of fertile planting media, such as marginal soil.

Several cocoa plantations in Jember have marginal land, meaning the soil's chemical, physical, and biological fertility is low. Organic fertilizers, such as compost at the seedling stage, can improve soil medium fertility (Mochtar *et al.*, 2018). Organic fertilizer is derived from plant and/or animal materials, which decompose into a simpler form so plants can easily absorb it (Eva *et al.*, 2020).

One organic fertilizer that can be used for cocoa seedlings is compost from dry leaves of walnut trees, which is widely available in Jember. According to Nurkhasanah *et al.* (2021), even dry leaves still contain quite a large amount of organic matter and contain a small amount of the nutrient N, so they have the potential to be used as compost fertilizers.

Mardiana *et al.* (2020) show that forage compost influences plant height, number of leaves, and dry weight of oil palm plant crowns. Meanwhile, Harahap *et al.* (2021) find that applying leaf compost fertilizer significantly affects the growth of oil palm seedlings on the number of leaves.

Arbuscular mycorrhizal fungi (AMF), one of soil's biological fertility components, are a form of symbiosis between fungi and plant roots. The mutually beneficial interaction between infected plant roots and AMF will help the absorption of non-mobile nutrients such as P more efficiently. In this association, the AMF found in the plant's rhizosphere will extract nutrients through hyphae, while the plant provides carbohydrates to the fungi for the survival of the AMF (Ida *et al.*, 2017). The presence of AMF at the seedling stage will help plants to grow more optimally. According to Padjung *et al.* (2019), giving AMF helps the growth and development of cocoa seedlings, increasing the height and fresh weight of shoots and roots in plants.

Providing dry leaf compost with AMF is hoped to improve the planting medium quality during the cocoa plant nursery period. Mardiana *et al.* (2020) mention that organic material is provided at the seedling stage in polybags because the available nutrients on land are insufficient to support plant seeds' growth. Therefore, the research aims to determine the effect of dry walnut leaf compost and mycorrhiza on the growth of cocoa seedlings.

MATERIALS AND METHODS

The research was conducted from March to June 2023 at the Agrotechnopark Greenhouse, Universitas Jember, Jember, Indonesia. The temperature in the greenhouse at night and during the day ranges from 26–30 ^{0}C with a relative humidity level of around 60%.

The soil used in this research came from Kemiri Village, Jember Regency, located on the slopes of Mount Argopuro. It is marginal due to its low nutrient content, as shown by Basuki *et al.* (2023), in which the pH is 7.10, the organic C is 2.17%, and the cation exchange capacity is 34.23 (cmol kg^{-1}), and the soil texture is sandy loam and the structure is massive. The soil was placed in a polybag measuring 20 cm x 30 cm with a soil weight of 1.5 kg. The planting material used in the research was open-pollinated ICCRI 08H cocoa seeds obtained from the Indonesian Coffee and Cocoa Research Institute, Jember. Before these seeds were used, their uniformity was selected. The AMF inoculum used in this study was sand grains containing 33 spores per gram. The compost material was dry walnut leaves obtained around the Agrotechnopark of Universitas Jember. The composting process lasted 21 days using commercial microorganisms. Then, the compost was air-dried for 30 minutes and chopped using a chopping machine until fine. Filtering was carried out to get a uniform compost size. Based on the results of analysis from the Soil Science Study Program Laboratory, Faculty of Agriculture, Universitas Jember, it was known that the chemical characteristics of the dry walnut leaf compost were pH 7.1, organic C 23.33%, N 1.26%, P_2O_5 1.43%, and K_2O 1.13%.

This research used the basic pattern of a completely randomized factorial design consisting of two factors: compost dose and mycorrhizal dose. This combination of treatments was repeated three times, so there were 27 experimental units.

The first factor was the dry walnut leaf compost dose consisting of three levels: without compost treatment (control), 60 g of compost per polybag, and 120 g of compost per polybag. The second factor was the dose of arbuscular mycorrhiza, which consisted of three levels: without AMF treatment (control), 15 g AMF polybag^{-1}, and 30 g AMF polybag^{-1}.

Before being put into polybags, the soil used in this research was sterilized by drying in the sun for three days, then sieved using a 2 cm diameter soil sieve. Then, 1.5 kg of soil was put into a polybag and mixed with compost and mycorrhiza according to the treatment. Mycorrhiza was applied to the soil near the plant roots (Wahyuni *et al.*, 2019).

Seeding was done by pointing the tip of the seed downwards. Seeding was done parallel in a damp burlap sack. After the cocoa sprouts were two weeks old, they were transferred to polybags with a distance between polybags of 24 cm x 24 cm. Plant maintenance activities included watering, weeding, replanting, and controlling pests and diseases according to plant needs.

Variables observed included the number of leaves, plant height measured from the soil surface to the tip of the seed, and stem diameter measured at a seedling height of 5 cm using a caliper once every two weeks. At the end of the observation, root volume was measured volumetrically, and fresh and dry weights of plants were measured gravimetrically. The degree of mycorrhizal infection was measured at the end of the observation using a microscopic method after the plant root samples were stained with 0.05% trypan blue. The level of mycorrhizal infection in plant roots was calculated based on the percentage of infected roots compared to the number of roots observed. The classification of mycorrhizal infections in this study was based on the method used by Widawati & Suliasih (2020): 0–5% infection (very low), 6-25% infection (low), 26–50% infection (medium), 51–75% infection (high), and 76–100% infection (very high).

The observation data were analyzed using analysis of variance, and if there were significant differences between treatments, further tests were carried out using Duncan's multiple range test at the 5% level.

RESULTS AND DISCUSSION

Table 1 presents an analysis of the influence of dry walnut leaf compost dose and mycorrhizal dose on several cocoa seedling growth variables. The data show that the combination of treatment with dry walnut leaf compost doses and mycorrhizal doses interacted and had a highly significant influence on the mycorrhizal infection variable and had a significant influence on the wet weight and root volume variables but had no significant effect on plant height, number of leaves, and stem diameter. This is also in line with Krisdayanti *et al.* (2020), who tested the effect of a combi–nation of endomycorrhizal *Trichoderma spp.* biofertilizer, with an inoculum of 10 mL of *Trichoderma spp.*, 150 endomycorrhizal spores, and 10 g of compost for each polybag on the growth of Sengon (*Paraserianthes falcataria* (L.) Nielsen) seedlings, including plant wet weight, plant dry weight, and root dry weight. The application of endomycorrhiza can increase the dry weight of candlenut plant seeds (*Aleurites moluccana*) because plant roots colonized with endomycorrhiza will expand the area of root absorption by the presence of external hyphae that grow and develop on the root hairs. Mycorrhizal

Table 1. Results of analysis of variance (F-calculated) of the effect of compost and AMF doses on plant height, number of leaves, stem diameter, root volume, mycorrhizal infection, and plant fresh and dry weight in cocoa seedlings aged 90 days after planting

Observed variables	F-calculated		
	Compost dose	Mychorriza dose	Interaction
Plant height (cm)	4.81*	7.77**	1.65 ns
Number of leaves (leaf)	4.36*	9.25**	0.20 ns
Stem diameter (mm)	4.58*	6.66**	1.76 ns
Root volume (mL)	5.35*	14.02**	3.02 *
Mycorrhizal infection (%)	36.07**	74.92**	7.95 **
Fresh weight (g)	5.43*	26.53**	4.01 *
Dry weight (g)	3.69*	22.69**	2.07 ns

Notes: (**) highly significant; (*) significant; (ns) insignificant; observation.

plants can absorb the P nutrient and other nutrients such as N, K, and Mg because mycorrhizae have root hyphae that will grow longer than plants without mycorrhizae, so plants with mycorrhizae have higher root weights (Ristiyanti *et al.*, 2014). Apart from that, an environment that supports the growth of mycorrhizae, including in the presence of compost, will create a good symbiosis with plant roots to create extensive external hyphae on plant roots (Nasrullah *et al.*, 2015).

Treatment with a dose of dry walnut leaf compost had a highly significant influence on the mycorrhizal infection and had a significant influence on the variables of plant height, number of leaves, stem diameter, root volume, fresh weight, and dry weight of the plant. This condition supports the results of Utami *et al.* (2019) that the provision of a single factor of 30 g of compost in each polybag had a significant effect on the growth of 90-day-old after planting cocoa seedlings, in terms of plant height, stem diameter, and leaf area. This is because plants will grow well if the nutrients in the soil are sufficient; in this case, it is provided by compost and can be absorbed by plants as a source of nutrition (Ngantung *et al.*, 2018). Mycorrhiza dose treatment had a highly significant influence on all observed variables; this supports Harahap *et al.* (2015), who tested the effect of giving 10 g of mycorrhizae for each polybag in pre-nursery oil palm seedlings aged 12 weeks after planting and shows a significant effect on the parameters of the degree of mycorrhizal infection, seedling height, stem diameter, number of leaves, total leaf area, greenness of the leaves, degree of infection, root volume, seed dry weight, and root dry weight. This is because the mycorrhiza fungus infects the root system of the host plant and then produces a network of external hyphae that are able to penetrate the subsoil layer, thereby increasing the root's capacity to absorb nutrients and water. The absorption of nutrients in the soil can increase plant growth (Nurhalimah *et al.*, 2014).

Interaction of Compost and Mycorrhiza Doses

Table 2 presents the interaction between compost dose and mycorrhiza on the root volume of cocoa seedlings. Based on the root volume of cocoa seedlings aged 90 days after planting, it can be seen that without compost, the root volume of plants treated with mycorrhiza was significantly higher than those without mycorrhiza. A similar thing was also observed in the treatment of 60 g polybag^{-1} compost. The compost treatment of 120 g polybag^{-1} produced a higher root volume than other treatments added with 30 g polybag^{-1} of mycorrhiza. Meanwhile, 120 g polybag^{-1} compost treatment without mycorrhiza produced the highest root volume compared to lower compost. Compost treatment had no significant effect on the treatment using mycorrhiza at 15 g polybag^{-1}. The combination of 120 g compost and 30 g mycorrhiza produced the highest root volume compared to all treatments. Because compost is an organic source rich in nutrients and good microorganisms, its use as fertilizer helps improve soil quality by improving soil structure, increasing water retention, and providing essential nutrients for plants; when combined with arbuscular mycorrhizal fungi (AMF), soil microbes that are in symbiosis with plant roots, plants will have an extensive root absorption area so that the nutrient absorption process becomes more efficient (Prayudyaningsih & Sari, 2016).

Table 3 presents the interaction between compost and mycorrhiza doses on mycorrhizal infection in cocoa seedlings. The level of mycorrhizal fungal infection on cocoa roots, which is influenced by the dose of mycorrhizal and compost, is presented in Table 3. From these results, it can be seen that plants that were not treated with mycorrhizal were not infected by this fungus. However, plants that were not given mycorrhiza but were given 60 g and 120 g of compost per polybag had mycorrhizal infection. This indicates the possibility of contamination from compost or the environment, considering that the planting medium used in this study was sterilized under sunlight, which might not be able to kill all microorganisms, including mycorrhizal fungi. From the results of this study, it appears that the highest mycorrhizal infection was obtained in the combination treatment of 120 g of compost and 30 g of mycorrhiza per polybag. The 15 g and 30 g mycorrhizal treatments per polybag showed higher mycorrhizal infection than without compost. This condition follows the results of previous research that using single mycorrhiza on peanut plants 12 weeks after planting resulted in significantly different root infections (66.60%) compared to those using a combination of mycorrhiza with 63.30%

Table 2. Interaction of compost doses and mycorrhizal doses on root volume of cocoa seedlings aged 90 days after planting

Compost (g polybag^{-1})	Mychorriza (g polybag^{-1})		
	0	15	30
	Root v-olume (mL)		
0	4.17 b B	6.33 a A	6.50 a B
60	5.17 b B	6.00 ab A	7.17 a AB
120	6.67 ab A	5.83 b A	8.00 a A

Note: Numbers followed by the same capital letter in the same column (mycorrhiza) are not significantly different, and numbers followed by the same lowercase letter in the same row (compost) are not significantly different in the Duncan 5% multiple range test.

Table 3. Interaction of compost doses and mycorrhizal doses on mycorrhizal infection of cocoa seedlings aged 90 days after planting

Compost (g polybag^{-1})	Mycorrhiza (g polybag^{-1})		
	0	15	30
	Mycorrhizal infection (%)		
0 g	0.00 b B	10.00 b B	21. 67 a C
60 g	8.33 b AB	31.67 a A	40.00 a B
120 g	13.33 c A	25.00 b A	65.00 a A

Note: Numbers followed by the same capital letter in the same column (mycorrhiza) are not significantly different, and numbers followed by the same lowercase letter in the same row (compost) are not significantly different in the Duncan 5% multiple range test.

organic fertilizer and 3.30% control. It is estimated that mycorrhiza has been able to infect plant roots; likewise, mycorrhiza has been able to adapt well to the environment and interact more with the roots of peanut plants (Hazra *et al.*, 2023).

Table 4 shows the fresh weight of cocoa seedlings 90 days after planting and treated with several doses of mycorrhiza and compost. These data confirm that plants treated with mycorrhiza combined with compost produced plants with a significantly higher fresh weight than those without compost and without mycorrhiza. Plants treated with compost but without mycorrhiza produced significantly different fresh weights than those without compost. On the other hand, compost treatment had no significant effect on the fresh weight of plants treated with mycorrhiza. From the data presented, plants treated with mycorrhiza at 30 g per polybag combined with compost produced the highest fresh weight of plants compared to other treatments. This condition is the same as the effect of giving a combination of phosphate fertilizer with mycorrhiza on the growth of sugarcane seedlings 80 days after planting in beach sand media. The combination treatment of phosphate fertilizer application with mycorrhiza results in significantly different fresh weights of sugarcane plants, namely 547 g compared to 378 g without mycorrhiza application. Mycorrhiza is suspected to increase the absorption of both macro and micronutrients, so the need for nutrients to support total fresh weight gain can be better met (Helena *et al.*, 2014).

Arbuscular mycorrhizal fungi in cocoa seedlings interact with each other on plant growth. According to Hasiholan *et al.* (2017), the organic material contained in compost provides many benefits for plants, including increasing plant productivity, influencing root development, and increasing soil fertility. The nutrients contained in compost, such as N, P, and K, can help fertilize and improve the physical, biological, and chemical properties of soil (Mochtar *et al.*, 2018). The nutrients in the planting media will stimulate the growth and development of cocoa plants, affecting root volume variables, plant fresh weight, and mycorrhizal infections.

This increase in plant fresh weight to the optimum point is thought to be related to the role of P as an essential activator in cell division, development, and elongation. Munawar (2011) states that the most essential function of phosphate is in the storage and transfer of energy in plants, which is an integral part of photosynthesis, carbohydrate metabolism, and cell formation, division, and multiplication.

Providing AMF can increase plant growth by inducing roots, which causes the root system to enlarge. This aligns with research conducted by Darlin *et al.* (2020) that giving AMF to the roots of cocoa plants can help plants absorb

Table 4. Interaction of compost doses and mycorrhizal doses on wet weights of cocoa seedlings aged 90 days after planting

Compost (g polybag^{-1})	Mycorrhiza (g polybag^{-1})		
	0	15	30
	Fresh- weight (g)		
0 g	7.83 b B	15.50 a A	17.57 a A
60 g	13.40 b A	14.13 b A	19.27 a A
120 g	15.13 b A	15.43 b A	18.93 a A

Note: Numbers followed by the same capital letter in the same column (mycorrhiza) are not significantly different, and numbers followed by the same lowercase letter in the same row (compost) are not significantly different in the Duncan 5% multiple range test.

nutrients by increasing physiological activity through mycorrhizal infection so that plants grow more perfectly.

The interaction occurs in root volume, mycorrhizal infection, and plant wet weight because dry leaf compost provides sufficient nutrient availability for plants. The main ingredients of the compost are dry leaves and cow dung, believed to contain mycorrhizal spores or other soil microorganisms that interact with arbuscular mycorrhizal fungi, making it easier for the roots to absorb nutrients. In line with Barita *et al.* (2018), providing P from fertilizer to cocoa plants plays a role in developing strong plant roots, helping plants absorb more nutrients from the soil. Mardiana *et al.* (2020) mention that it is necessary to provide dry leaf compost and mycorrhiza at the seedling stage because the amount of nutrients in polybags is limited, and the roots of infected cocoa plants will more easily absorb nutrients such as N, P, and K, elements crucial for the growth and development of cocoa plants so that it affects the wet weight of the plant.

The availability of AMF can help increase nutrient absorption for plants even though the nutrient content in compost is low. According to Suharno *et al.* (2020), applying mycorrhiza and organic fertilizer to the roots of cocoa plants plays a vital role in increasing nutrients, especially phosphorus, which helps plant growth by absorbing nutrients on marginal land.

The Effect of Compost Doses

The parameters of plant height, number of leaves, stem diameter, and plant dry weight are presented in Figure 1. From these data, it can be seen that giving 120 g of compost per polybag resulted in significantly different plant heights compared to 60 g of compost per polybag and the control. Meanwhile, 60 g of compost per polybag had no significant effect on plant height. The number of leaves of cocoa plants treated with 120 g of compost per polybag was significantly different compared to 60 g of compost per polybag but not significantly different from the control. The same trend is also seen in stem diameter. Compost treatment of 120 g significantly affected the dry weight of cocoa plants 90 days after planting. However, the 60 g compost treatment was not significantly different from the control.

The nutrients contained in dry leaf compost are 1.26% nitrogen, 1.43% phosphorus, and 1.13% potassium, where according to Wenda *et al.* (2017), compost containing N, P, and K is essential for plant growth. Adequate nutrition in cocoa seedlings will stimulate the growth of shoots and leaves to be more optimal; cocoa seedlings that receive enough nutrition tend to have a better number of leaves and growth, as Waskito *et al.* (2017) mention that an increase in plant height is always followed by an increase in the number of leaves.

Aziza *et al.* (2022) state that plants need a high supply of nitrogen in the growth process of roots, stems, and leaves. Healthy plant roots will help absorb water and nutrients to support good stem growth. Providing fertilizer according to the dose and needs can increase plant growth, whereas giving too much fertilizer will affect the concentration of the soil solution, making it difficult for plant roots to absorb.

The dry weight of a plant is determined mainly by the activity of the roots in transporting water and nutrients into the plant. The number of leaves can influence the increase in plant dry weight because leaves are the place to accumulate plant photosynthesis results (Nurdin, 2011). The nutrients in dry leaf compost play a role in increasing the dry weight of plants because dry leaf compost helps optimize the development and photosynthesis process of plant metabolism (Lizwati *et al.*, 2014).

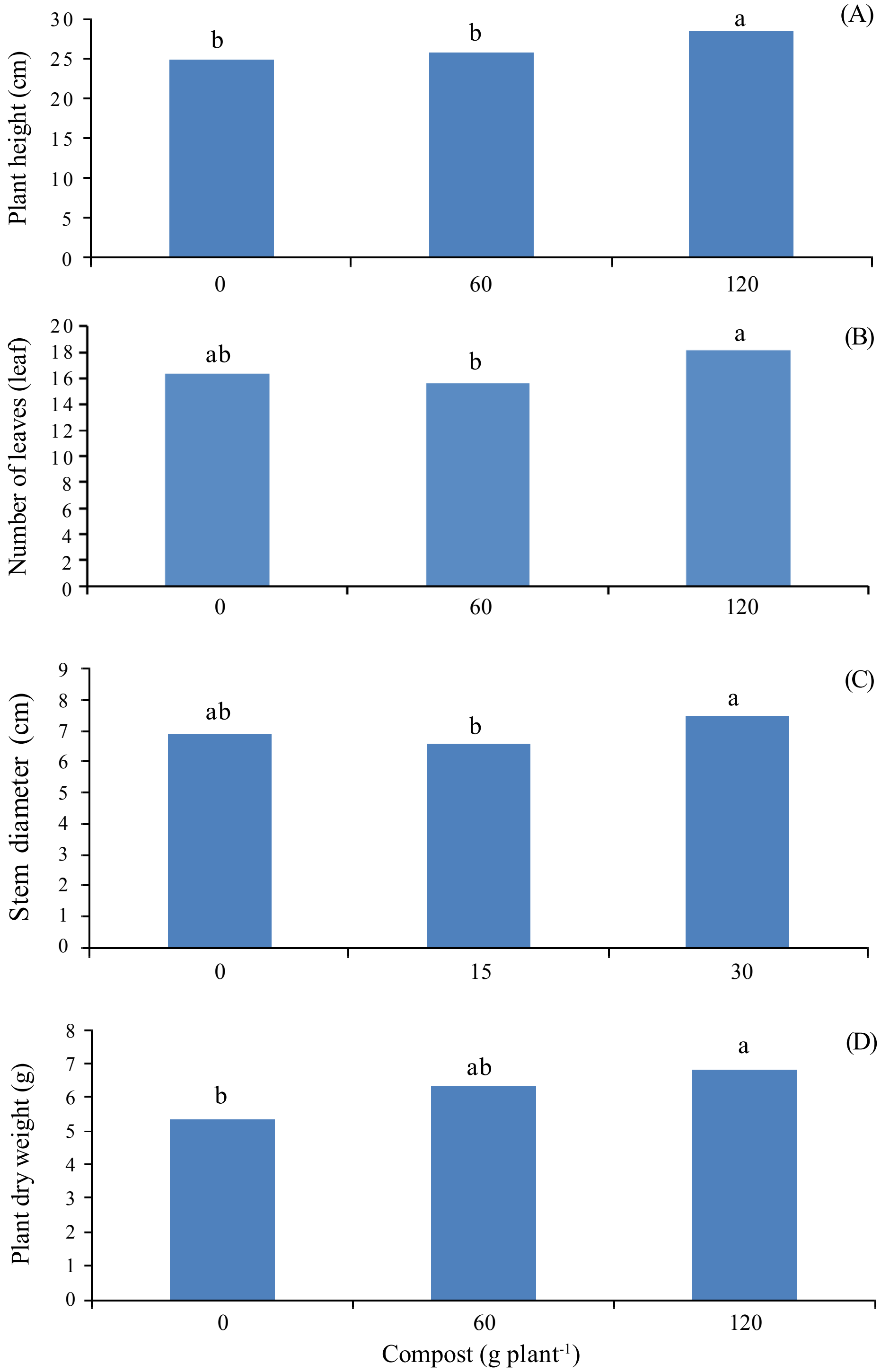

Figure 1. The effect of compost doses on plant height (A), number of leaves (B), stem diameter (C), and plant dry weight (D)

Note: Bars followed by the same letter on the same parameters indicate that the treatments are not significantly different in Duncan's multiple range test at the 5% level.

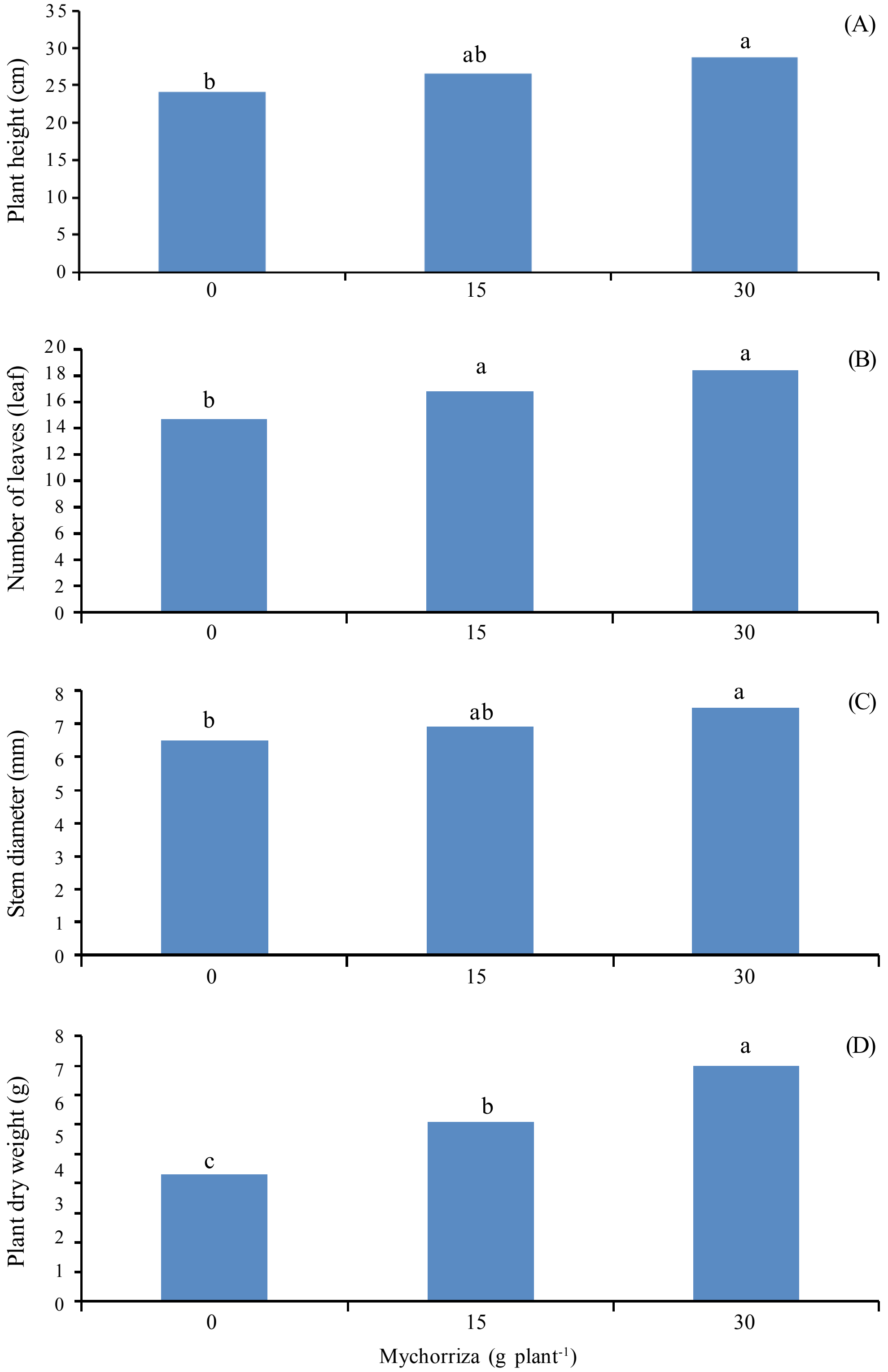

Figure 2. The effect of mycorrhizal doses on plant height (A), number of leaves (B), stem diameter (C), and plant dry weight (D)

Note: Bars followed by the same letter on the same parameters indicate that the treatments are not significantly different in Duncan's multiple range test at the 5% level.

The Effect of Mycorrhiza Doses

The main effect of mycorrhizal treatment on plant height, number of leaves, stem dia-meter, and plant dry weight is presented in Figure 2. From these data, it can be seen that giving mycorrhizae as much as 30 g per polybag significantly affected plant height. However, the mycorrhiza treatment of 15 g per polybag had no significant effect. On the other hand, for the l21eaf number parameter, the mycorrhizal treatment had a significant effect, but there was no difference between the 30 g and 15 g mycorrhizal treatment per polybag on the number of leaves. Providing 30 g of mycorrhiza per polybag significantly affected the stem diameter of cocoa plants 90 days after planting. However, the stem diameter of plants treated with mycorrhiza at 15 g per polybag was not significantly different compared to the control. The dry weight of plants treated with mycorrhiza was significantly higher than the control. A higher mycorrhizal dose produced a higher plant dry weight; in other words, the dry weight of cocoa plants 90 days after planting showed a positive correlation with the mycorrhizal dose.

These results are due to the relationship between mycorrhiza and plant roots, which forms a symbiotic relationship where AMF is able to increase plant growth by inducing roots, which causes enlargement of the root system so that the area on the root surface will absorb the nutrient P to a greater extent. Providing arbuscular mycorrhizal fungi to the roots of cocoa plants will increase the physiological activity of the plant, which can help the cocoa plant's organs to grow better, such as plant height (Darlin *et al.*, 2020).

AMF contains auxin, cytokinin, and gibberellin; these ingredients are growth regulators to stimulate cell enlargement. Cocoa plants with mycorrhiza will produce a higher number of leaves, while plants that do not have mycorrhiza will have a lower number of leaves. According to Nasrullah *et al.* (2015), roots in symbiosis with AMF will stimulate root growth and help plants absorb nutrients and water for plant growth. The higher the dose of AMF given to plants, the better the plant growth in the photosynthesis process. Darli *et al.* (2020) confirm that giving mycorrhiza to cocoa seedlings gives significantly different results for stem diameter.

CONCLUSIONS

There was a highly significant interaction between the application of compost and mycorrhiza on root infections, as well as a significant interaction on root volume and wet weight of cocoa seedlings. The best treatment combination for the growth of cocoa plants is 120 g plant^{-1} compost and 30 g plant^{-1} mycorrhiza. Compost treatments had a significant effect on plant height, number of leaves, stem diameter, and dry weight of cocoa plants; where the best treatment was at a compost dose of 120 g plant^{-1}. Mycorrhizal treatments had a highly significant effect on plant height, number of leaves, stem diameter, and dry weight of cocoa plants; where the best treatment was at a mycorrhizal dose of 30 g plant^{-1}.

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