

Coffee Plants' Endomycorrhizae Potential to increase the growth and nutrient uptake of Arabica Coffee (*Coffea arabica* L.) under Field Condition

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Abstract

Inorganic fertilizers utilization is the most common way to increase plant productivity. However, the intensive use of organic fertilizer can harm the environment. Therefore, alternative fertilization by utilizing soil microorganisms to provide plant nutrients is needed. Endomycorrhizae is known as a microorganism that can increase the availability of nutrients and plant growth. This study aimed to determine the potential of endomycorrhizae to increase the growth of arabica coffee seedlings under field conditions. A Randomized Complete Block Design (RCBD) with 5 replications was used with four treatments, i.e., (P0) control: without endomycorrhizae and fertilizer, (P1) inorganic fertilizer: NPK recommended dose for seedlings nine months after sowing (N 184 kg/ha, P₂O₅ 72 kg/ha, and K₂O 120 kg/ha from 400kg/ha urea, 200 kg/ha SP-36, and 200 kg/ha KCl, respectively), (P2) endomycorrhizae: 1:1 (w/w basis) endomycorrhizal inoculum-planting medium, and (P3) endomycorrhizae + organic fertilizer: 1:1 (w/w basis) endomycorrhizal inoculum-planting medium with the addition of chicken manure at a dose of 10 tons/ha. The results showed that endomycorrhizae could increase the growth of arabica coffee seedlings by increasing plant height, plant dry weight, and plant N, P, and K uptake by 15.4%, 23.3%, 52.5%, 90.8%, and 75.6%, respectively compared to the control with 67.5% of root colonization at 16 weeks after transplanting (WAT). In conclusion, endomycorrhizae can potentially increase the growth of arabica coffee seedlings under field conditions.

Keywords: *endomycorrhizae, growth, arabica coffee, fertilizer*

1. Introduction

Indonesia is the fourth largest coffee-producing country in the world after Brazil, Vietnam, and Columbia (1). Coffee is a commodity that has a vital role in the economy of Indonesia.

The area of coffee plantations in Indonesia is around 1.245 million hectares, with average productivity is 794 kg/ha in 2019 (2). However, it is relatively low compared to coffee productivity in Vietnam which reached 2.78 tons/ha in the same year (3).

One of the factors that cause low productivity of coffee in Indonesia is suboptimal maintenance, especially fertilization (4,5). Fertilization is a critical process for growing coffee plants because it replaces nutrients lost from the soil due to sedimentation, volatilization, and absorption; thus, the plant's nutrients can be fulfilled. Plant's nutrient deficiencies can affect growth, such by inhibiting metabolic processes and

negatively affecting crop productivity (6). Coffee is an annual plant that requires a lot of nutrients during its life cycle. A hectare of coffee plantation requires 53.2-172.0 kg N, 10.5-36.0 kg P₂O₅, and 80.7-180.0 kg K₂O annually. Thus, fertilization needs to be applied annually to fulfill the nutrient needs of coffee plants (7,8).

Inorganic fertilizers are the most common way to supply plant nutrients and increase plant productivity (9). However, the intensive use of inorganic fertilizers can harm the environment and other organisms, e.g., soil and water pollution through nutrient leaching, destruction of soil physical characteristics, accumulation of toxic chemicals in water bodies, as well as causing loss of biodiversity (10–12). Alternative fertilization by utilizing soil microorganisms that have specific activities in providing nutrients for the plant is needed in coffee cultivation (13).

Arbuscular mycorrhizal fungi (AMF) or endomycorrhizae is a well-known biofertilizer that usually replaces inorganic fertilizer because it can increase plant productivity by optimizing the absorption of nutrients in the soil (11). It can also improve soil fertility, such as soil aggregation, nutrient availability, microbial activities, nitrogen, carbon, and phosphorus cycling (14). AMF can recruit Phosphate Solubilizing Bacteria (PSB) that produce phosphatase to mineralize insoluble phosphate in the soil and release soluble P that can easily be assimilated by plants (14,15). Endomycorrhizae have a mutualistic symbiosis with plant roots by forming intra-extraradical mycelium, spores, vesicles, and arbuscular structures that provide additional nutrients for plants (16). More than 150 endomycorrhizal species can be symbiotic, with 90% of vascular plant species and 80% of terrestrial plants, including coffee (17,18). The results of previous studies reported that endomycorrhizal inoculation in coffee plants improved the growth of arabica coffee plants at the initial production phase (1.5 years after planting) by increasing plant height, stem diameter, and the number of branches compared to the plant without endomycorrhizae application (8). Moreover, endomycorrhizae also potentially reduce the dose of NPKMg fertilizer by up to 50% (4).

Based on previous research conducted by Sari (19), isolates of endomycorrhizal, *Fusarium oxysporum*, from Arabica coffee plants in Malabar coffee plantations, Bandung Regency, and Genteng Village, Sumedang Regency, have enzymatic activities such as hydrolysis of starch and cellulose, phosphate solubilization, and IAA production. It also can hasten the time of arabica coffee seed germination from 31 to 20 days after sowing. However, the potential of endomycorrhizal isolates in the growth of Arabica coffee seedlings under field conditions was not known yet. Thus, this study was carried out to determine the potential of endomycorrhizal *F. oxysporum* in increasing Arabica coffee seedlings' growth and nutrient uptake under field conditions.

2. Methodology

2.1. Preparation of Endomycorrhizae Inoculum

The endomycorrhizal starter inoculum, *Fusarium oxysporum*, was obtained from the Microbiology Laboratory, SITH-ITB, Bandung, West Java, Indonesia, in a solid media containing sterile soil and potato extract 1:1 (w/v basis) as a carrier medium. The endomycorrhizal starter inoculum was multiplication in the soil-compost substrate (1:1 w/w basis) with the ratio of 1:10 (w/w basis) and incubated for 72 hours to obtain the endomycorrhizal density of 2×10^6 CFU/g (20).

2.2. Experimental Design, Endomycorrhizal Inoculation, and Planting

The experiment was carried out in Suntenjaya Village, Lembang District, West Java (6°49'19" S, 107°42'18" E, and an altitude of 1,307 masl) from October 2020 to February 2021. The experiment was carried out under field conditions with an average temperature of around 22.3-24.5°C, relative humidity of around 57.1-84.4%, and rainfall of about 2,000 mm/year. A Randomized Complete Block Design (RCBD) with five replications was used with four treatments, i.e., (P0) control: without endomycorrhizae and fertilizer, (P1) inorganic fertilizer: NPK recommended dose for seedlings nine months after sowing (N 184 kg/ha, P₂O₅ 72 kg/ha, and K₂O 120 kg/ha from 400kg/ha urea, 200 kg/ha SP-36, and 200 kg/ha KCl, respectively), (P2) endomycorrhizae: 1:1 (w/w basis) endomycorrhizal inoculum-planting medium, and (P3) endomycorrhizae + organic fertilizer: 1:1 (w/w basis) endomycorrhizal inoculum-planting medium with the addition of chicken manure at a dose of 10 tons/ha.

Polybags 25 cm x 25 cm were filled with planting medium. P0 and P1 treatments were filled with 5 kg soil as a planting medium. In comparison, P2 and P3 treatments were filled with 2.5 kg soil and 2.5 kg endomycorrhizal inoculum (1:1 w/w basis) as a planting medium, resulting in the medium weight of 5 kg for each treatment. In the P3 treatment, 28.41 g of chicken manure (dose of 10 tons/ha) was added to the planting media at the same time with endomycorrhizal inoculum application. All polybags containing planting media were incubated for seven days before transplanting Arabica coffee seedlings (21). Application of NPK fertilizer for P1 treatment was carried out once at 4 weeks after transplanting. The chemical properties of the soil used in this study are listed in Table 1.

Table 1. Chemical characteristics of the soil in the experimental area

Soil Properties	Value	Criteria*
pH H ₂ O	5,6	Slightly acid
C-Organic (%)	3,37	High
Total N (%)	0,41	Moderate
C/N Ratio	8	Moderate
Available P (mg/kg)	491,1	Very high
Available K (mg/kg)	60,4	Very high

*Criteria for Soil Research Hall in Technical Guidelines for Chemical Analysis of Soil, Plants, Water, and Fertilizers (2009)

Arabica coffee seedlings used in this study were eight months old after sowing. The planting technique was done according to the Technical Guidelines for Good Coffee Cultivation (22). According to the treatment, each polybag containing planting media was planted with an Arabica coffee seedling with a straight-down root position, then the coffee plants were watered. Watering and controlling plant pest organisms was carried out manually for 16 weeks during the experiment (23). Plant's height and diameter were measured after initial planting as initial data (day 0) for this experiment.

2.3. Growth Parameters of Arabica Coffee Seedlings

The growth parameters of arabica coffee seedlings were observed and collected four times during the 16 weeks experiment. Plant height was measured using a ruler from the base of the stem to the tip of the shoot. The stem diameter was measured using a digital caliper of 2 cm from the planting medium (23).

2.4. Endomycorrhizal Root Colonization

Fine root samples from each treatment and replication were collected randomly at 16 weeks after transplanting. Root samples were carefully washed with distilled water and cut into 1 cm long segments, then soaked in 10% (w/v) KOH for 12 hours, rinsed in tap water, acidified in 2% HCl (v/v) overnight, and stained with 0.05% (w/v) trypan blue in lactoglycerol. Excess solution on the roots was removed with lactoglycerol solution (24). The stained root segments were arranged on the object-glass and observed under a microscope at 400 times magnification. The root colonization rate was calculated using the following formula (25) :

$$\begin{aligned} \text{Root colonization rate (\%)} \\ = \frac{\text{number of infected roots}}{\text{numbers of all observed root}} \times 100\% \end{aligned} \quad (1)$$

2.5. Plant Analysis

The seedlings from each treatment and replication were harvested randomly at 16 weeks after transplanting (WAT), then cleaned from the soil particles. The dry weight of plants was determined by drying them in the oven at 70°C to a constant weight (about 48 hours) and weighed as plant dry weight (21). The dried plants were ground to a fine powder (<2 mm). Fine plant powder was used for the analysis of plant nutrient contents. The plant nutrient contents were determined as follows: N content was determined by distillation after digestion by the Kjeldahl method, and a spectrophotometer determined P content at 889 nm. In contrast, K content was determined by atomic absorption spectrophotometer after wet digestion (26). Plant nutrient uptake was calculated using the following formula:

$$\begin{aligned} \text{Nutrient uptake (g/plant)} \\ = \text{plant dry weight (g)} \\ \times \text{plant nutrient content (\%)} \end{aligned} \quad (2)$$

2.6. Statistical Analysis

The obtained data were analyzed with one-way analysis of variance (ANOVA) after checking the normality of data and homogeneity of variances. It was then followed by Duncan Multiple Range Test (DMRT) at a significant level of 0.05 using IBM SPSS Statistics version 25.0 (20).

3. Result and discussion

3.1. Seedlings Growth

Figure 1 shows plant height at 0 to 16 weeks after transplanting. This study showed that endomycorrhizal (P2) increased plant height by 15.4% at 16 WAT compared to the control (P0), although not statistically significant ($p > 0.05$). The application of endomycorrhizae and chicken manure (P3) increased the plant height by 32.2% at 16 WAT. These differences were statistically significant ($p \leq 0.05$) compared to the control (P0). This result showed that a combination of organic fertilizer and biofertilizers gives better growth than a single use of biofertilizers and has almost similar growth with a recommended NPK fertilizer (P1) as standard fertilization. It might be because organic fertilizer contains organic sources that are useful for biofertilizers' growth and increase the soil's activity (27). In addition, using chicken manure as organic fertilizer increases the mineral content in the soil compared to without chicken manure, so the plant's nutrients can be fulfilled and produce optimal growth (28).

Figure 2 shows the stem diameter of the plant from 0 to 16 weeks after transplanting. All treatments in this study did not have any significant differences based on one-way ANOVA statistical analysis ($p > 0.05$). However, plants treated with endomycorrhizae, either P3 or P2 in this study, had a larger diameter than the control (P0) during 16 weeks of the experiment by 3.51 mm and 3.43 mm, respectively. The highest stem diameter was found in the P1 treatment (3.80 mm), while the lowest stem diameter was found in the P0 treatment (3.28 mm). This finding was in line with the study by Daras et al. (8) and Wang et al. (27). They mentioned that the growth of the plants inoculated with endomycorrhizal was increased compared to the non-inoculated plants. It might be because endomycorrhizae can increase plant nutrient uptake, resulting in increased growth. Clark and Zeto (28) and Heijden et al. (18) stated that various plant nutrients such as P, N, K, Ca, Mg, Cu, and Zn can be absorbed efficiently by endomycorrhizae through the role of extraradical mycelium (29).

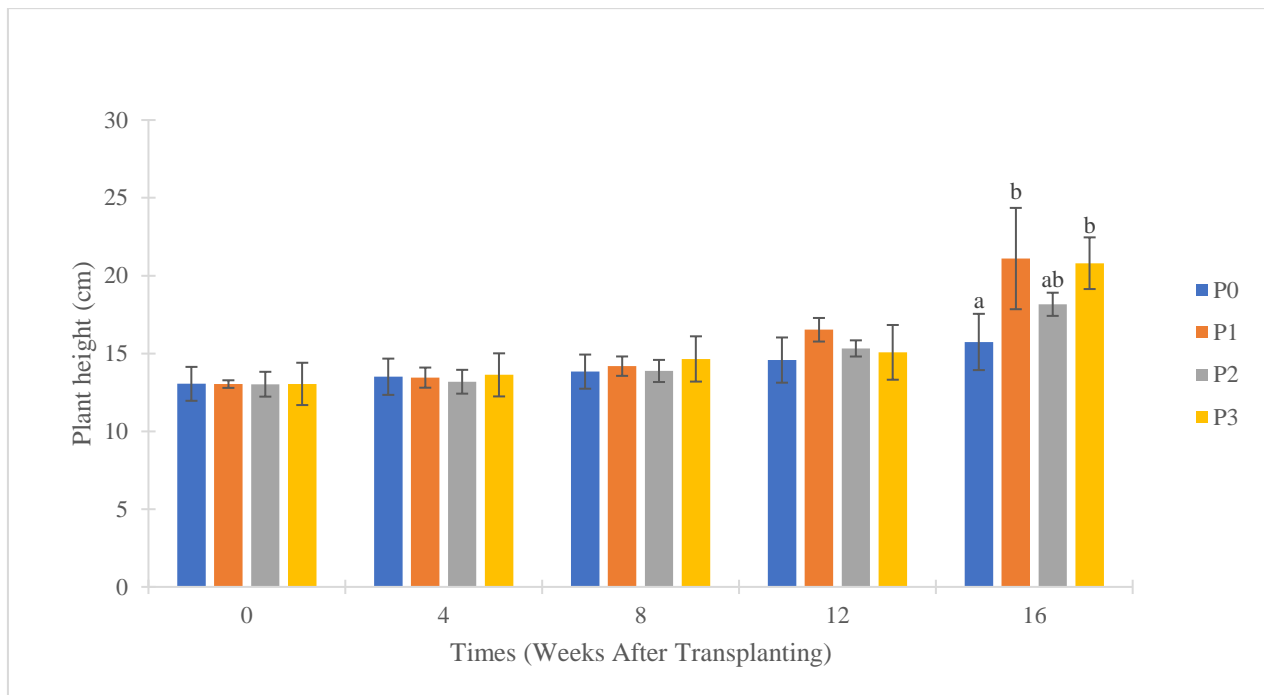


Figure 1. Arabica coffee plant height at 0, 4, 8, 12, and 16 weeks after transplanting (MSP). P0: control (without endomycorrhizae and fertilizers), P1: inorganic fertilizer (NPK recommended dose for seedlings nine months after sowing (400 kg/ha urea, 200 kg/ha SP-36, and 200 kg/ha KCl)), P2: endomycorrhizae (1:1 (w/w basis) endomycorrhizal inoculum-planting medium), P3: endomycorrhizae + organic fertilizer (1:1 (w/w basis) endomycorrhizal inoculum-planting medium) with the addition of chicken manure at a dose of 10 tons/ha. The vertical bar represents the standard deviation. Different letters represent significant differences on the Duncan Multiple Range Test at a significant level of 0.05.

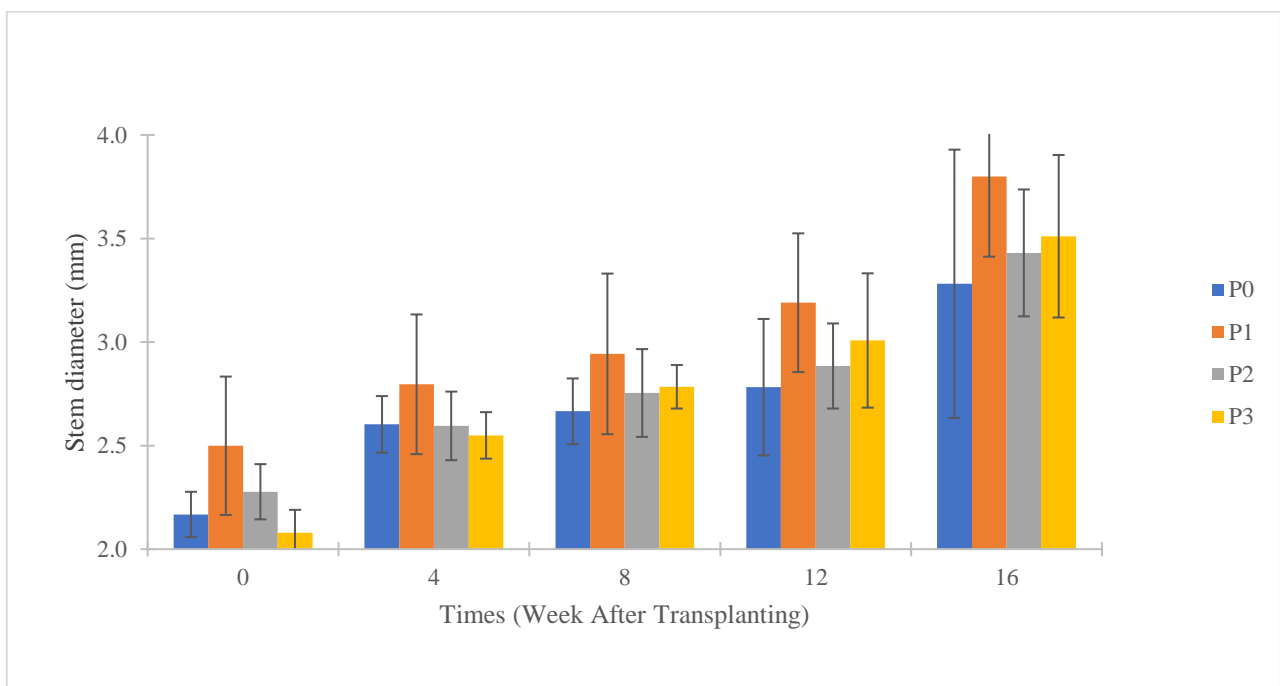


Figure 2. Stem diameter of arabica coffee plants at 0, 4, 8, 12, and 16 weeks after transplanting (MSP). P0: control (without endomycorrhizae and fertilizers), P1: inorganic fertilizer (NPK recommended dose for seedlings nine months after sowing (400 kg/ha urea, 200 kg/ha SP-36, and 200 kg/ha KCl)), P2: endomycorrhizae (1:1 (w/w basis) endomycorrhizal inoculum-planting medium), P3: endomycorrhizae + organic fertilizer (1:1 (w/w basis) endomycorrhizal inoculum-planting medium) with the addition of chicken manure at a dose of 10 tons/ha. The vertical bar represents the standard deviation.

3.2. Endomycorrhizal Root Colonization

The colonization of endomycorrhizal on coffee plant root was observed in all treatments. It was characterized by intercellular and intracellular mycelium, arbuscules, and vesicles in the root tissue. Analysis of variance indicated a significant effect of treatments ($p \leq 0.05$) at 16 WAT (Figure 3). P2 and P3 treatments significantly had higher root colonization than the treatment without endomycorrhizal inoculation (P0, P1). The highest root colonization (67.5%) was found in the endomycorrhizal treatment (P2), while the

lowest root colonization (2.7%) was found in the recommended dose of NPK treatment (P1). This finding was in line with the study by Juntahum et al. (20), who mentioned that the plant inoculated with endomycorrhizal had higher root colonization than without inoculation. Low endomycorrhizal colonization on plant roots in fertilizer treatment, both organic and inorganic, was due to the inability of endomycorrhizae to colonize plant roots in a high concentration of soil nutrients. Verbruggen and Kiers (30) stated that fertilizer application, especially the application of mineral-P and N fertilizer, causes the reduction of the resource allocation for mycorrhizae by the host plant, thereby reducing the rate of root colonization.

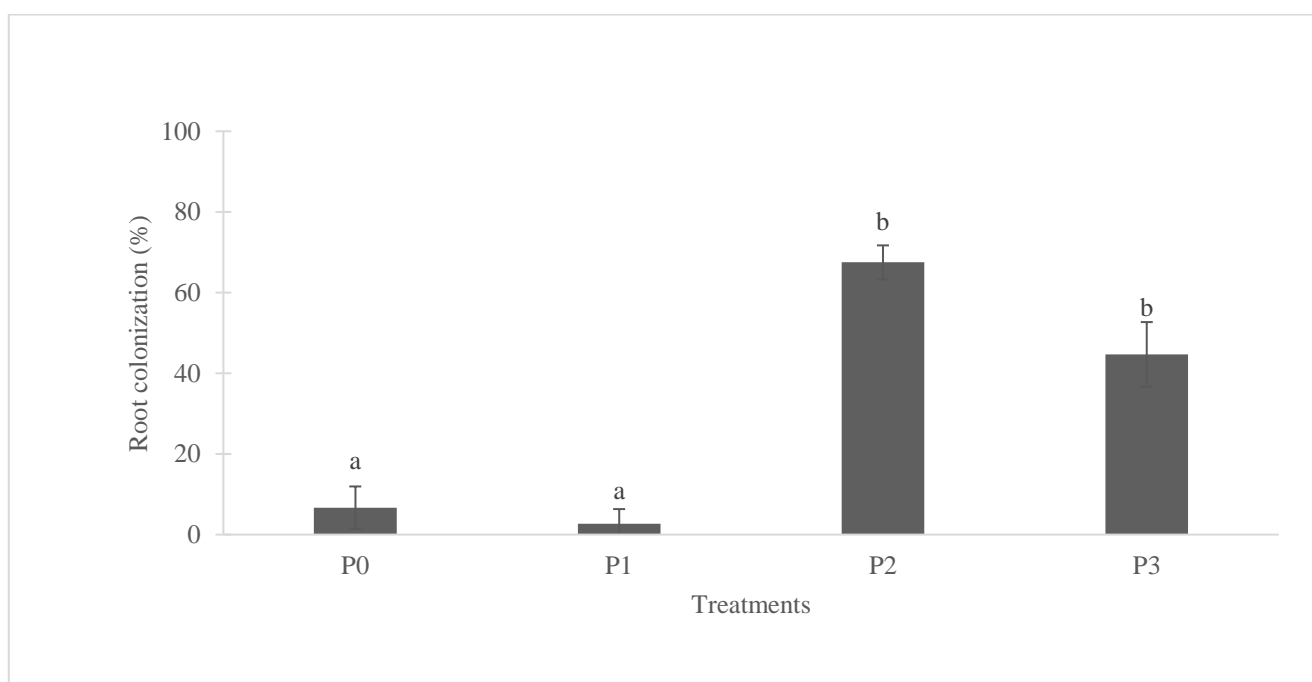


Figure 3. Percentage of root colonization at 16 weeks after transplanting (MSP). P0: control (without endomycorrhizae and fertilizers), P1: inorganic fertilizer (NPK recommended dose for seedlings nine months after sowing (400 kg/ha urea, 200 kg/ha SP-36, and 200 kg/ha KCl)), P2: endomycorrhizae (1:1 (w/w basis) endomycorrhizal inoculum-planting medium), P3: endomycorrhizae + organic fertilizer (1:1 (w/w basis) endomycorrhizal inoculum-planting medium) with the addition of chicken manure at a dose of 10 tons/ha. The vertical bar represents the standard deviation. Different letters represent significant differences on the Duncan Multiple Range Test at a significant level of 0.05.

3.3. Plant Dry Weight and Nutrient Uptake

Analysis of variance indicated a significant effect of treatment ($p \leq 0.05$) to plant dry weight and nutrient uptake at 16 WAT (Table 2). Application of endomycorrhizal with the addition of chicken manure (P3) and recommended dose of NPK fertilizer (P1) treatments significantly enhanced plant dry weight than the control (P0). Meanwhile, endomycorrhizal inoculation (P2) slightly increased plant dry weight by 16.6% compared to the control (P0); thus, it was not significant statistically ($p > 0.05$). The plant dry weight showed a higher value, an increase of around 38.7% compared to the control (P0) in endomycorrhizal treatment with the addition of

chicken manure (P3). These differences were statistically significant ($p \leq 0.05$), suggesting that the endomycorrhizal application needs to be supplemented with organic fertilizer to increase plant dry weight. This finding was in line with the study by Abreu et al. (31). They mentioned that the application of endomycorrhizae with the addition of organic fertilizer could increase plant dry weight due to better physiological performance by root colonization than without endomycorrhizal application. Yang et al. (32) mentioned that the addition of organic matter had a beneficial effect in stimulating endomycorrhizae hyphal growth, sporulation, and root colonization. The production of endomycorrhizae extraradical mycelia can increase the plant's capacity to uptake

nutrients and water efficiently for biomass accumulation, increasing plant dry weight (33).

Plant nutrient uptake was increased in the recommended dose of NPK fertilizer (P1) and endomycorrhizal (P2 and P3) treatments compared to the control (P0) (Table 2). Generally, plant nutrient uptake in the control treatment (P0) always shows a minor performance and recommended dose of NPK fertilizer (P1) was more effective than the other treatments in increasing N uptake. However, P and K uptake were more effective under endomycorrhizal with the addition of chicken manure (P3) treatment. Meanwhile, endomycorrhizal

treatment (P2) was significant statistically ($p \leq 0.05$) to N and K uptake compared to control (P0) with an increase by 52.5% and 75.6%, respectively, but was not significant statistically ($p > 0.05$) to P uptake. This finding was in line with the study by Juntahum et al. (20), who mentioned that N and K nutrient uptake in endomycorrhizal inoculated plants was higher than in the control treatment. P uptake by endomycorrhizae was not significantly different compared to control due to a very high soil available P (Table 1), thereby endomycorrhizae reducing the capacity of P uptake in P-rich soils (30).

Table 2. Effect of endomycorrhizal inoculation and fertilization on dry weight and nutrient uptake of arabica coffee plants at 16 weeks after transplanting

Treatments**	Plant dry weight (g)*	Nutrient uptake (g/plant) *		
		N	P	K
P0	12,47 ± 2,07 ^a	29,26 ± 2,84 ^a	7,30 ± 1,57 ^a	13,75 ± 0,97 ^a
P1	20,61 ± 2,13 ^c	57,56 ± 15,60 ^c	18,34 ± 8,52 ^b	34,87 ± 11,73 ^c
P2	14,54 ± 2,68 ^a	44,66 ± 1,39 ^b	13,93 ± 2,45 ^{ab}	24,15 ± 1,13 ^b
P3	17,30 ± 2,32 ^b	49,42 ± 5,31 ^{bc}	20,70 ± 4,65 ^b	39,81 ± 1,80 ^c

*Values represent mean ± SD (n=5). Values with different letters in each row indicate significant differences between treatments (p -value ≤ 0.05 ; DMRT test).

**P0: control (without endomycorrhizae and fertilizers), P1: inorganic fertilizer (NPK recommended dose for seedlings nine months after sowing (400 kg/ha urea, 200 kg/ha SP-36, and 200 kg/ha KCl)), P2: endomycorrhizae (1:1 (w/w basis) endomycorrhizal inoculum-planting medium), P3: endomycorrhizae + organic fertilizer (1:1 (w/w basis) endomycorrhizal inoculum-planting medium) with the addition of chicken manure at a dose of 10 tons/ha.

Generally, plants with endomycorrhizal treatment have a better NPK uptake because endomycorrhizal form a network of very massive mycelium on the roots of the host plant and the rhizosphere area, allowing mycorrhizal plants to exploit large volumes of soil, which results in increased nutrient uptake (4). The increase in nutrient uptake occurs because endomycorrhizae have inorganic phosphate (Pi) *P14* transporters with high affinity on the surface of extraradical mycelium; thus, it can accumulate polyphosphate (polyP) along their mycelium, which then enters the host plant tissue (34). In addition, plants colonized by endomycorrhizae also have nitrogen transporter *AMT2*, which is active in absorbing NH_4^+ and NO_3^- (35). Meanwhile, plant K uptake increased along with P uptake because plants absorb K^+ as a counter ion of short-chain polyP granules in mycorrhizal vacuoles to regulate Pi homeostasis (36).

4. Conclusion

Endomycorrhizae treatment (P2) have the potential to increase the growth of arabica coffee seedlings under field condition with an increase in plant height, plant dry weight, and plant N, P, and K uptake by 15.4%, 16.6%, 52.5%, 90.8%, and 75.6%, respectively compared to the control (P0) with 67.5% of root colonization at 16 WAT. Plant growth increment is due to the ability of endomycorrhizae to efficiently absorb water and plant nutrients through the role of

extraradical mycelium. However, added endomycorrhizae with chicken manure increases plant growth and nutrient uptake more than using endomycorrhizae as a biofertilizer.

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