




# Application of Arbuscular Mycorrhizal Fungi (AMF) to Enhance Biomass of *Uncaria gambir* (Gambier) in Ex-Coal Mining Soil

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## Abstract

Gambier cultivation faces limitations due to decreasing land availability; however, utilizing ex-coal mining land presents a promising alternative for sustainable expansion. Ex-coal mining land has the problem of low available nutrients because of bonded nutrients. Arbuscular Mycorrhiza Fungi (AMF) can help release bound nutrients into available nutrients, so that on ex-coal mining land, there is an increase in the amount of available nutrients that can be absorbed by plants for their growth. The study aimed to determine the effect of different types of arbuscular mycorrhizal fungi (AMF) and their doses on the biomass of gambier seedlings planted in ex-coal mining soil. An experimental design was employed using a randomized block design with two treatments: the types of arbuscular mycorrhizal fungi (AMF) (*Acaulospora* sp., *Glomus* sp., and *Gigaspora* sp.) and the AMF dosages (0, 5, 10, 15, and 20 g per seedling). All treatments were replicated three times. The parameters observed were soil chemical analysis, root colonization by arbuscular mycorrhizal fungi (AMF), shoot and root fresh and dry weights, net assimilation rate, and relative growth rate. The results showed that AMF type and dose treatment had a significant effect on all parameters, except for the relative growth rate, and there was no interaction between the two treatments. *Glomus* sp. at a dose of 20 g per seedling provided the best influence in increasing gambier seedlings' biomass under the ex-coal mine soil.

Keywords: extensification, *Glomus* sp., marginal, seedling, root colonization

## Introduction

Gambier (*Uncaria gambir* Roxb.) is a plantation crop that grows widely in Southeast Asia, especially in Indonesia. Gambier is a major plantation export commodity for Indonesia, serving as an important source of foreign exchange. Dried gambier gum, produced from processed and molded extracts of crushed twigs and leaves, is widely utilized as an ingredient in the cosmetics, pharmaceutical, food, clothing, and textile dye industries (Lidar et al., 2018). Indonesia is the world's leading exporter of gambier, meeting global demand with nearly 80% of its national production originating from West Sumatra Province (Suharman, 2018).

Although Indonesia offers excellent marketing and development opportunities for gambier, accounting for the world's primary production, the volume and export value of gambier in Indonesia do not exhibit stable conditions every year (Indonesian Ministry of Trade, 2017). The problem that farmers often face in their development is the low productivity of the gambier. One alternative way to increase the low productivity of gambier is to utilize an ex-mining area that can be diverted for agricultural activities. This diversion can also mitigate the negative impacts of mining and expedite the rehabilitation of abandoned former mining land. There are at least three of the largest coal-producing islands in Indonesia: Kalimantan, Java, and Sumatra. Notably, Sumatra Island has coal resource reserves of 55.8 billion tons (Fajri, 2022). West Sumatra is one of the provinces on the island of Sumatra that has a coal mining area spread across several Regency. One of them is in the South Coast Regency, which has a coal mining area of 192.08 hectares (ATM Promining, 2024).

The primary challenge to agricultural cultivation in ex-coal mining areas is the poor physical, chemical, and biological quality of the soil. These soils often contain toxic levels of heavy metals such as Al, Fe, Pb, Hg, and Cd, which are harmful to both plants and humans (Permana, 2010; Sarie, 2019; Pramaditya and Nilawati, 2022). One promising approach for restoring these degraded lands is the use of arbuscular mycorrhizal fungi (AMF). AMF are soil-borne microorganisms that form symbiotic relationships with plant roots, enhancing the absorption of essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K), as well as water. Their extensive hyphal networks allow plant roots to access soil regions that would otherwise be unreachable (Sun et al., 2018; Zai et al., 2021). Remarkably, AMF can establish symbiosis with nearly 90% of all plant species and has a high rate of dispersal (Kumalawati et al., 2018).

The benefits of AMF extend beyond improved nutrient uptake; they also help protect plants against environmental stresses such as low pH, heavy metal toxicity, and pathogen attacks (Suharno and Sancayaningsih, 2013; Basri, 2018; Husein et al., 2022). According to Prayoga and Prasetya (2021), AMF is particularly effective in marginal environments, such as former coal mine sites, due to its adaptability and ability to ameliorate polluted soils. Therefore, it is essential to evaluate both the type and dosage of arbuscular mycorrhizal fungi for their effects on the growth of gambier in ex-coal mining soils.

## Materials and Methods

The experiment was conducted from February to June 2024 at the Faculty of Agriculture Experimental Station, Andalas University, Padang, Indonesia, at an altitude of approximately 200 meters above sea level. The tyndallization process was carried out at the Soil Laboratory, Faculty of Agriculture, Andalas University. Plant weight measurement and observation of root colonization were performed at the Plant Physiology Laboratory, Faculty of Agriculture, Andalas University. The research was conducted using a two-factor completely randomized design, which was repeated three times. The first factor was the arbuscular mycorrhizal fungi (AMF) types (*Acaulospora* sp., *Glomus* sp., *Gigaspora* sp.), and the second factor was AMF dosages, which were in the form of spores and sand mixes, at 0, 5, 10, 15, 20 g per plant. There were 45 experimental units, each consisting of four sample plants: three designated for destructive sampling and one for a non-destructive sample, resulting in a total of 180 sample plants.

The plant material used in this experiment is a 2-month-old “Cubadak” variety gambier seedling (Figure 1) from Gambier plant brood stock that has been more than ten years old, obtained from the South Coast Regency. AMF *Acaulospora*, *Glomus*, and *Gigaspora* sp. were prepared in sand media. Ex-coal mining soil was collected from an ex-mining area in Tambang Village, Jurai IV District, South Coast Regency.



Figure 1. A two-month-old gambier “Cubadak” seedling

### Soil Sampling Procedure

Soil samples were taken in the ex-coal mining area of the mining company PT Atoz Nusantara Mining using the stratified random sampling method. Soil sampling was carried out at three sampling location points using a soil drill with a depth of  $\pm 30$  cm from the ground surface. Then the samples were placed in plastic buckets. All soil samples at each sampling location point were mixed and stirred evenly in the plastic bucket. The soil samples were then placed into labeled plastic containers and taken to the laboratory for analysis.

### Experimental Procedure

Ex-coal mining soil was soaked with water for 3 days and then air-dried. The soil was then sifted using a 5-mesh sieve. The sifted soil and cow manure are sterilized using the tyndallization method (Cahyani, 2009; Alfiah et al., 2016). Water is put into a container just below the boiler and then heated to boil for 2 hours. The soil and cow manure are placed separately into a plastic bag and then into a boiler to be tyndallized for 2 hours. The soil and cow manure, which had been thoroughly treated with tyndallization, were then mixed in a 1:1 volume ratio and incubated for one week before planting. Incubated planting media were placed into a 15 cm x 20 cm polybag.

AMF application is carried out by spreading sand media that contains AMF spores into the planting hole,  $\pm$  5 mm from the seedling roots. Then, 2-month-old gambier plant seedlings with the criteria of having three pairs of leaves, similar stem sizes, and the visual appearance of healthy growing seedlings (free from pests and diseases) were planted one seedling per polybag and kept under 70% shading at the nursery until 30 days after planting (DAP) and moved to open area until observation ended. Plant maintenance activities included watering, weeding, pests and diseases control, and follow-up fertilizer application using 3.3 g N, 4.2 g P<sub>2</sub>O<sub>5</sub>, and 3 g K<sub>2</sub>O per plant at 30 days after planting.

The observed parameters included ex-coal mining chemical soil analysis (pH, C-Organic, available P, total N, P, and K), conducted at the beginning of the study. The percentage of root colonization by arbuscular mycorrhizal fungi (AMF) was observed at 84 days after planting (DAP) using the Mycorrhizal root staining method (Phillips and Hayman, 1970). The stained root was observed under a microscope (400x magnification) to identify the appearance of AMF intraradical structures (hyphae, arbuscules, vesicles, and spores). The percentage of root colonization by arbuscular mycorrhizal fungi (AMF) was determined using the formula of Nusantara et al. (2012). The following formula was used to calculate root colonization:

$$\text{Root colonization (\%)} = \frac{\text{The total field of view infected}}{\text{The total field of view observed}} \times 100\%$$

Classification of percentage colonization was according to Rajapakse and Miller (1992), namely: (a) lowest: 0-5%, (b) low: >5-25%, (c) medium: >25-50%, (d) high: >50-75%, and (e) highest: >75-100% colonization value.

At 84 days after planting, the gambier seedlings were cleaned and separated to examine the fresh weight of the shoots and roots. Shoot and root dry weight were measured after drying for 24 hours in an oven at 70°C. Net assimilation and relative growth rate according to Sudhakar et al. (2016) was calculated at 42 and 84 DAP using the following formula:

$$\text{Net assimilation rate (g.cm}^{-1}\text{.day}^{-1}) = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\text{Log}_e A_2 - \text{Log}_e A_1}{A_2 - A_1}$$

$$\text{Relative growth rate (g.g}^{-1}\text{.day}^{-1}) = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{t_2 - t_1}$$

Where  $t_2$  and  $t_1$  are the times of the second and first sample observation,  $W_2$  and  $W_1$  are the total plant dry weights at times  $t_2$  and  $t_1$ ,  $\text{Log}_e$  is the natural logarithm, and  $A_2$  and  $A_1$  are the plant leaf areas at times  $t_2$  and  $t_1$ .

## Data Analysis

Analysis of variance (ANOVA) was performed to determine the significant differences in treatment effects across all variables, except for chemical soil analysis. Root colonization was analyzed descriptively. ANOVA analysis was followed by Duncan's Multiple Range Test (DMRT) at a 95% confidence level ( $\alpha = 0.05$ ), if a significant difference was detected. All stage of statistical data analysis was performed using the Statistical Tool for Agricultural Research (STAR) application.

## Results and Discussion

### Ex-Coal Mining Chemical Soil Analysis

The soil analysis results in Table 1 show that ex-coal mining soil pH is classified as very acidic. The total N, available P, total P, and total K are low, and the organic C content is moderate. Soil conditions that are too acidic can inhibit plant growth because they bind essential nutrients such as phosphorus, making them unavailable to plants. According to Havlin et al. (2005), acid soil conditions can inhibit microbial activity, including N mineralization from organic matter and nitrification. Low total N content indicates low nitrogen availability in the soil. Nitrogen is an essential nutrient for plant vegetative growth. Low available phosphorus (P), total phosphorus (P), and total potassium (K) content will negatively affect plant growth. Phosphorus deficiency in plants causes them to become stunted, and Potassium deficiency affects the photosynthesis process and nutrient transportation. C-organic content is essential for improving soil structure, increasing water-holding capacity, and providing a food source for soil microorganisms.

The application of AMF to soil and successful root colonization by AMF are expected to increase the availability of previously unavailable nutrients. So that the availability of low nutrients, such as nutrient P, which plays a role in the photosynthesis process, can be increased. Indirectly, mycorrhiza-infected roots facilitate the absorption of water and nutrients essential for plant growth.

### Percentage of Root Colonization by Arbuscular Mycorrhizal Fungi (AMF)

Table 2 shows that gambier seedlings without AMF treatment had no root colonization (0%) due to the sterilized soil. According to Sagala et al. (2013), in sterilized media, roots are unlikely to be colonized by naturally occurring arbuscular mycorrhizal fungi

(AMF), resulting in inactive mycorrhizal associations. This indicates that the sterilization process using the Tyndallization method was effective. Therefore, the mycorrhizal colonization observed in gambier roots is attributable solely to the applied mycorrhiza treatment.

The AMF applied was symbiosis with the roots of gambier seedlings, as indicated by a colonization percentage of 60% to 93.33%. *Glomus* sp. can colonize roots with a rate of up to 80% even though it is only applied with a dose of 5 g per plant, while other types can only achieve the same percentage of colonization when used with a dose of 15 g per plant. The lowest colonization percentage was 60% for *Gigaspora* sp. at a dosage of 5 g per plant, which is classified as high according to Nusantara et al. (2012). Conversely, the highest colonization rate was 93.33% for *Glomus* sp. at 20 g plant, falling into the very high colonization category. These AMF colonization results on gambier seedling roots are illustrated in Figure 2. The symbiotic relationship between plants and arbuscular mycorrhizal fungi (AMF) is quantified

by the colonization percentage in the host roots; higher colonization indicates a stronger mutualistic symbiosis and greater effectiveness of the AMF.

The very high colonization rate of *Glomus* sp. aligns with previous research on mycorrhizal communities in ex-mining areas. For instance, Margarettha (2011) reported a denser population of *Glomus* spores compared to *Acaulospora* in such environments. Similarly, Kartika et al. (2012) identified three AMF genera—*Glomus*, *Acaulospora*, and *Gigaspora*—with *Glomus* being dominant in ex-coal mine soils. Lubis (2021) documented five indigenous arbuscular mycorrhizal fungus (AMF) genera at the PT Nan Rieng coal mine, including 16 species of *Glomus*, five of *Acaulospora*, three of *Scutellospora*, one of *Gigaspora*, and two of *Entrophospora*. Recent work by Wisnubroto et al. (2024) confirmed that *Glomus* sp. is more prevalent across five ex-coal mining slopes than *Acaulospora* and *Gigaspora*, indicating its superior adaptability and reproductive capacity in degraded soils.

Table 1. The soil chemical properties of ex-coal mining soil

| Soil parameter                           | Analytical methods | Value                        | Status*     |
|--|--------------------|------------------------------|-------------|
| pH H <sub>2</sub> O (1:5)                | Electrometry       | 4.42                         | Very acidic |
| C-Organic                                | Walkley and Black  | 2.64 %                       | Medium      |
| Total N                                  | Kjeldahl           | 0.12 %                       | Low         |
| Available P                              | Bray 1             | 6.12 ppm                     | Low         |
| Total P (P <sub>2</sub> O <sub>5</sub> ) | HCl 25%            | 17.21 mg.100 g <sup>-1</sup> | Low         |
| Total K (K <sub>2</sub> O)               | HCl 25%            | 11.09 mg.100 g <sup>-1</sup> | Low         |

Notes: \*Soil chemical property status based on Eviati et al. (2023).

Table 2. Percentage of roots colonized by AMF in gambier seedlings at 84 days after planting

| AMF types              | Percentage of roots colonized at different AMF dosages* |       |       |       |       |
|------------------------|---|-------|-------|-------|-------|
|                        | 0 g   | 5 g   | 10 g  | 15 g  | 20 g  |
| <i>Acaulospora</i> sp. | 0.00  | 66.67 | 73.33 | 80.00 | 86.67 |
| <i>Glomus</i> sp.      | 0.00  | 80.00 | 86.67 | 86.67 | 93.33 |
| <i>Gigasporas</i> sp.  | 0.00  | 60.00 | 66.67 | 80.00 | 80.00 |

Notes: \*AMF dosages in grams per plant.

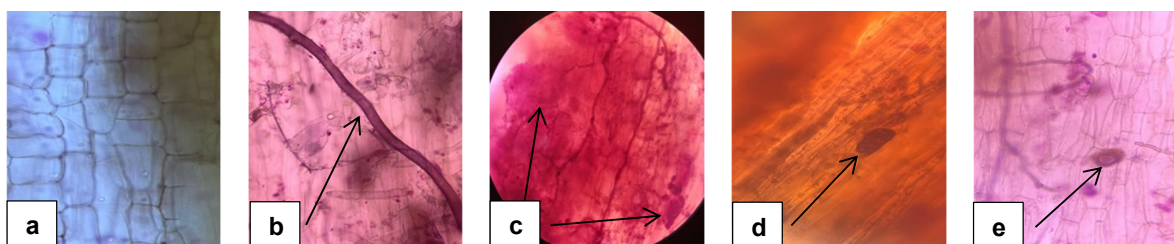


Figure 2. AMF colonization in gambier seedlings at 84 days after planting under a 400x microscope magnification: (a) uninfected roots, (b) internal hyphae, (c) arbuscula, (d) vesicula, (e) spores.

Mycorrhizal colonization begins with the formation of an appressorium on the root surface, which then penetrates the epidermal cells of the root. Subsequently, hyphae grow either intracellularly or extracellularly within the root cortex, sometimes forming hyphal coils in specific hosts. These hyphae absorb nutrients from the soil and transport them into root cells. Phosphorus movement through the hyphae follows cytoplasmic flow within the plant root cells, with nutrient transfer estimated to occur via arbuscules (Rokhminarsi et al., 2020).

External hyphae absorb phosphate ions from the soil and convert them into polyphosphate compounds. These compounds are transported through the hyphae and broken down into organic phosphate forms that plant cells can absorb (Simanjuntak et al., 2023). The fine diameter of hyphae, smaller than root hairs, enables them to penetrate micro-pores in the soil, facilitating water absorption even under low moisture conditions.

Plants colonized by mycorrhiza exhibit enhanced water uptake, which also facilitates the absorption of nutrients transported via mass flow, such as nitrogen (N), potassium (K), and sulfur (S) (Zulkoni et al., 2020). Additionally, mycorrhizal hyphae release phosphatase enzymes that mobilize phosphorus from soil compounds, increasing its availability to plants (Maulana and Harahap, 2023).

### Effects of AMF Types and Dosages on Gambier Seedling Biomass

Both AMF type and dosage significantly influence the fresh weight of shoots and roots, although no significant interaction effect between these factors was detected (Table 3). *Glomus* sp. inoculation resulted in the highest average shoot and root fresh weights, i.e., 15.95 g and 7.93 g, respectively. However, these values were not significantly different from those observed with *Acaulospora* sp. Increasing AMF dosage corresponded with increased fresh weights, with the 20 g per seedling treatment producing the heaviest shoots (20.28 g) and roots, comparable to those of the 15 g per seedling dosage.

The superior symbiosis of *Glomus* sp. with gambier seedlings, as reflected by root colonization percentages (Table 1), enhances water and nutrient absorption, which supports vigorous plant growth. Shoot fresh weight, which comprises stems and leaves, reflects the accumulated photosynthates and is strongly influenced by nutrient and water availability. Effective nutrient uptake facilitated by arbuscular mycorrhizal fungi (AMF) promotes photosynthesis, with the resulting assimilates being translocated to growing tissues, thereby increasing shoot biomass.

Root fresh weight is influenced by root moisture content; increased water uptake raises turgor pressure, contributing to greater fresh weight. Manurung et al. (2018) noted that mycorrhizal inoculation improves turgor pressure uniformity, which is linked to increased water potential in the roots.

Table 3. Biomass of gambier seedlings with different types of AMF and AMF dosages at 84 days after planting

| Treatments                          | Shoot fresh weight (g) | Root fresh weight (g) | Shoot dry weight (g) | Root dry weight (g) |
|-------------------------------------|------------------------|-----------------------|----------------------|---------------------|
| <b>AMF types</b>                    |                        |                       |                      |                     |
| <i>Acaulospora</i> sp.              | 14.55 ab               | 7.53 ab               | 2.68 b               | 1.22                |
| <i>Glomus</i> sp.                   | 15.95 a                | 7.93 a                | 3.57 a               | 1.20                |
| <i>Gigasporas</i> sp.               | 13.89 b                | 6.85 b                | 2.48 b               | 1.18                |
| <b>AMF dosages (g per seedling)</b> |                        |                       |                      |                     |
| 0                                   | 8.81 d                 | 3.19 d                | 1.39 d               | 1.09 c              |
| 5                                   | 12.29 c                | 6.23 c                | 2.42 c               | 1.15 bc             |
| 10                                  | 15.45 b                | 7.52 b                | 3.29 b               | 1.23 ab             |
| 15                                  | 17.16 b                | 9.61 a                | 3.12 b               | 1.23 ab             |
| 20                                  | 20.28 a                | 10.62 a               | 4.33 a               | 1.28 a              |
| AMF types                           | *                      | *                     | *                    | ns                  |
| AMF dosages                         | *                      | *                     | *                    | *                   |
| AMF types x dosages                 | ns                     | ns                    | ns                   | ns                  |

Notes: \*= significant, ns= not significant at  $\alpha=0.05$ ; values followed by the same letter within the same column are not significantly different at  $\alpha=0.05$  according to the DMRT.

This suggests that *Glomus* sp. effectively enhances water absorption in gambier seedling roots, positively impacting root biomass.

AMF colonization induces morphological and physiological changes in roots, including altered growth hormone levels and increased photosynthate production, which collectively enhance overall plant growth. The increase in root fresh weight is mainly due to the extension of external hyphae into the soil, which maximizes nutrient (especially phosphorus) and water uptake.

Shoot dry weight reflects the efficiency of photosynthesis, and results show that increasing AMF dosage significantly enhances both shoot and root dry weights. Meanwhile, AMF type significantly affects only root dry weight, with no significant interaction observed between AMF type and dosage on either shoot or root dry weight (Table 3). Among the AMF types tested, *Glomus* sp. produced the highest average shoot dry weight of 3.57 g. Both shoot and root dry weights of gambier seedlings increased progressively with higher AMF dosages. The 20 g per seedling yielded the heaviest shoot dry weight (4.33 g), which was comparable to root dry weight but not significantly different from the 10 and 15 g per seedling dosages.

Greater photosynthetic activity results in increased dry biomass accumulation. The sustained symbiotic relationship between plants and mycorrhiza enhances metabolic processes, ensuring optimal

photosynthesis and sufficient nutrient availability for growth. Since *Glomus* sp. exhibited the highest root colonization rate in gambier seedlings (Table 2), photosynthesis was likely more efficient in this treatment, leading to greater shoot dry weight compared to other AMF types.

The effectiveness of the 20 g per seedling dose in increasing shoot dry weight aligns with findings by Ali et al. (2023), who reported that *Glomus* sp. application at 20 and 25 g per seedling produced the highest dry weight in Robusta coffee seedlings. Similarly, Ramadhano (2022) found that a 20 g per plant dose of AMF maximized shoot dry weight in *Citronella* grown on ex-coal mining land. In dosage treatments, the 20 g per seedling AMF application resulted in the highest average shoot dry weight of 1.28 g, indicating a positive correlation between AMF dose and shoot biomass accumulation.

Root dry weight represents the accumulation of photosynthates in the roots. The continuous symbiotic association with arbuscular mycorrhizal fungi (AMF) influences plant metabolic processes that promote root formation and increase root membrane permeability. Moreover, roots colonized by mycorrhiza contain higher auxin levels, stimulating root growth (Budi and Hardhani, 2020). Melan (2019) also reported that AMF inoculation increases root number, branching, and diameter. Enhanced root development improves nutrient uptake, thereby supporting overall plant growth. Consequently, mycorrhizal infection leads to increased root volume and dry weight.

Table 4. Net assimilation and relative growth rate of gambier seedlings with AMF types and dosages at 84 days after planting

| Treatments                   | Net assimilation rate (mg.cm <sup>-1</sup> per day) | Net relative growth (mg.cm <sup>-1</sup> per day) |
|------------------------------|---|---|
| AMF types                    |   |   |
| <i>Acaulospora</i> sp.       | 7.0 b   | 56.4  |
| <i>Glomus</i> sp.            | 8.6 a   | 56.9  |
| <i>Gigasporas</i> sp.        | 6.2 b   | 52.4  |
| AMF dosages (g per seedling) |   |   |
| 0                            | 5.2 b   | 55.6  |
| 5                            | 7.4 a   | 56.7  |
| 10                           | 8.3 a   | 54.4  |
| 15                           | 7.2 a   | 53.3  |
| 20                           | 8.2 a   | 56.2  |
| AMF types                    | *   | ns  |
| AMF dosages                  | *   | ns  |
| AMF types x dosages          | ns  | ns  |

Notes: \*= significant, ns= not significant at  $\alpha=0.05$ ; values followed by the same letter within the same column are not significantly different at  $\alpha=0.05$  according to the DMRT.

### Net Assimilation and Relative Growth Rate

AMF type and dosage significantly affect the net assimilation rate and have an insignificant effect on net relative growth. Also, there was no significant interaction effect on the net assimilation rate and net relative growth between AMF type and dosage. *Glomus* sp. gave the highest net assimilation rate value, 8.6 mg.cm<sup>-1</sup> per day. The AMF dosage treatment of 10 g per seedling yielded the highest value of net assimilation rate, which was 8.3 mg.cm<sup>-1</sup> per day. Still, it was not significantly different from other dosages, except for the control (Table 4).

The treatment with *Glomus* sp. resulted in the highest average net assimilation rate, which correlates with its superior root colonization in gambier seedlings. The effective colonization by *Glomus* sp. enhances water and nutrient uptake, promoting optimal growth of the seedlings. Rahman (2021) noted that healthy plants with well-developed leaves and organs exhibit higher net assimilation rates.

Net assimilation rate reflects a plant's photosynthetic capacity. A larger leaf area allows for greater light interception, thereby increasing photosynthesis and dry weight accumulation. Safitri et al. (2018) emphasized that wider leaves correspond to higher net assimilation rates. Adequate water and nutrient supply slow leaf senescence and sustain assimilation rates. Conversely, insufficient resources lead to competition between old and young leaves, resulting in reduced water and nutrient availability for both.

Regarding the effects of AMF dosage on the net assimilation rate in Gambier seedlings, treatments ranging from 5 to 20 g per seedling all significantly improved net assimilation compared to the control/without AMF. However, increasing the AMF dose beyond 5 g per seedling did not result in further significant increases in net assimilation rate. This suggests that while AMF inoculation enhances photosynthetic efficiency, higher doses beyond a threshold may not provide additional benefits.

### Conclusions

The application of various types of arbuscular mycorrhizal fungi at different doses resulted in a percentage of root colonization by AMF ranging from 60% to 93%. *Glomus* sp. at 5 g per plant has produced a root colonization percentage of 80% and achieved 93% when applied at a rate of 20 g per plant. AMF type and dosage treatment significantly increased shoot and root fresh weight, shoot and root dry weight, net assimilation rate, and relative growth

rate. *Glomus* sp. at a dose of 20 g per seedling increased the biomass of gambier seedlings under ex-coal mine soil.

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