

GROWTH AND PHYSIOLOGICAL RESPONSE OF GAMBIER (*UNCARIA GAMBIR* ROXB.) SEEDLINGS TO FIELD CAPACITY AND APPLICATION OF ARBUSCULAR MYCORRHIZAL FUNGI ESSENTIAL

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Abstract. Optimizing field capacity and Arbuscular Mycorrhizal Fungi (AMF) are essential to improve the growth and drought resistance of gambier seedlings. The study used a completely randomized factorial design with four field capacities (100%, 75%, 50%, and 25%) and three AMF doses (5, 10, and 15 g/plant). The experiment was conducted in the greenhouse and Plant Physiology Laboratory of Universitas Andalas, with five replications and a total of 540 seedlings. Field capacity and AMF significantly affected the growth and physiology of gambier seedlings. Significant interactions occurred in root colonization, root length, root volume, root weight, and proline content. Application of at 5 g/plant resulted in the highest root colonization (68.4%) and root dry weight (1.42 g), and reduced proline accumulation at 75% field capacity. Seedlings at 75% field capacity showed higher plant height (28.6 cm), leaf area (52.3 cm²), shoot dry weight (2.15 g), and net assimilation rate (0.031 g cm² week⁻¹) compared to other treatments. These results confirm that the combination of 75% field capacity and at 5 g/plant can increase growth and improve physiological adaptation of gambier seedlings to drought stress.

Keywords: *colonization, physiology, mycorrhiza, growth, seedlings*

Introduction

The secondary metabolites of gambier (*Uncaria gambir* Roxb.), a tropical plantation commodity, are used in the food, health, and biopharmaceutical sectors, making it a resource. According to the Coordinating Ministry for Economic Affairs of the Republic of Indonesia (2021), Indonesia is the world's largest producer and exporter of gambier, accounting for approximately 80% of global demand (Zainal et al., 2023; Aprizal et al., 2024). Partly due to poor seedling success, productivity tends to decline even though gambier plantations are still growing. Limited water availability exacerbates this issue, particularly during the dry season, when it causes drought stress and hinders seedling growth. For gambier plants in particular, sustainable cultivation requires 'a seedling strategy enhances the seedlings' resistance to environmental stressors, especially drought.

Access to water is essential, particularly during the early phases of gambier nursery development. To ensure ideal seedling growth, adequate water availability promotes photosynthesis, increases cell turgor pressure, and accelerates root development. Drought stress, especially during the dry season, is a significant obstacle in the nursery process. Plants that are unable to adjust to these circumstances may die or sustain irreversible harm (Munns and Millar, 2023; Hartati et al., 2021; Yunita et al., 2020). Drought stress

mitigation strategies are crucial considering climate change, which increases the frequency and intensity of droughts. The use of Arbuscular Mycorrhizal Fungi (AMF) is an innovative solution that can be applied to gambier nurseries to overcome drought stress. Arbuscular mycorrhizal fungi are soil microorganisms that form a symbiotic relationship with plant roots, playing a crucial role in enhancing nutrient uptake, promoting growth, and increasing plant tolerance to drought stress.

In addition to promoting plant growth, mycorrhizal-based technology offers financial benefits, including reduced irrigation expenses on large plantations. The use of mycorrhizae can reduce water requirements during the dry season by enhancing their water-absorbing efficiency, thereby promoting more sustainable management of water resources (Zhou et al., 2024). On marginal soils, including ultisols, it has been demonstrated that applying AMF in solid form by sprinkling it on the planting medium prior to seedling planting improves plant growth and increases root colonization (Zainal et al., 2024; Rahmad et al., 2025).

To improve seedling adaptation to drought conditions, it is essential to comprehend how these two factors interact. Reducing the field capacity in the growing medium is one technique used to test how plants react to drought stress. Using this technique, drought conditions are created by lowering the soil's water content below the field capacity threshold. For instance, four different levels of field capacity were used in the experiment conducted by Rehman et al. (2024): 100%, 75%, 50%, and 25%. Morphological and physiological parameters, including photosynthetic rate, stomatal conductance, and proline concentration, were used in this study to evaluate plant responses to drought stress. These parameters all demonstrated notable differences among *S. ioclados* populations.

The growth of gambier seedlings will depend on the proper dosage of mycorrhizae being applied. According to Yusnawati's (2016) research, the best results for the growth of gambier plants in the young phase occurred 16 weeks after planting on Ultisol planting media when a dose of Arbuscular Mycorrhizal Fungi of 10 g/plant was applied in conjunction with 50 g/plant of gambier leaf pulp compost. The best kind of mycorrhizal fungi to boost gambier seedling growth on former coal mining soil media is *Glomus* sp.

To determine how the application of Arbuscular Mycorrhizal Fungi biological agents influences the application of drought stress on gambier plant seedlings, more research is required on the use of Arbuscular Mycorrhizal Fungi on drought stress conditions in gambier plant seedlings. The study "Growth and Physiological Response of Gambier (*Uncaria gambir* Roxb.) Seedlings to Field Capacity and Application of Mycorrhizal Arbuscular Fungi" was thus carried out by the author. It is anticipated that the findings of this study will significantly contribute to the development of more sustainable and effective gambier nursery practices, thereby promoting higher gambier production and quality on the international market.

Methodology

The study was carried out between February and May 2025 at the Arif Hidrofarm greenhouse in Padang and the Plant Physiology Laboratory at Andalas University's Faculty of Agriculture. The research materials included Udang gambier seeds (Muarapaiti, Lima Puluh Kota), sterile ultisol soil, NPK fertilizer, Arbuscular Mycorrhizas (*Glomus* sp.), and supporting chemicals (KOH 10%, HCl 2%, trypan blue). The experiment employed a factorial Completely Randomized Design (CRD) with two factors: field capacity (100%, 75%, 50%, 25%) and AMF dose (5, 10, 15 g/plant), each

with five replications. There were 60 experimental units, each containing nine plants (3 selective and six destructive), for a total of 540 plants.

Seedlings were planted in polybags filled with ultisol soil, and soil moisture was kept at field capacity through a gravimetric method. Pests and diseases were managed both mechanically and chemically using insecticides (cypermethrin, emamectin benzoate, and abamectin) and fungicides (Dithane M-45). The insecticide used in this study was INDOMA 300SC. The dosage was 1.5 ml per liter of water. Applications were made twice daily in the morning throughout the study. Then the fungicide used in this research was Dithane M-45 with the active ingredient 80% mangkozeb. The dose used is 6 grams per liter of water. How to apply: dissolve 6 grams of Dithane M-45 in 1 liter of water, stir well, add 1 ml of Agristik adhesive, after the mixture is even, spray the fungicide solution onto the parts of the plant that are attacked by the disease. Observations were made every week for four to twelve weeks after the plants were established.

AMF root colonization, plant height (measuring height to growing point), leaf number (count all the leaves that have opened completely), leaf area (measure the total leaf area), root length (maximum root length), and volume were some of the growth parameters observed. The physiological parameters studied were proline content, stomatal density, seedling water content, net assimilation rate (NAR), relative growth rate (RGR), and shoot-to-root ratio. Root colonization was assessed using the root staining method and NAR and LTR were calculated using the formula provided by Gardner et al. (1991).

Results and discussion

Arbuscular mycorrhizas colonization

The analysis results (*Table 1*) show no interaction between field capacity and A dosage on the percentage of AMF root colonization in gambir seedlings 12 weeks after planting. Nonetheless, both factors had a significant impact. AMF root colonization ranged from 40 to 86.66%, classified as moderate to very high (Salim et al., 2020), indicating that all treatments established a root-mycorrhizal symbiosis to varying degrees of success.

Table 1. Percentage of arbuscular mycorrhizal fungal root colonization in gambier seedlings aged 12 weeks post planting

Field capacity	Arbuscular mycorrhizal fungi									Rate
	5 g/plant			10 g/plant			15 g/plant			
	-----%-----									
100%	60.00	±	0.46	53.33	±	1.08	40.00	±	0.44	51.11 d
75%	86.66	±	0.40	80.00	±	0.20	73.33	±	0.38	80.00 a
50%	73.33	±	0.29	73.33	±	0.38	66.66	±	0.45	71.11 b
25%	66.66	±	0.40	73.33	±	0.40	53.33	±	0.21	64.44 c
Rate	71.66			70.00			58.33			
	A			B			C			

Numbers followed by the same capital letter in the same row and lowercase letter in the same column are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

At 75% field capacity, the highest colonization was achieved with AMF doses of 5 g/plant (86.66%) and 10 g/plant (80.00%), indicating that soil moisture at this level is

ideal for hyphal development and arbuscular formation. Colonization decreased at 25% and 50% field capacity due to drought stress, which inhibited hyphal growth, and at 100% field capacity due to overly wet soil conditions, which reduced oxygen in the root zone.

In comparison to higher doses (10 g or 15 g/plant), 5 g of AMF/plant resulted in the highest colonization rate. Excessive doses can lead to spore competition and increase the host plant's carbon load, thereby reducing the effectiveness of colonization.

In general, colonization success is determined by the suitability of AMF species, soil physical and chemical conditions, and moisture levels. These findings are consistent with those of Chareesri et al. (2020) and Wang et al. (2024), who found that optimal moisture promotes external hyphal spread while conditions that are too dry or wet inhibit colonization. Root staining with Trypan Blue revealed the presence of typical AMF structures (hyphae, arbuscules, vesicles, and spores) (Phillips and Hayman, 1970), indicating successful mycorrhizal infection in all treatments.

Root length

The results (Table 2) show a significant interaction between field capacity and AMF dose on the root length of gambier seedlings 12 weeks after planting, indicating that soil moisture levels have a significant impact on mycorrhizal effectiveness. The best treatment was achieved at 50% field capacity with an AMF dose of 10 g/plant, yielding the most extended root length (33.42 cm). This field capacity condition results in a moderate level of water stress, which is consistent with Smith and Read's (2010) discovery that mycorrhizae form an optimal symbiosis in soil conditions that are neither too dry nor too saturated. External hyphal development is maximized under these conditions, resulting in significantly higher water and nutrient absorption.

Table 2. Root length of gambier seedlings with various levels of field capacity and AMF doses at 12 weeks after planting

Field capacity	Arbuscular mycorrhizal fungi		
	5 g/plant	10 g/plant	15 g/plant
	-----cm-----		
100%	19.05 b A	16.84 c B	12.74 c B
75%	17.00 b A	10.67 d B	19.50 ab A
50%	24.79 a B	33.42 a A	21.73 a B
25%	20.19 b A	23.22 b A	17.01 b B
KK =	16.93%		

Numbers followed by the same capital letter in the same row and lowercase letter in the same column are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

Relatively long root lengths were also obtained at 25% field capacity with an AMF dose of 10 g/plant (23.22 cm) and 50% field capacity with an AMF dose of 5 g/plant. This suggests that at low to moderate humidity levels, a sufficient or moderate dose of mycorrhizae is effective in stimulating root growth. However, at a high dose (15 g/plant),

particularly at 50% field capacity (21.73 cm), its effectiveness declines. This phenomenon indicates a saturation point, at which excessive colonization increases the plant's metabolic burden or causes competition among hyphae (Widyaningrum et al., 2023; Brundrett, 2002).

In contrast, 100% field capacity produced shorter root lengths than other field capacities. Saturated conditions reduce oxygen availability in the root zone, inhibiting root respiration and mycorrhizal activity. The 100% field capacity treatment with 15 g/plant AMF produced the shortest root length at 12.74 cm. A similar condition was observed at 75% field capacity with an AMF dose of 10 g/plant (10.67 cm), indicating that suboptimal humidity can reduce the effectiveness of mycorrhizal symbiosis even at a relatively high dose

Root volume

The results (Table 3) revealed a significant interaction between field capacity and AMF dosage on root volume in gambier seedlings grown for 12 weeks after planting. The most effective treatment was obtained at 50% field capacity with an AMF dosage of 10 g/plant, resulting in the highest root volume of 7.35 ml. This result suggests that moderate mycorrhizal dosages applied under ideal soil moisture conditions can significantly improve root system development. A similar effect was observed at 25% field capacity with a dosage of 10 g/plant (6.73 ml), indicating that mycorrhizal fungi can still assist plants in adapting to mild water stress.

Table 3. Root volume of gambier seedlings with various levels of field capacity and AMF doses at 12 weeks post planting

Field capacity	Arbuscular mycorrhizas		
	5 g/plant	10 g/plant	15 g/plant
	-----ml-----		
100%	4.45 b B	5.57 c A	5.23 b A
75%	6.73 a B	5.91 bc B	6.97 a A
50%	6.24 a B	7.35 a A	5.87 b B
25%	6.22 a A	6.73 ab A	5.45 b B
KK =	13.66%		

Numbers followed by the same capital letter in the same row are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

However, even with increased AMF dosage, 100% field capacity resulted in lower root volume, indicating that water-saturated conditions limit the effectiveness of mycorrhizal colonization. At a high dosage (15 g/plant) and 75% field capacity, root volume was relatively high (6.97 ml), but not significantly different from several other treatments, indicating a colonization saturation point that can reduce nutrient uptake efficiency. Physiologically, increased root volume is linked to mycorrhizae's ability to expand the root exploration zone and increase water and nutrient absorption, particularly under moderate water stress (Smith and Read, 2010).

Proline content

The findings (Table 4) reveal a significant interaction between field capacity and AMF dosage on the proline content of gambier seedlings 12 weeks after planting. The highest proline content was recorded at 50% field capacity with an AMF dosage of 15 g/plant (364.49 $\mu\text{mol/g}$), and the lowest value was at 100% field capacity with a dosage of 15 g/plant (4.81 $\mu\text{mol/g}$). The rise in proline content as field capacity falls suggests a plant adaptation mechanism to water stress. AMF promotes proline metabolism by increasing water and nutrient uptake, allowing plants to withstand stress.

Table 4. Proline content of gambier plant seedlings with various levels of field capacity and AMF doses at 12 weeks post-harvest age

Field capacity	Arbuscular mycorrhizal fungi		
	5 g/plant	10 g/plant	15 g/plant
	---- $\mu\text{mol/g}$ fresh weight-----		
100%	24.3848 c B	71.2294 b A	4.8128 c C
75%	75.7213 bc B	123.5284 b A	75.4005 b B
50%	128.9829 b C	131.5497 b B	364.4890 a A
25%	247.0568 a B	205.0251 a C	329.4329 a A
KK =	13.08%		

Numbers followed by the same capital letter in the same row are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

Proline content increased as field capacity decreased, indicating the adaptive response of gambier plants to water stress. High AMF treatment at low field capacity (50-25%) resulted in greater proline accumulation than other treatments, indicating that AMF can optimize plant metabolism under stress conditions. The increased proline accumulation in AMF-inoculated plants demonstrates the critical role of mycorrhizae in maintaining osmotic balance, expanding root uptake, and increasing metabolic efficiency during water stress.

The interaction between AMF dose and field capacity significantly affected the proline content of gambier seedlings. The 15 g/plant AMF treatment at 50% field capacity resulted in the highest accumulation (364.49 $\mu\text{mol/g}$). The increase in proline content at low field capacity suggests that AMF supports a physiological adaptation mechanism in plants to water stress by increasing nutrient and water uptake. This finding is consistent with previous research on various plants that has confirmed the role of mycorrhizae in increasing plant resistance to abiotic stresses.

Stomata density

The results (Table 5) show that field capacity and AMF dose individually had a significant effect on the stomatal density of gambier seedlings 12 weeks after planting. However, there was no interaction between the two. Stomatal density was highest at 100% field capacity (175.05 mm^2) and lowest at 25% field capacity (103.74 mm^2). The AMF dose

treatment of 10 g/plant resulted in the highest stomatal density (155.20 mm²), which differed significantly from the dose of 15 g/plant but not from the dose of 5 g. The decrease in stomatal density at low field capacity is a plant adaptation mechanism that reduces water loss through transpiration, although it may also reduce photosynthesis. The role of AMF is evident in increasing water and nutrient absorption, thereby supporting stomatal formation at optimal doses; however, excessive doses can burden plant metabolism.

Table 5. Stomata density of gambier seedlings with various levels of field capacity and AMF doses at 12 weeks post planting

Field capacity	Arbuscular mycorrhizal fungi									Rate	n
	5 g/plant			10 g/plant			15 g/plant				
	-----mm ² -----										
100%	201	±	18	185	±	24	138	±	13	175.05 a	3
75%	166	±	16	158	±	23	123	±	29	148.82 b	3
50%	104	±	10	153	±	29	116	±	41	124.44 b	3
25%	91	±	20	109	±	16	111	±	40	103.75 c	3
Rate	140.6			151.5			122.0				
	A			A			B				
KK=	3.34%										

Numbers followed by the same capital letter in the same row are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

The stomatal density of gambier seedlings at 12 weeks post-plant age was significantly affected by field capacity and AMF dose, but there was no interaction between the two. Stomatal density was highest at 100% field capacity (175.05 mm²) and lowest at 25% field capacity (103.74 mm²). This difference suggests that water availability influences stomatal formation, whereas water stress at low field capacity inhibits the differentiation of leaf epidermal cells. AMF at a dose of 10 g/plant resulted in the highest density (155.20 mm²), indicating that moderate doses can improve nutrient and water absorption without causing excessive metabolic burden.

These findings are consistent with previous research by Arniputri et al. (2025), who found that mycorrhizal application increases plant physiological activity and water use efficiency. Under low field capacity conditions, partial stomatal closure is a physiological adaptation that reduces water loss through transpiration. In contrast, increased AMF colonization helps maintain cell turgor pressure, promoting stomatal opening. These findings highlight the importance of a suitable AMF dosage in enabling plant adaptation to water stress and maintaining photosynthetic efficiency.

Seed water content

The results (Table 6) show that there was no significant interaction between AMF dosage and field capacity on the water content of gambier seedlings, implying that the observed effects are due to individual treatments for each factor. Field capacity had a significant impact on seedling water content, with 75% producing the highest water content (78.30%). This value was significantly different from 100% field capacity, which was achieved at only 73.56%. Field capacities of 50% and 25% were at intermediate levels (75.51% and 74.67%, respectively).

Table 6. Water content of gambier plant seeds with various levels of field capacity and AMF doses at 12 weeks after planting

Field capacity	Arbuscular mycorrhizal fungi									Rate	n
	5 g/plant			10 g/plant			15 g/plant				
	-----(%)----										
100%	75.13	±	3.44	72.42	±	3.23	73.12	±	4.00	73.56 c	5
75%	79.99	±	3.22	80.01	±	2.93	74.88	±	1.19	78.29 a	5
50%	75.50	±	1.40	76.84	±	1.40	74.19	±	1.82	75.51 b	5
25%	74.37	±	1.92	75.56	±	0.13	74.07	±	2.58	74.67 b	5
Rate	76.25 A			76.21 A			74.07 C				
KK=	3.34%										

Numbers followed by the same capital letter in the same row and lowercase letter in the same column are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

This difference suggests that moderate to moderate water availability (75% KL) provides the best physiological conditions for gambier plants. Due to the maintained balance of water and oxygen in the root zone, these conditions enable more efficient nutrient absorption, photosynthesis, and transpiration. Conversely, at 100% field capacity, overly saturated soil conditions inhibit root respiration due to low oxygen availability (hypoxia), thus disrupting the stability of plant tissue water content.

Zeyliger and Ermolaeva (2025) supported these findings, stating that optimal soil moisture distribution is crucial to the success of precision farming systems, as excessive soil moisture can lead to a moisture imbalance that impairs the stability of plant tissue water content. On the other hand, AMF dosage treatments had no significant effect on water content; however, data trends indicated that a dosage of 10 g/plant produced the highest average water content (76.21%), compared to doses of 5 g (76.25%) and 15 g (74.07%). This result suggests that the presence of AMF in moderate doses can still help to maintain plant water status. According to Manurung et al. (2015), excessively high soil moisture can reduce the effectiveness of mycorrhizae in supporting nutrient and water absorption due to limited oxygen in the root zone. Conversely, Amrizal et al. (2021) emphasized that AMF application under appropriate moisture conditions can increase the efficiency of water and nutrient absorption, ultimately supporting optimal plant growth.

The optimal tissue water content in gambier seedlings is influenced by a combination of appropriate soil moisture conditions and the presence of AMF at the appropriate dose. A 75% field capacity provides the best conditions because it balances water availability and oxygen supply. At the same time, moderate doses of AMF promote efficient water uptake without putting an excessive metabolic burden on the plant. This suggests that precise soil moisture management, combined with appropriate AMF applications, can be an effective strategy for enhancing the physiological status of gambier seedlings, enabling optimal growth under various environmental conditions.

Plant height

The analysis results showed no interaction between field capacity and AMF dosage on gambier seedling height increase at 12 weeks after planting (Table 7). However, the single

AMF dose treatment had a significant effect, while the field capacity treatment did not show a significant effect. However, there was a trend toward increased seedling height at low field capacity, with the highest average value at 25% field capacity (26.77 cm).

The increase in seedling height at the 5 g AMF dose/plant (28.78 cm) indicates that mycorrhizal inoculation at the appropriate dosage level can increase root colonization, expand nutrient uptake areas, and improve water distribution within plant tissues. On the other hand, application of higher doses (10–15 g/plant) has the potential to reduce the effectiveness of symbiosis due to competition between spores, disruption of physiological balance, and metabolic imbalance in the root tissue (Smith and Read, 2010).

Plant morphological and physiological parameters also contribute to increased seedling height. A larger leaf number and leaf area expand the photosynthetic surface area, increasing photosynthate accumulation and supporting stem elongation (Chen et al., 2021). Furthermore, a root system with greater length and volume enhances the plant's ability to optimally absorb water and nutrients, which in turn enhances vegetative growth (Bengough et al., 2011). Therefore, the combination of appropriate field capacity and the correct AMF dosage is crucial for supporting gambier seedling development during the early stages of growth.

Table 7. Height of gambier seedlings with various levels of field capacity and AMF doses at 12 weeks post planting

Field capacity	Arbuscular mycorrhizal fungi						Rate	n
	5 g/plant		10 g/plant		15 g/plant			
	-----cm-----							
100%	28.87	± 2.03	22.53	± 1.73	21.87	± 3.58	24.42	15
75%	29.13	± 4.98	25.20	± 3.88	22.47	± 1.92	25.60	15
50%	29.60	± 4.20	25.53	± 4.50	23.07	± 1.75	19.55	15
25%	30.27	± 1.83	25.73	± 1.68	24.33	± 2.09	26.78	15
Rate	29.47		24.75		22.93			
KK=	A		B		C			
	10.73%							

Numbers followed by the same capital letter in the same row are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

Based on (*Fig. 1*), the growth graph of gambier seedlings from the 4th to the 12th week after planting shows that all treatments, both field capacity and dosage of Arbuscular Mycorrhizas, produced a continuously increasing growth trend. This indicates that the vegetative growth phase has not yet reached its optimal point, so that growth is likely to continue if the observation is extended. However, the growth rate between treatments shows quite significant variation. The 25% field capacity treatment with an AMF dose of 5 g/plant showed the most significant and consistent increase in height, exceeding that of the other treatments throughout the observation period. In contrast, the treatment with an AMF dose of 15 g/plant, especially at 50% and 100% field capacity, showed a relatively slower growth rate, indicating that increasing AMF doses is not always directly proportional to increased seedling growth.

This graphic visualization provides a more comprehensive picture of the growth response of gambier seedlings to the combination of field capacity and AMF doses. These

findings align with Erdayana's (2021) report, which states that excessive application of AMF to cocoa seedlings can reduce root colonization efficiency, thus providing no additional benefit to plant growth. Furthermore, low field capacity (25%) combined with low AMF doses has been shown to provide an optimal growth response. This phenomenon can be explained by the plant's adaptation mechanism to mild water stress, where physiological stress, to a certain extent, can stimulate root and stem. Therefore, this graph confirms the interpretation of the table data that an AMF dose of 5 g/plant is the most effective treatment in supporting gambier seedling height growth, especially under low field capacity conditions.

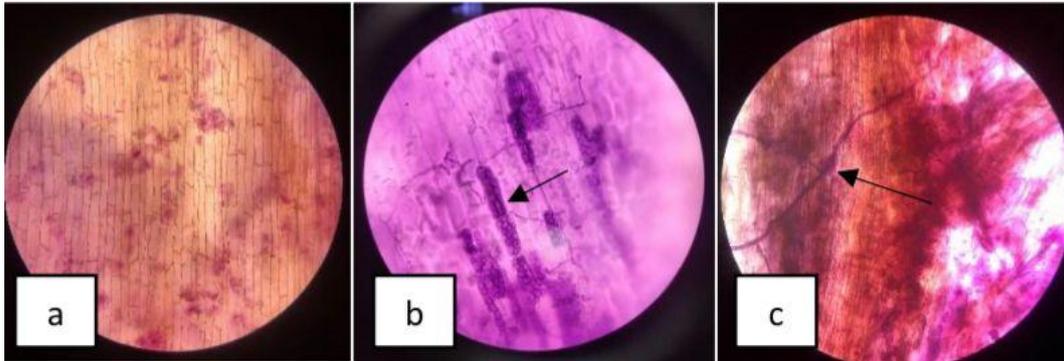


Figure 1. AMF colonization on the roots of 12-week-old gambier seedlings at various field capacities and AMF doses. Hyphae, arbuscules, and vesicles are visible, indicating successful colonization. a) Uncolonized roots, b) Spores, and c) Internal hyphae. and the applied stain should be indicated; 400 x magnification microscope. stained by immersing it in 0.05% lactophenol trypan blue for a few moments

Number of leaves

Leaf number is a crucial indicator in assessing a plant's photosynthetic capacity, as it directly influences light absorption, gas exchange, and transpiration. The analysis showed no significant interaction between field capacity and the dose AMF on leaf number in gambier seedlings at 12 weeks after planting. This indicates that plant responses to these two factors are independent.

Table 8 shows that the single-field capacity treatment had no significant effect on leaf number. However, the highest average leaf number was obtained at 75% field capacity (33.49 leaves) compared to 25%, 50%, or 100%. This condition indicates that soil moisture at 75% field capacity is at an optimal level to support physiological activities, including leaf cell differentiation and enlargement, without causing water saturation that could disrupt root aeration (Mergby et al., 2021). Balanced moisture conditions are believed to contribute to better photosynthetic performance (Patel et al., 2022).

The AMF dosage treatment showed a significant effect on the number of leaves in gambier seedlings. Application of a dose of 5 g/plant produced the highest yield, at 34.18 leaves, significantly higher than doses of 10 g/plant (28.45 leaves) and 15 g/plant (26.56 leaves). The optimal AMF dosage can increase root colonization, improve nutrient absorption, and support vegetative growth. Appropriate dosage can also prevent the dominance of particular mycorrhizal species, thus maintaining microbial balance in the root zone (Hazra et al., 2021) and increasing plant physiological efficiency, especially in soil media with low fertility (Alfarabbi, 2024).

Table 8. Number of leaves of gambier seedlings with various levels of field capacity and AMF doses at 12 weeks post planting

Field capacity	Arbuscular mycorrhizal fungi									Rate	n
	5 g/plant			10 g/plant			15 g/plant				
	-----sheet-----										
100%	33.19	±	2.70	26.92	±	2.59	26.43	±	4.37	28.85	15
75%	35.05	±	7.37	30.74	±	4.73	25.70	±	3.24	30.50	15
50%	34.49	±	6.44	29.77	±	6.37	24.40	±	2.26	22.16	15
25%	34.00	±	6.12	26.35	±	4.04	29.69	±	2.55	30.01	15
Rate	34.18 A			28.45 B			26.56 B				
KK=	15.09%										

Numbers followed by the same capital letter in the same row are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

Total leaf area

The analysis results (Table 9) show no interaction between field capacity and Arbuscular Mycorrhizal Fungi (AMF) dosage on the leaf area of gambier seedlings at 12 weeks after planting. However, a single AMF dosage treatment had a significant effect. Application of AMF at a dosage of 5 g/plant resulted in the highest leaf area, with an average value of 207.87 cm², while doses of 10 g/plant and 15 g/plant yielded lower results. Meanwhile, the single field capacity treatment showed no significant effect on this parameter.

Table 9. Total leaf area of gambier seedlings with various levels of field capacity and AMF doses at 12 weeks post planting

Field capacity	Arbuscular mycorrhizal fungi									Rate	n
	5 g/plant			10 g/plant			15 g/plant				
	-----cm ² -----										
100%	217.55	±	25.39	185.77	±	10.13	166.39	±	20.78	189.90	5
75%	207.54	±	41.12	204.48	±	16.68	161.70	±	12.36	191.24	5
50%	204.50	±	33.19	199.26	±	35.80	153.16	±	5.01	139.23	5
25%	201.89	±	33.66	180.98	±	18.93	175.36	±	8.07	186.08	5
Rate	207.87 A			192.62 B			164.15 C				
KK=	13.08%										

Numbers followed by the same capital letter in the same row are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

The increase in leaf area in the 5 g/plant AMF treatment was due to increased mycorrhizal colonization of the roots, which improved nutrient uptake, particularly phosphorus, and water use efficiency. Lopita et al. (2024) reported that AMF colonization can increase phosphate uptake severalfold compared to non-mycorrhizal plants, while Sylvia et al. (2005) stated that optimal AMF infection is positively related to increased

leaf area and plant biomass. Phosphorus plays a crucial role in the formation of ATP and other essential organic compounds for cell division and growth (Brundrett, 2009).

Arbuscular mycorrhizal fungi are also known to increase the production of growth hormones such as cytokinins and auxins, which support leaf development (Tang et al., 2022). The observed improvements in root length and volume in the AMF treatment support the hypothesis that increased leaf area is closely related to root system development. This mechanism also helps plants mitigate the impact of water stress by maintaining cell turgor, particularly at medium field capacity (75% and 50%), which showed higher leaf area values compared to the extreme treatments.

Net assimilation rate

The analysis results (Table 10) indicate that there was no interaction between the dosage of Arbuscular Mycorrhizal Fungi (AMF) and field capacity on the net assimilation rate (NAF) of gambier seedlings at 12 weeks after planting. However, the single-field capacity treatment had a significantly different effect, whereas the AMF dosage did not show a significant difference. NAF is a crucial parameter for assessing the net photosynthetic efficiency of plants in producing dry biomass per unit leaf area per unit time.

Table 10. Net assimilation rate of gambier plant seeds with various levels of field capacity and AMF doses at 12 weeks post planting

Field capacity	Arbuscular mycorrhizal fungi									Rate	n
	5 g/plant			10 g/plant			15 g/plant				
	-----mg/cm ² /week-----										
100%	0.20373	±	0.03583	0.20700	±	0.02600	0.17336	±	0.02140	0.1947 c	5
75%	0.26153	±	0.03461	0.23867	±	0.04469	0.21938	±	0.01231	0.2399 a	5
50%	0.23180	±	0.04337	0.22377	±	0.02883	0.22068	±	0.01074	0.2254 b	5
25%	0.21003	±	0.01677	0.23024	±	0.02742	0.21069	±	0.01925	0.2170 b	5
Rate	0.22677			0.22492			0.20603				
	A			A			B				
KK=	13.16%										

Numbers followed by the same capital letter in the same row and the same lower-case letter in the same column are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

The 75% field capacity treatment produced the highest NAF, at 0.2399 mg/cm²/week, followed by the 50% field capacity treatment (0.2254 mg/cm²/week) and the 25% field capacity treatment (0.2170 mg/cm²/week). While 100% field capacity showed the lowest value (0.1947 mg/cm²/week). This indicates that moderate soil moisture provides optimal conditions for photosynthetic activity. At 100% field capacity, excess water saturation can potentially cause hypoxia in the root zone, which inhibits root respiration and reduces photosynthetic efficiency (Flexas et al., 2006). Conversely, too low a field capacity can cause drought stress, reduce cell turgor, close stomata, and inhibit CO₂ diffusion into the leaf mesophyll.

These results align with research by Lutfi et al. (2023), which states that moderate soil water content can increase photosynthetic rates in C₃ and C₄ plants, while conditions that

are too dry or too wet reduce photosynthetic activity. Furthermore, conditions at 75% field capacity also support mycorrhizal activity, which increases nutrient uptake, particularly phosphorus, necessary for ATP formation and energy transport in photosynthesis (Brundrett, 2009).

Graphs of the net assimilation rate (NAB) of gambier seedlings from 4 to 12 weeks after planting are shown in *Figures 2 and 3*. Generally, there are variations in the trend of increasing and decreasing NAB during the observation period, although the plant leaf area experienced relatively stable growth dynamics. In some field capacity treatments and AMF doses, NAB decreased in the initial phase (4–6 to 8–10 weeks after planting) and increased again towards the end of the observation period. This phenomenon indicates that increases in leaf area are not always directly proportional to increases in NAB.

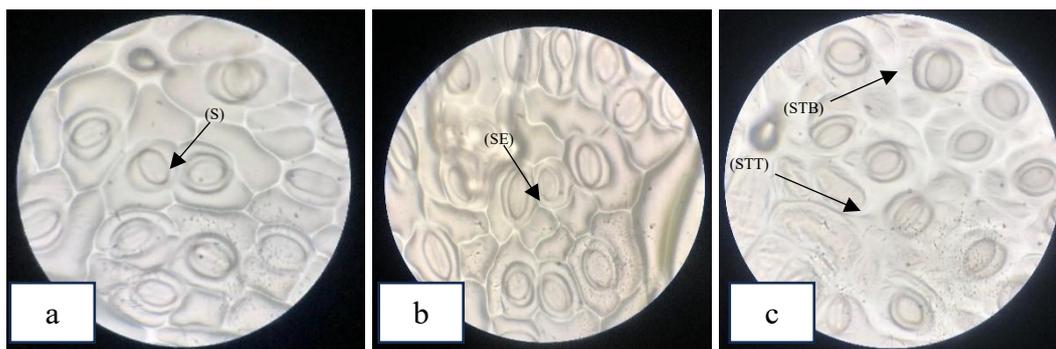


Figure 2. The stomata shape on 12-week-old gambier seedling leaves was observed under 40× microscope magnification at various levels of field capacity and AMF doses. Stomata (S), Epidermal Cells (SE), Open Stomata (STB), Closed Stomata (STT)

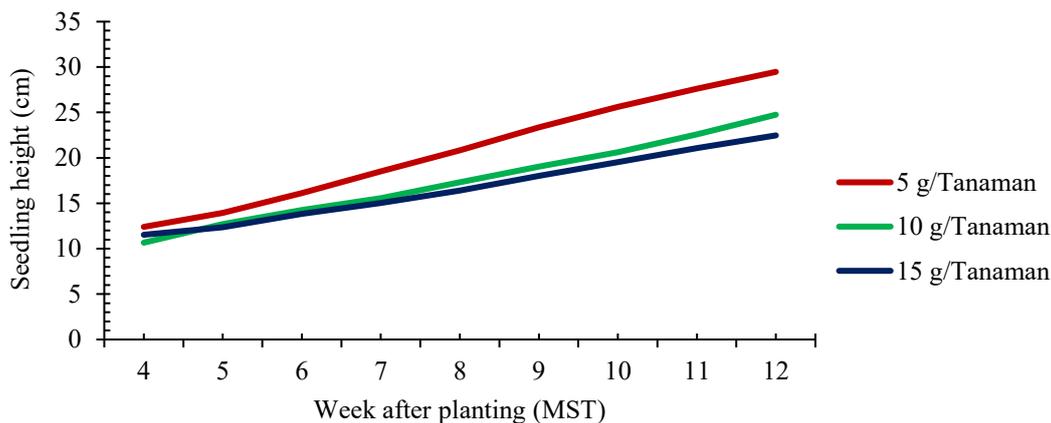


Figure 3. The growth rate of gambier plant seedlings at various AMF dose levels started from 4 MST to 12 MST

From weeks 10 to 12 after planting (WAP), the LAB increased again. This can be attributed to the physiological maturity of the leaves formed in the previous phase, where photosynthetic capacity reaches optimal levels as chlorophyll content and chloroplast efficiency increase. Furthermore, colonization by Arbuscular Mycorrhizal Fungi (AMF)

begins to reach a functional stage after passing through the pre-symbiotic and early colonization phases, which typically occur from weeks 8 to 10 after planting (Bücking and Kafle, 2015). At this stage, arbuscules and vesicles in the roots have played an active role in increasing the absorption of phosphorus, an essential element in the formation of ATP, which is required for the regeneration of RuBP in the Calvin cycle.

The balance between photosynthate sources and sinks also influences fluctuations in photosynthate production. At moderate soil moisture levels (50–75% of field capacity) and optimal AMF doses, leaves can serve as efficient sources of photosynthate, while sink organs, such as stems and roots, can optimally utilize carbohydrates for growth. Conversely, stress conditions caused by extreme humidity or inappropriate AMF doses lead to physiological disturbances, resulting in decreased photosynthate efficiency, even when the leaf area is large.

Relative growth rate

The results of the analysis of variance (*Table 11*) indicate that there was no significant interaction between AMF dosage and field capacity on the relative growth rate (LTR) of gambier seedlings at the 5% significance level. However, the field capacity treatment had a significant effect, while the AMF dosage treatment did not show a significant effect.

Table 11. Relative growth rate of gambier seedlings with various levels of field capacity and AMF doses at 12 weeks post planting

Field capacity	Arbuscular mycorrhizal fungi			Rate	n
	5 g/plant	10 g/plant	15 g/plant		
	----g/week----				
100%	0.54755 ± 0.10532	0.51205 ± 0.06000	0.45435 ± 0.05325	0.5046 c	5
75%	0.70568 ± 0.07088	0.61095 ± 0.09918	0.51932 ± 0.02591	0.6120 a	5
50%	0.59283 ± 0.09667	0.55459 ± 0.06481	0.53217 ± 0.02409	0.5599 b	5
25%	0.53018 ± 0.03935	0.56278 ± 0.06175	0.53675 ± 0.04558	0.5432 b	5
Rate	0.59406 A	0.56009 A	0.51064 B		
KK=	12.16%				

Followed by the same capital letter in the same row and the same lower-case letter in the same column are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

The highest LTR was achieved at 75% field capacity, with a value of 0.6120 g/week, significantly higher than the 100% field capacity, which reached only 0.5046 g/week. Field capacities of 50% and 25% showed lower LTR values, and were not significantly different from each other, at 0.5599 g/week and 0.5432 g/week, respectively. This indicates that balanced soil moisture conditions at 75% field capacity can optimally support plant physiological processes by providing sufficient oxygen availability in the root zone, maintaining cell turgor pressure, and maximizing the distribution of photosynthates for vegetative growth.

The decrease in LTR values at 100% field capacity is caused by water-saturated conditions that inhibit root respiration, reduce nutrient uptake efficiency, and reduce photosynthetic activity. Meanwhile, a 25–50% field capacity causes drought stress that

limits water availability, inhibits stomatal opening, and reduces cell turgor, resulting in slowed cell division and elongation. These findings emphasize that soil moisture balance is a key factor in supporting the vegetative growth of gambier seedlings, especially in the early phase when the plant's metabolic needs for water and nutrients are at a high level (Figs. 4 and 5).

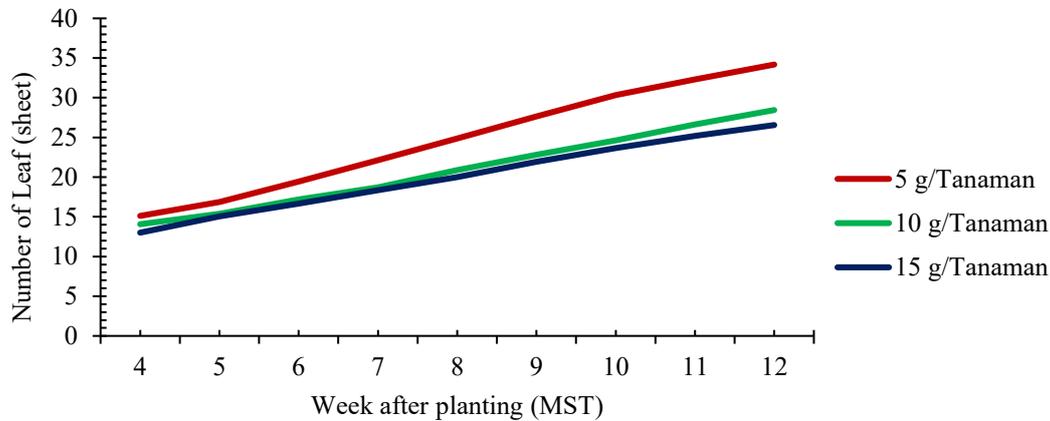


Figure 4. Growth rate of the number of leaves of gambier seedlings in various AMF dose levels, starting from 4 MST to 12 MST

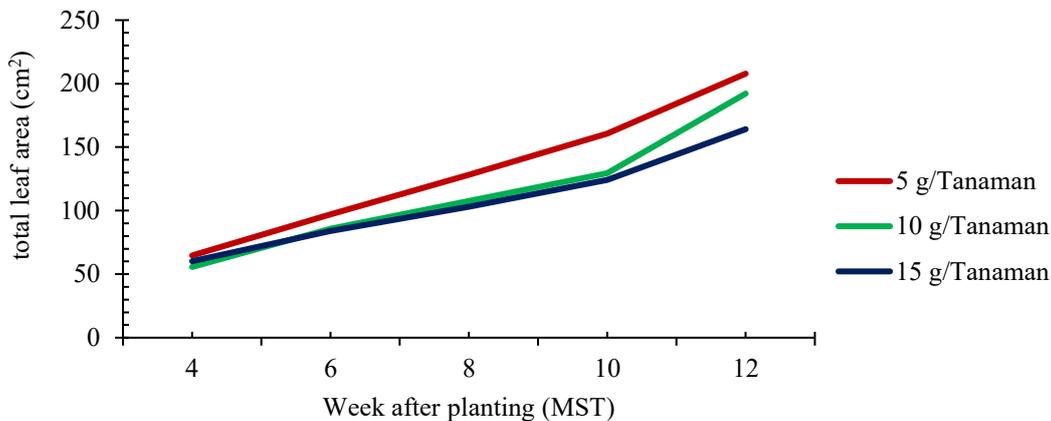


Figure 5. The growth rate of leaf area of gambier seedlings in treatments of various levels of field capacity and multiple levels of AMF doses from 4 MST to 12 MST

The effectiveness of AMF is significantly influenced by the balance of soil moisture and the applied dose. At medium field capacity with moderate doses, root colonization, nutrient supply, and photosynthetic activity are optimal, resulting in increased leaf area, canopy weight, and the accumulation of new biomass. Moderate humidity also maintains stomatal openness and efficient CO₂ assimilation, thus supporting chlorophyll formation and leaf expansion. Conversely, extreme humidity conditions (too low or too high) cause physiological stress, inhibit cell division and enlargement, and reduce the efficiency of AMF symbiosis. These findings confirm that proper soil moisture management is a key factor in maximizing the role of AMF in supporting the vegetative growth of gambier seedlings.

Root crown ratio

Statistical analysis at the 5% significance level showed no interaction between field capacity and the dose of Arbuscular Mycorrhizal Fungi (AMF) on the shoot-root ratio of gambier seedlings at 12 weeks after planting (Table 12). However, a single AMF dose treatment had a significant effect, while field capacity did not. The highest average shoot-root ratio was obtained at an AMF dose of 5 g/plant (4.48), followed by a dose of 10 g/plant (4.45). In contrast, a dose of 15 g/plant showed the lowest value (4.22). Significant differences occurred between low-medium and high doses, confirming that moderate AMF application is more effective in supporting balanced biomass allocation between shoots and roots.

A balanced shoot-root ratio reflects the plant's physiological efficiency in distributing photosynthetic output and resources to support stable growth. At low to moderate doses, AMF colonization occurs optimally without placing an excessive metabolic burden on the plant. The external hyphae of AMF play a crucial role in expanding the root's absorption surface area, thereby ensuring that water and nutrient needs are met without requiring excessive root growth. As a result, a greater proportion of biomass can be allocated to canopy growth, which supports the expansion of the photosynthetic area and optimal formation of young leaves.

This finding aligns with the report by Hardiyanti et al. (2025), which stated that the application of AMF at low doses can enhance the vegetative growth efficiency of oil palm seedlings under drought stress conditions. Similar results were also reported by Rizki et al. (2025) on mini lamtoro plants (*Desmanthus virgatus*) in saline soil, where AMF play a role in maintaining a stable shoot-root ratio by increasing nutrient absorption. Thus, in the context of Gambier nurseries, the application of AMF at low to moderate doses has been proven to increase the efficiency of biomass allocation, support photosynthetic activity, and maintain the balance of vegetative growth, thereby supporting plant productivity in the next phase.

Table 12. Root crown ratio of gambier seedlings with various levels of field capacity and AMF doses at 12 weeks post harvest

Field capacity	Arbuscular mycorrhizal fungi									Rate	n
	5 g/plant			10 g/plant			15 g/plant				
	----gram----										
100%	4.29	±	1.04	4.30	±	0.03	4.14	±	0.12	4.24 b	5
75%	4.71	±	0.23	4.40	±	0.17	4.23	±	0.38	4.45 a	5
50%	4.41	±	0.31	4.66	±	1.00	4.42	±	0.52	4.49 a	5
25%	4.51	±	0.47	4.45	±	0.24	4.10	±	0.06	4.35 a	5
Rate	4.48			4.45			4.22				
	A			A			B				
KK=	7.19%										

Numbers followed by the same capital letter in the same row and lowercase letter in the same column are not significantly different according to the Duncan Multiple Range Test at the 5% significance level

Conclusion

Significant interactions occurred in root colonization, root length, root volume, root weight, and proline content. Application of AMF 5 g/plant resulted in the highest root

colonization (68.4%) and root dry weight (1.42 g), and reduced proline accumulation at 75% field capacity. Seedlings at 75% field capacity showed higher plant height (28.6 cm), leaf area (52.3 cm²), shoot dry weight (2.15 g), and net assimilation rate (0.031 g cm² week⁻¹) compared to other treatments. These results confirm that the combination of 75% field capacity and AMF 5 g/plant can increase growth and improve physiological adaptation of gambier seedlings to drought stress.

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