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Original Article

Arbuscular mycorrhizal fungi, phosphorus and organic residues in *Peltophorum dubium* (Spreng.) Taub. seedlings

Fungos micorrízicos arbusculares, fósforo e resíduos orgânicos em mudas de *Peltophorum dubium* (Spreng.) Taub.

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Abstract

Studies have underscored a growingdemand for innovative practices in the cultivation of seedlings from forest species, with a notable emphasis on the utilization of organic waste, inoculation with arbuscular mycorrhizal fungi (AMF), and phosphate fertilization. This study aimed to evaluate the impact of organic residues, inoculation with AMF, and phosphorus on the growth and quality of *Peltophorum dubium* (Spreng.) Taub. seedlings. Two independent experiments were conducted. In Experiment I, treatments included inoculation with various AMF species: control (without inoculation), *Clareoideoglomus etunicatum*, *Rhizophagus heterosporum*, *Rhizophagus clarum*, and MIX (a combination of the three AMF species), all in conjunction with varying doses of phosphorus (0, 60, 120, 180, and 240 mg kg^{-1} soil). In Experiment II, treatments comprised a control group (without AMF) and inoculation with *Glomus clarum*, *Gigaspora margarita*, *Gigaspora albida*, *Clareoideoglomus etunicatum*, and MIX (a combination of the four AMF species), each associated with four substrates: S1) soil + coarse sand – SCS, S2) SCS + poultry manure, S3) SCS + cattle manure, and S4) SCS + sheep manure. *Peltophorum dubium* seedlings exhibited heightened growth with the inoculation of *R. heterosporum*, *R. clarum*, and MIX. Positive responses were observed in seedlings when exposed to organic residues, particularly sheep manure, resulting in increased biomass production and enhanced Dickson quality index. The AMF inoculation, specifically with *R. heterosporum*, *R. clarum*, and MIX, provided optimal growth conditions for *P. dubium* seedlings. Remarkably, the utilization of organic residues, notably substrates with chicken manure and cattle manure, exerted substantial positive effects on both growth and quality of *P. dubium* seedlings.

Keywords: alternative substrates, '*canafístula*', Fabaceae, poultry manure, symbiosis.

Resumo

Os trabalhos têm mostrado demanda crescente na busca de práticas para produção de mudas de espécies florestais, destacando tendências no aproveitamento de resíduos orgânicos, inoculação com fungos micorrízicos arbusculares (FMA) e adubação fosfatada. Objetivou-se por meio deste estudo avaliar o efeito de resíduos orgânicos, inoculação com fungos micorrízicos arbusculares (FMA) e fósforo no crescimento e qualidade de mudas de *Peltophorum dubium* (Spreng.) Taub. Dois experimentos foram realizados independentes. Para o Experimento I, os tratamentos consistiram na inoculação com as espécies de FMA: controle (sem-inoculado), *Clareoideoglomus etunicatum*, *Rhizophagus heterosporum*, *Rhizophagus clarum* e MIX (mistura das três espécies de FMA), todos associados a doses de fósforo (0, 60, 120, 180 e 240 mg kg^{-1} de solo). Para experimento II, os tratamentos consistiram em uma testemunha (sem FMA) e inoculação com *Glomus clarum*, *Gigaspora margarita*, *Gigaspora albida*, *Clareoideoglomus etunicatum* e MIX (mistura das quatro espécies de FMA), associados a quatro substrato: S1) solo + areia grossa – SCS, S2) SCS + esterco de aves, S3) SCS + esterco bovino e S4) ECS + esterco ovino. Mudas de *P. dubium* apresentaram maior crescimento com a inoculação de *R. heterosporum, R. clarum* e MIX. As mudas responderam positivamente ao uso de resíduos orgânicos, principalmente esterco ovino, aumentando a produção de biomassa e o índice de qualidade de Dickson. Mudas de *P. dubium* se beneficiaram da inoculação com FMA, sendo que os inóculos *R. heterosporum*, *R. clarum* e MIX proporcionaram as melhores condições de crescimento. Mudas de *P. dubium* responderam expressivamente no crescimento e qualidade ao uso de resíduos orgânicos, principalmente ao esterco de galinha e bovino.

Palavras-chave: substratos alternativos, canafístula, *Fabaceae*, cama de frango, simbiose.

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1. Introduction

Seedling production plays a pivotal role in determining forest quality, serving objectives that span commercial exploitation and conservation initiatives. Generally, areas designated for cultivating forest species often exhibit low fertility and suboptimal soil management practices, thereby compromising the productive potential inherent in these species. Araujo et al. (2018) underscore the necessity for studies aimed at producing high-quality seedlings and reducing costs, particularly in response to the escalating demand for environmental projects involving native species to rehabilitate degraded areas.

Among the myriad promising practices associated with the production of seedlings for forest species, noteworthy trends include the utilization of organic residues, inoculation with arbuscular mycorrhizal fungi (AMF), and phosphate fertilization. Arbuscular mycorrhizal fungi (AMF) present a viable alternative for seedling production, enhancing plant capacity to absorb water and nutrients from the soil (Silva et al., 2022; Nardi et al., 2023). Additionally, when coupled with the host species, they augment the root surface area, thereby fostering heightened metabolism and overall plant growth (Pereira et al., 2022; Nardi et al., 2022).

Within mycorrhizal associations, one of the primary soil nutrients absorbed by AMF is phosphorus (Lima et al., 2020), a vital element for metabolism and growth but often limited in availability to plants due to its fixation in soils of tropical regions (Pereira et al., 2021). However, research on the association between AMF and phosphorus remains inadequate for *P. dubium* seedlings. Studies have been undertaken to elucidate the dependency of AMF inoculation for various tree species, particularly in the context of seedling formation (Silva et al., 2017).

Another sustainable practice in nursery management involves incorporating organic residues for formulating substrates. When introduced, these residues contribute to physicochemical attributes of the soil, thereby favoring plant nutrition and growth (Araújo et al., 2018; Santos et al., 2020a). The choice of substrate is critical for the growth and quality of seedlings in the nursery (Santos et al., 2020b). However, the literature lacks registered information on the association of AMF with substrates based on organic materials for *P. dubium* seedlings.

We hypothesize that the use of bioinputs, represented here by AMF, associated with phosphate fertilization or organic residues, can augment the initial growth and quality of *P. dubium* seedlings. Consequently, our objective is to evaluate the effect of AMF associated with organic residues and varied phosphorus doses on the growth of *P. dubium* seedlings.

2. Material and Methods

2.1. Seeds collection, seedlings formation and general conditions

The species employed in this experiment was *Peltophorum dubium*, and seeds were gathered from various matrices in the municipality of Dourados, State of Mato Grosso do Sul. Seed dormancy was overcome by immersing the seeds in hot water at 80 °C for 24 hours (Davide and Silva, 2008). Subsequently, the seeds were sown in polyethylene tubes (120 cm^3 capacity) filled with a commercial substrate (Bioplant®) and remained in these tubes until the transplanting date, 50 days after sowing. No fertilization was applied to the seedlings during this initial period.

The experiments were conducted in the seedling nursery of the Faculty of Agrarian Sciences, Federal University of Grande Dourados (22°11'53.2" S; 54°56'02.3" W), located in the municipality of Dourados, Mato Grosso do Sul. The nursery was covered with a 150-micron-thick transparent low-density polyethylene film, with a black nylon screen on the sides providing 70% shading. Experiment I took place from July to December 2020, while Experiment II occurred from June to November 2019.

2.2. Experiment I – AMF and P in P. dubium seedlings

The experimental design was completely randomized, and treatments involving AMF and P (phosphorus) were combined in a factorial arrangement. Five levels of AMF and five levels of P fertilizer were applied, each with four replicates. Each experimental unit consisted of one pot containing two plants. The mycorrhizal treatments included inoculation with AMF species (control, *Clareoideoglomus etunicatum*, *Rhizophagus heterosporum*, *Rhizophagus clarum*, and MIX - a combination of the three AMF species). The AMF strains (registration number: A27569B) were obtained from Embrapa Maize and Sorghum. Phosphorus was applied at doses of 0, 60, 120, 180, and 240 mg P kg^{-1} of soil, using KH_2PO_4 as the phosphorus source.

The substrate for this experiment was Oxisols (USDA classification), corresponding to Dystrophic Red Latosol (Brazilian classification) (Santos et al., 2018), with the following initial chemical attributes (before correction): pH H_2O = 5.2, P (Melich-1) = 17.6 mg/dm^3, S-SO_4 = 14.70 mg/dm^3, K = 0.60 $cmol_c$ dm^3, Ca = 3.58 $cmol_c$ dm^3, Mg = 1.38 $cmol_c$ dm^3, Al = 0.17 $cmol_c$ dm^3, H + Al = 6.41 $cmol_c$ dm^3, Organic matter = 22.24 g/dm^3, organic carbon = 12.90 g/dm^3, sum of bases = 5.56 $cmol_c$ dm^3, CEC = 11.97 $cmol_c$ dm^3, V (%) = 46.45, m (%) = 2.97, B = 0.41 mg/dm^3, Cu = 14.60 mg/dm^3, Fe = 47.10 mg/dm^3, Mn = 77.00 mg/dm^3, and Zn = 2.80 mg/dm^3, and a mixture with coarse sand in a 2:1 (v/v) ratio. Soil correction and fertilization were performed by applying one gram of soil for each dm^3 of limestone per cubic meter of soil. To ensure the presence of only mycorrhizal fungi after inoculation, the substrate was autoclaved at 121º C and 1 atm pressure for one hour and later placed in plastic pots (7 dm^3 capacity).

2.3. Experiment II – AMF and organic residues in P. dubium seedlings

The experimental design was completely randomized, in a 6 x 4 factorial arrangement, with four replicates, and one pot containing two plants per experimental unit. Treatments included a control group (without AMF) and inoculation with AMF species *Rhizaphagus clarum*, *Gigaspora margarita*, *Gigaspora albida*, *Clareoideoglomus etunicatum*, and MIX (a combination of the four AMF species).

These treatments were combined with four substrates containing organic residues: S1) (soil + coarse sand – SCS) in a 2:1 (v/v) ratio, S2) SCS + poultry manure, S3) SCS + cattle manure, and S4) SCS + sheep manure, all in a proportion of 2:1:1 (v/v). The soil used in this experiment was classified as Oxisols (USDA), corresponding to Dystrophic Red Latosol (Santos et al., 2018), with the same chemical attributes as in Experiment I.

Soil correction and fertilization were conducted by applying one gram of soil for each dm^3 of limestone and 700 g of phosphorus for each m^3 of soil, in the form of organic residues. The quantities used were 68.93 g/dm^3 poultry manure, 68.63 g/dm^3 cattle manure, and 72.95 g/dm^3 sheep manure, corrected for 60% availability in the first year. Substrates were packed into 0.08-micron-thick autoclave bags (20 L capacity) made of high-density polyethylene and sterilized in an autoclave at 121º C and 1 atm pressure for one hour. Subsequently, these bags were placed in plastic pots (7 dm^3 capacity).

2.4. Inoculation of AMF in the substrates

The inoculation of arbuscular mycorrhizal fungi (AMF) took place at the seedlings' transplanting date. AMF inoculum, comprising a mixture of soil, spores, and roots colonized with AMF, was applied by adding 50 cm^3 of inoculum in proximity to the root system of seedlings (approximately 3 cm from the roots and hyphae). The control treatment did not receive any inoculation. The AMF isolates, *Clareoideoglomus etunicatum* (donated by the Laboratory of Organic Matter and Soil Microbiology at the State University of Mato Grosso do Sul), *Rhizophagus heterosporum*, and *Rhizophagus clarum* (registration number: A27569B, obtained from Embrapa Maize and Sorghum), were multiplied in a substrate composed of a 1:1 (v/v) mixture of soil and coarse sand. The substrate was sterilized in an autoclave at 121 °C and 1 atm pressure for 1 hour before being placed in plastic trays with a capacity of 20 dm^3. A layer of soil containing AMF propagules was then applied over the sterile substrate, with *Brachiaria ruziziensis* as the host plant. After 150 days, when the host plants were dehydrated and died, the substrates were stored in plastic bags and later used as a source of inoculum for AMF.

2.5. Assessments

In both experiments, the following characteristics were evaluated at 30, 60, 90, 120, and 150 days after transplanting (DAT): plant height (PH) measured with a graduated ruler (cm), stem diameter (SD) measured with a digital caliper (mm), and height: diameter ratio (HDR) calculated. At 150 DAT, seedlings were removed from the pots, and shoots (leaves + stem) and roots were separated to determine dry mass. Shoot and root fresh mass (SFM and RFM) were weighed using a precision analytical balance (0.001 g). The collected material was then dried in a forced air circulation oven at 60° ± 5 for 72 hours to obtain shoot dry matter (SDM) and root dry matter (RDM). SDM and RDM were summed to obtain the total dry matter (TDM). The shoot: root ratio (SRR) was calculated as SRR = (SDM/RDM), and Dickson's quality index (DQI) was calculated using the formula: DQI = TDM/ (HDR + SRR) (Dickson et al., 1960). Mycorrhizal dependence (MD) and mycorrhizal efficiency (ME) were obtained from the data of dry matter of mycorrhizal seedlings (DMM) and dry matter of non-mycorrhizal seedlings (DMN) using the formulas: MD (%) = ((DMM – DMN) / DMM) x 100 and ME (%) = ((DMM – DMN) / DMN) x 100, proposed by Plenchette et al. (1983).

2.6. Data analysis

For Experiment I, all data underwent ANOVA (F test, $p < 0.05$). When significant, means were compared using the Tukey's test ($p \leq 0.05$). Pearson's correlation analysis and cluster analysis were performed at $p \leq 0.05$. Statistical analyses were conducted in the R software R core team 2019 (Pinheiro et al., 2020) using generalized linear mixed models (GLMM), and means were obtained by Tukey's test at a 5% probability, using the R software.

For Experiment II, all data underwent ANOVA (F test, $p < 0.05$), and means were compared using the by Tukey's test at a 5% ($p \leq 0.05$). In both experiments, data for periods of evaluation were subjected to linear, quadratic, or exponential regression analyses. Statistical analyses were conducted in the R software (Pinheiro et al., 2020).

3. Results and Discussion

3.1. Experiment I – AMF and P in P. dubium seedlings

The biometric characteristics were evaluated based on the isolated effects of arbuscular mycorrhizal fungi (AMF) and phosphorus (P). This trend aligns with the findings of Bassan et al. (2018), who similarly did not observe an interaction between P doses and mycorrhizal fungi on the growth of *P. dubium* seedlings ($p<0.05$), indicating only isolated effects. Conversely, different results were obtained in the development of *Schizolobium amazonicum* seedlings concerning P doses associated with AMF species, with Brito et al. (2017) observing a positive interaction between these factors.

It is noteworthy that varying results may emerge when evaluating seedling production of native species in association with different AMF, given that the benefits derived from symbiosis depend on the intricate interaction between microorganisms and hosts, as well as prevailing environmental conditions (Carvalho et al., 2022). Plant responses to inoculation are contingent upon the physiological and biochemical compatibility between the plants and AMF, which is intricately linked to the inherent behavior of each species (Frey, 2019).

Analyzing the isolated effects of P doses without AMF inoculation, a significant impact was observed for stem diameter (SD) and height/diameter ratio (HDR), although not for plant height (PH). Conversely, in the absence of phosphate doses but with AMF inoculation, a significant effect was observed for PH and SD. Significant interactions between AMF species and evaluation periods were noted for PH, SD, and HDR, as well as significant interactions between P doses and evaluation periods for SD and HDR.

However, no interaction was observed for PH, indicating that phosphate fertilization did not influence height increases in *P. dubium* seedlings.

Exploring the relationship between different AMF at each evaluation period (30, 60, 90, 120, and 150 DAT) with PH revealed no significant differences between treatments at 30 and 60 DAT. Yet, at 90, 120, and 150 DAT, a significant difference emerged, with the control (without inoculum), *R. clarum*, and MIX yielding superior performance in terms of plant height. In contrast, seedlings inoculated with *R. heterosporum* and *C. etunicatum* exhibited the lowest plant height, with *C. etunicatum* displaying the poorest performance (Figure 1A).

Examining the variable SD, no significant difference was observed at 30 DAT between treatments (Figure 1B). At 60 DAT, however, a significant difference emerged between the control treatment (without inoculum), *R. clarum*, and MIX compared to treatments with *R. heterosporum* and *C. etunicatum*.

The height of *P. dubium* seedlings was influenced by different AMF and evaluation periods. A linear trend was observed, with the control, MIX, and *R. clarum* exhibiting the highest average growth at 150 DAT (Table 1). Seedling height is a crucial parameter for the classification, selection, and commercialization of forest species (Baldin et al., 2015). All seedlings, with heights ranging from 24.65 cm to 43 cm at the end of 150 DAT, were deemed suitable for field use, falling within the optimal range of 15 to 30 cm (Paiva and Gomes, 2000).

Stem diameter was also influenced by AMF inoculation across evaluation periods, demonstrating a linear response. Treatments using *R. clarum*, without inoculation, MIX, and *R. heterosporum* exhibited a larger mean diameter at 150 DAT (Table 2). A larger stem diameter is indicative of a seedling's enhanced chances of survival in the field after planting Andivia et al., (2021).

Table 1. Equation of Height of *Peltophorum dubium* seedlings at different evaluation periods as a function of different AMF´s.

Planta height (cm)		
AMF	**Equation**	**R^2**
AMF: C. etunicatum	Y= 6.7+0.122x	0.99
AMF: MIX	Y=3.86+0.25x	0.95
AMF: *R. clarus*	Y=4.56+0.243x	0.95
AMF: *R. heteros*	Y=6.15+0.192x	0.92
Without AMF	Y=4.54+0.272x	0.94

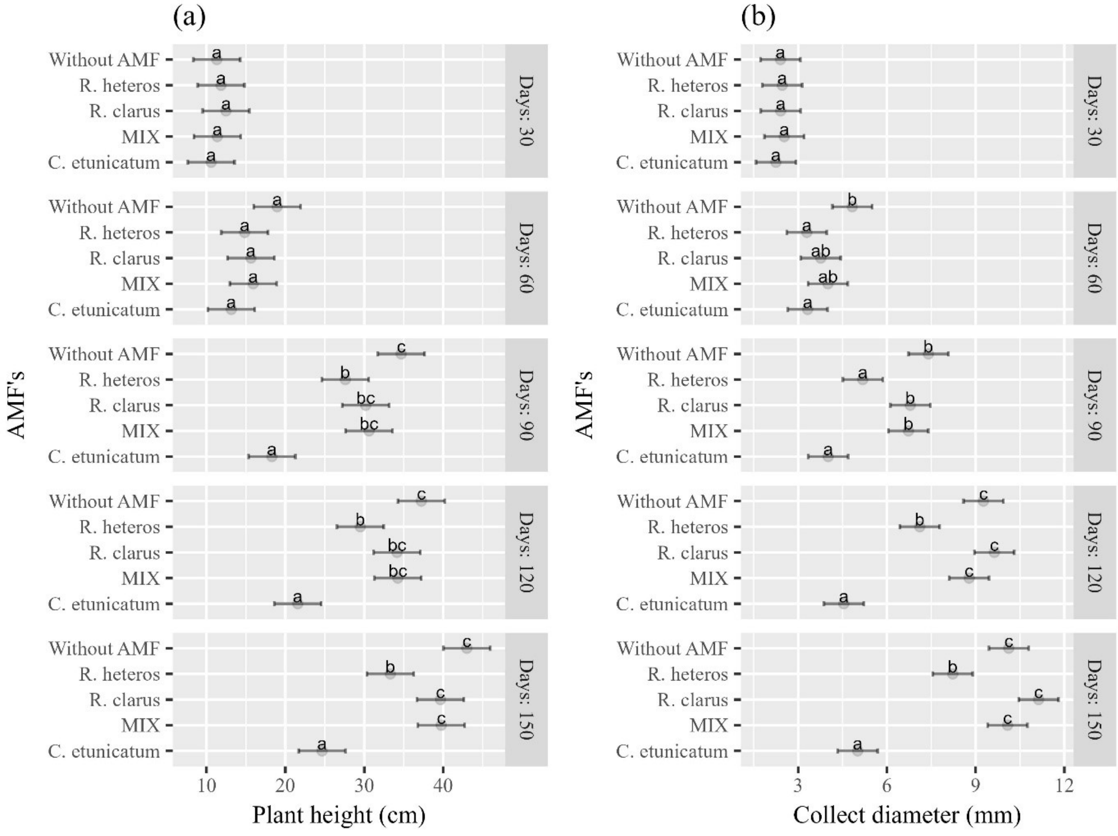

Figure 1. AMF within each evaluation period for the characteristics height (A) and stem diameter (B) in *Peltophorum dubium* seedlings. Means followed by the same letter do not differ statistically from each other by Tukey test ($p > 0.05$).

Comparing evaluation periods for each P dose revealed a significant effect of the doses for SD, represented by a linear function. The dose of 60 mg kg^{-1} performed better at all evaluation periods compared to other treatments, highlighting the highest values at 30 and 150 DAT. Conversely, the highest dose of 240 mg kg^{-1} presented the lowest values of SD. This suggests that *P. dubium* seedlings, in the absence of phosphate fertilization, exhibited superior development for SD compared to the dose of 240 mg kg^{-1}, indicating the detrimental effects of this high dose on seedling development.

Phosphorus, integral to various metabolic processes, influenced the development of *P. dubium* seedlings. Lower P doses promoted superior plant development, while higher doses exhibited a deleterious effect. Phosphorus plays an important role in photochemical activities, enhancing growth through increased ATP production, thereby contributing to biomass production.

Seedling height and stem diameter, vital morphological parameters, are indispensable for analyzing the quality of forest seedlings. However, these factors should not be assessed in isolation to accurately evaluate seedling quality (Gonçalves et al., 2013).

Correlation analysis results revealed positive and significant correlations between the variables studied, except for the correlation between SRR and RFM and RDM, which, although positively correlated, were not statistically significant (Figure 2). The variable HDR exhibited a negative correlation with the other variables and is used as a characteristic to evaluate seedling quality. The highest HDR observed was 7.0 in the treatment with 120 mg kg^{-1} using *R. heterosporum.*

The highest positive correlations were observed between SDM and SFM and between TDM and SFM (0.99 and 0.97, respectively). This positive correlation between dry matter (RDM and SDM) and seedling quality (DQI) indicates that biomass production of *Peltophorum dubium* seedlings is directly related to their quality. Plants inoculated with *C. etunicatum* were grouped in the lower left quadrant of principal component analysis, suggesting that *C. etunicatum* exhibited similar behavior across different P doses compared to the other AMF species, with HDR influencing this behavior (Figure 3A).

The correlation was exceptionally high for the evaluated characteristics, with the first component (Axis 1) explaining 78.2% of data variability, while the second component (Axis 2) explained 11.3%. The negative correlation of HDR with other characteristics was evident. The dendrogram from multivariate analysis divided the treatments into three groups, with treatments involving *C. etunicatum* for all doses forming a large cluster, indicating similarity and greater dissimilarity compared to other treatments (Figure 3B).

Inoculation with AMF significantly enhanced the growth variables analyzed, although this effect was not observed for AMF *C. etunicatum*, which yielded lower growth values in the present experimental conditions. The benefits of symbiosis extend beyond mere growth, encompassing nutritional enhancements, improved root system utilization and conservation, heightened tolerance to biotic or abiotic stresses, and physiological and biochemical changes (Pereira et al., 2022), such as increased photosynthetic rates and root production.

3.2. Experiment II - AMF and organic residues in P. dubium seedlings

The inoculation of mycorrhizal fungi and the incorporation of organic residues into substrate formulations to produce *P. dubium* seedlings elicited distinct effects.

Table 2. Equation of evaluation periods as a function of each AMF (A) and dose of P (B) for the characteristic stem diameter (SD) in *Peltophorum dubium* seedlings.

Stem diameter (mm)		
A		
AMF	**Equation**	**R^2**
AMF: C. etunicatum	Y= 1.8+0.0225x	0.97
AMF: MIX	Y=0.462+0.662x	0.99
AMF: *R. clarus*	Y=0.25+0.0777x	0.98
AMF: *R. heteros*	Y=0.651+0.0512x	0.98
Without AMF	Y=0.842+0.0662x	0.97
B		
P doses	**Equation**	**R^2**
0	Y= 0.668+0.0585x	0.99
60	Y=0.48+0.0655x	0.98
120	Y=0.575+0.0589x	0.99
180	Y=0.641+0.0587x	0.98
240	Y=1.14+0.0423x	1

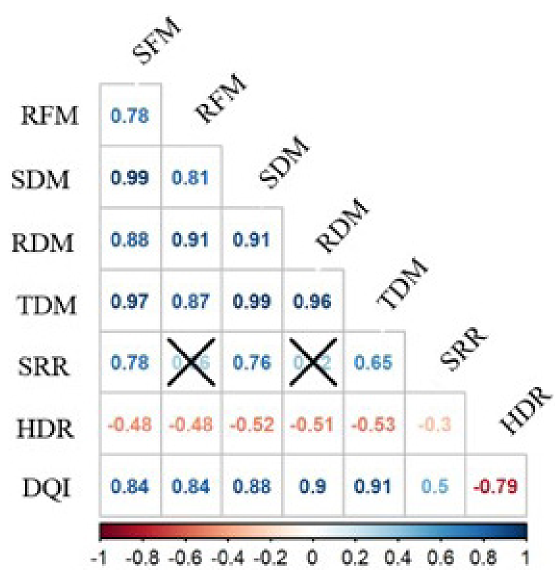

Figure 2. Pearson's correlation for quantitative and qualitative characteristics in *Peltophorum dubium* seedlings inoculated with AMF and under different doses of P.

Noteworthy interactions were observed between AMF and the evaluation period for stem diameter, as well as between substrate composition and the evaluation period for plant height, stem diameter, and height/diameter ratio (HDR).

P. dubium seedlings cultivated in substrates enriched with organic residues exhibited enhanced growth compared to those without such additions, signifying that the inclusion of organic residues positively contributed to increased growth characteristics. This effect was particularly prominent at 120 and 150 days after transplanting (DAT), with seedlings grown in substrate amended with sheep manure displaying the most substantial growth (50.86 cm) compared to those in the substrate lacking organic residues, which achieved values of 23.18 cm at 150 DAT (Table 3).

Stem diameter of *P. dubium* seedlings was significantly influenced by the interaction between substrates containing organic residues and evaluation period (Table 3). The diameter exhibited substantial increases from 60 days after transplanting onward. Seedlings grown in substrate with cattle manure presented the highest diameter (9.42 cm), a statistically superior result compared to seedlings grown without organic residues, which displayed a diameter of 5.03 cm (Table 3). Padilha et al. (2018) reported similar findings when assessing the growth of *P. dubium* seedlings in commercial and organic substrates.

The HDR of *P. dubium* seedlings showed no differences until 60 days after transplanting (Table 3). Subsequently, an increase in HDR was observed from 60 to 150 DAT for seedlings grown in substrate with sheep manure. According to Araujo et al. (2020), HDR is linked to seedling endurance, with low values indicating more tender or lignified plants that possess a greater chance of survival after field transplanting. Accordingly, seedlings produced in substrates S1, S2, and S3 exhibited optimal characteristics. Generalized mixed models generated estimation equations for plant height (PH), stem diameter (SD), and HDR, providing a foundation for future studies with *P. dubium* under similar conditions aiming to predict measurements of these variables from 0 to 150 DAT (Table 4).

AMF species, such as *G. albida* and *C. etunicatum*, contributed to an increase in the fresh and dry mass of roots compared to *G. clarum* and *G. margarita*, although not differing significantly from the control treatment (Table 5). The inoculation of AMF positively impacted the physiological and nutritional characteristics of the plants, given the vital role these organisms play in nutrient absorption (Wang et al., 2017) and the stimulation of phytohormone synthesis (Pons et al., 2020).

Although there was no significant difference in root dry matter values between seedlings produced without AMF and those inoculated with *G. albida*, it is noteworthy that *G. albida* provided 24% higher values compared to the control without AMF. Silva et al. (2018), in their evaluation of *Calophyllum brasiliense* Cambess. seedlings, reported that AMF inoculation favored increased plant biomass. Lima et al. (2020) similarly found a positive effect of AMF inoculation on seedling performance in *Toona ciliata* M. Roem, highlighting the importance of AMF inoculation in promoting seedling growth.

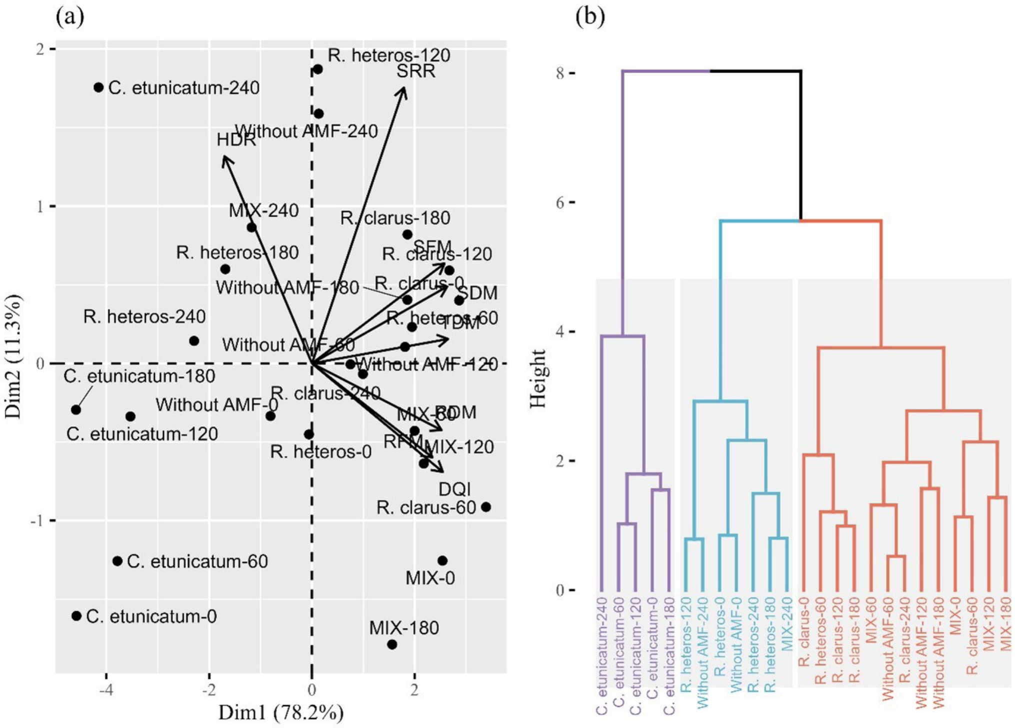

Figure 3. Principal component analysis (A) and cluster analysis (B) in *Peltophorum dubium* seedlings inoculated with AMF and under different doses of P.

Table 3. Height, stem diameter, and height: diameter ratio (HDR) of *Peltophorum dubium* seedlings grown in different substrates in function of evaluation periods.

Plant Height (cm)					
Substrates	**30 DAT**	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**
S1	7.32 a	12.60 a	15.07 c	19.88 b	23.18 b
S2	6.85 a	11.30 a	18.26 bc	35.42 a	46.43 a
S3	6.80 a	11.79 a	19.86 b	34.60 a	48.43 a
S4	7.29 a	13.91 a	26.20 a	43.12 a	50.86 a
Stem diameter (mm)					
Substrates	**30 DAT**	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**
S1	1.92 a	3.17 a	4.15 a	4.78 b	5.03 b
S2	1.71 a	2.56 b	4.25 a	7.40 a	9.18 a
S3	1.76 a	2.66 b	4.25 a	7.06 a	9.42 a
S4	1.83 a	2.84 ab	4.67 a	7.05 a	8.85 a
Height-diameter ratio (HDR)					
Substrates	**30 DAT**	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**
S1	4.15 a	4.26 a	3.94 c	4.44 c	4.90 c
S2	4.34 a	4.74 a	4.57 bc	5.17 b	5.63 bc
S3	4.23 a	4.69 a	4.86 b	5.32 b	5.63 bc
S4	4.32 a	5.24 a	5.96 a	6.46 a	6.09 a

Means followed by the same letter do not differ from each other by the Tukey test ($p < 0.05$). S1) Soil + sand – SCS, S2) SCS + chicken manure, S3) SCS + cattle manure, and S4) SCS + sheep manure.

Table 4. Equations of height, diameter, and height:diameter (HDR) of *Peltophorum dubium* seedlings in different substrate at different evaluation periods.

Substrates	Height plant	Stem diameter	HDR
S1	e(1.721 +0.009 ^{t})	e(1.008 +0.008 ^{t})	e(1.427 -0.003 ^{t})
S2	e(1.328 +0.016 ^{t})	e(1.015 +0.014 ^{t})	e(1.301+0.001 ^{t})
S3	e(1.335 +0.016 ^{t})	e(1.014 +0.014 ^{t})	e(1.269+0.002 ^{t})
S4	e(1.407 +0.017 ^{t})	e(1.014 +0.016 ^{t})	3(1.073 +0.010 ^{t})

S1: Soil + sand – SCS; S2: SCS + chicken manure; S3: SCS + cattle manure; S4: SCS + sheep manure. e (exponential).

Table 5. Shoot fresh mass (SFM), root fresh mass (RFM), total fresh mass (TFM), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM) of *Peltophorum dubium* seedlings inoculated with AMF. C.E: *C. etunicatum*, G.A: *G. albida*, G.C: *G. clarum*, G.M: *G. margarita*, and MIX: mixture of C.E + G.A + G.C + G.M in different substrates.

AMF	SFM	RFM	TFM	SDM	RDM	TDS
			g plant^{-1}			
C. etunicatum	61.87 a	16.49 a	78.36 a	22.79 a	6.28 b	29.07 a
G. albida	69.37 a	19.29 a	88.67 a	27.11 a	8.01 a	35.12 a
G. clarum	61.62 a	10.65 b	72.28 a	22.74 a	4.64 b	27.38 a
G. margarita	50.56 a	10.98 b	61.55 a	19.96 a	4.52 b	24.48 a
MIX	70.62 a	19.00 a	89.63 a	25.83 a	6.75 a	32.58 a
Without AMF	63.12 a	20.60 a	83.73 a	22.98 a	6.44 a	29.42 a

Means followed by the same letter do not differ from each other by the Scott-Knott test ($p < 0.05$).

In general, seedlings produced with three organic residues exhibited the highest values of shoot fresh and dry mass (Table 6). This indicates that the presence of organic matter is important for biomass production compared to the control, which solely contained soil and sand. Notably, seedlings responded significantly to the application of organic residues, with the highest production of shoot fresh matter recorded at 86.25 g/plant in seedlings grown in sheep manure, a statistically significant difference from seedlings produced in the substrate with only soil and sand.

Native and arboreal species typically respond positively to the addition of organic residues to cultivation substrates, especially in soils with low natural fertility, as these materials improve the chemical attributes of the substrate (Santos et al., 2020b; Goelzer et al., 2020). It is worth emphasizing that organic residues can also contribute to the substrate's physical characteristics, such as porosity and density, thereby enhancing water retention and promoting better root system exploration, as observed in *Alibertia edulis* Rich. (Santos et al., 2023).

In a study by Faria et al. (2016), evaluating the production of *Mimosa setosa* Benth. seedlings in different substrates based on cattle manure, poultry manure, quail manure, and commercial substrate, the best results for shoot dry mass (SDM), root dry mass (RDM), and total dry mass (TDM) were obtained with a substrate containing 25% commercial substrate + 35% poultry manure and 40% soil. Similarly, Gonzaga et al. (2016), in their evaluation of different substrates and AMF sporulation in *Joannesia princeps* Vell. LC., *Hymenaea courbaril* L., and *Hevea brasiliense* seedlings, reported positive results for SDM, RDM, and TDM with the use of poultry and cattle manure, echoing the findings for P. dubium.

P. dubium seedlings did not exhibit mycorrhizal dependence (Figure 4). Abreu et al. (2018) found mycorrhizal dependence of 25.53% for *G. margarita* in *Enterolobium contortisiliquum* seedlings. Symbiotic efficiency was observed for AMFs *G. albida* and *C. etunicatum*, with values of 19.64% and 5.5%, respectively (Figure 5). It was observed that *C. etunicatum* and MIX exhibited minimal symbiotic efficiency, while *G. albida* displayed the best symbiotic efficiency. In a study by Bassan et al. (2018) involving the inoculation of mycorrhizal fungi and phosphorus doses, *P. dubium* seedlings exhibited high dependence and mycorrhizal efficiency for the species *G. albida* and *C. etunicatum*, while symbiosis with *G. clarum* and *G. margarita* was ineffective.

The response to AMF inoculation varies with the species and its interactions with the environment, considering edaphoclimatic conditions and the compatibility of the symbiotic organism. In this study, *P. dubium* seedlings exhibited a more pronounced response to organic residues than to AMF, possibly because these organic materials fulfilled the nutritional needs of the species.

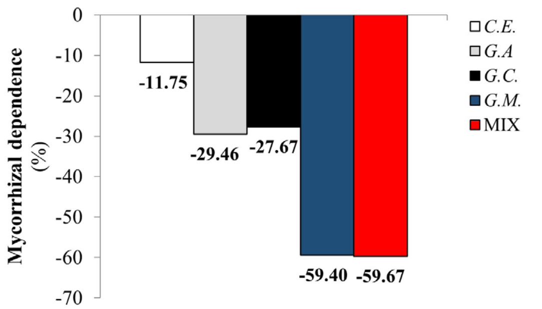

Figure 4. Mycorrhizal dependence in *Peltophorum dubium* seedlings inoculated with AMF. C.E: *C. etunicatum*, G.A: *G. albida*, G.C: *G. clarum*, G.M: *G. margarita*, and MIX: mixture of C.E + G.A + G.C + G.M.

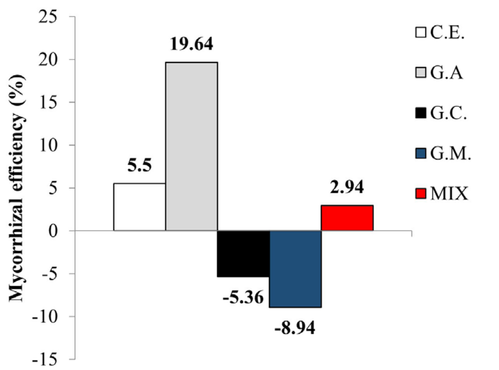

Figure 5. Mycorrhizal efficiency in *Peltophorum dubium* seedlings inoculated with AMF. C.E: *C. etunicatum*, G.A: *G. albida*, G.C: *G. clarum*, G.M: *G. margarita*, and MIX: mixture of C.E + G.A + G.C + G.M.

Table 6. Shoot fresh mass (SFM), root fresh mass (RFM), total fresh mass (TFM), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM). of *Peltophorum dubium* seedlings inoculated with AMF.

Substrates	SFM	RFM	TFM	SDM	RDM	TDM
			g plant^{-1}			
S1	10.45 b	3.75 c	14.29 b	4.29 b	1.03 c	5.33 b
S2	71.66 a	24.51 a	69.18 a	27.49 a	9.16 a	36.61 a
S3	83.00 a	19.63 b	102.63 a	32.04 a	8.11 a	40.16 a
S4	86.25 a	16.79 b	103.04 a	30.44 a	6.11 a	36.56 a

Means followed by the same letter do not differ from each other by the Tukey test ($p < 0.05$). S1: Soil + sand – SCS; S2: SCS + chicken manure; S3: SCS + cattle manure; S4: SCS + sheep manure.

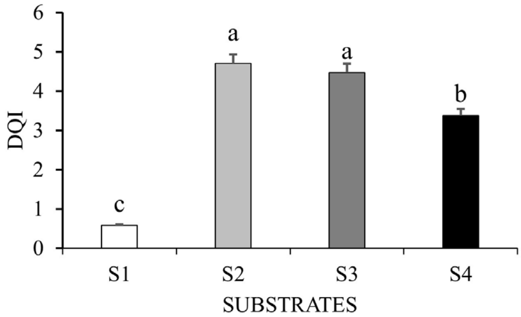

Figure 6. Dickson's quality index of *Peltophorum dubium* seedlings in different substrate with organic residues. S1) Soil + sand – SCS, S2) SCS + chicken manure, S3) SCS + cattle manure, and S4) SCS + sheep manure.

Additionally, when organic residues were added to the soil, microbial activity in the substrate increased, potentially leading to lower AMF efficiency. According to Carvalho et al. (2022), mycorrhizal colonization should not be the sole parameter for assessing efficiency, as certain AMF species can be highly infective but inefficient in nutrient provision, failing to enhance plant physiology and growth.

The Dickson's Quality Index (DQI) of *P. dubium* seedlings was higher in seedlings produced with poultry and cattle manure (Figure 6). This suggests an improvement in the chemical attributes of the substrate, favoring seedling nutrition and growth. Similar results were reported by Kratka and Correia (2015) when testing different substrates in the production of *Myracrodruon urundeuva* Allemão seedlings, where optimal results were achieved in substrates with organic residues. The DQI serves as an indicator for evaluating the quality of forest seedlings, considering important parameters such as height, diameter, and biomass through HDR (Medeiros et al., 2018). A higher DQI value correlates with better seedling quality (Melo et al., 2018).

The information gleaned from this study may support sustainable practices in commercial nurseries, guiding ex situ cultivation. Future studies should further characterize the nutritional status of *P. dubium* seedlings under similar conditions, providing additional insights for refining the propagation protocol of this species.

In conclusion, *Peltophorum dubium* seedlings derived significant benefits from AMF inoculation, with *R. heterosporum*, *R. clarum*, and MIX yielding optimal growth conditions. The response of *P. dubium* seedlings to organic residues was substantial, especially in substrates enriched with chicken manure and cattle manure. While the use of mycorrhizal fungi did not produce a significant effect on *P. dubium* seedlings, positive symbiotic efficiency was observed for the species *C. etunicatum* and *G. albida*.

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