

Germination and Initial Growth Performance of *Anadenanthera macrocarpa* (Benth.) in Different Substrates

ABSTRACT

Seed germination is considered the crucial phase in the establishment and development of plants. Knowledge about the appropriate conditions for seed germination is of fundamental importance, mainly due to the influence and different responses that they can present depending on several factors such as seed viability, light, water, oxygen, temperature, and substrate, among others. Therefore, the main objective of the work was to evaluate the germination and the initial development of the *A. macrocarpa* in the function of different substrates. The experimental design adopted was completely randomized, where 6 different substrates were used in 4 replications. The treatments consisted of S₁ (Commercial Substrate); S₂ (Washed Sand); S₃ (Commercial Substrate 50% + Vermiculite 50%); S₄ (Commercial substrate 50% + Rice straw 25% + Pine bark powder 25%); S₅ (Black Soil); S₆ (Black Soil 75% + Sawdust 25%). The evaluated characteristics were germination percentage (%), emergence speed index, shoot and root length (cm), shoot and root fresh mass (gram), and shoot and root dry mass (gram). The use of commercial substrate + vermiculite stood out for most of the analyzed characteristics, being germination (78.57%), aerial part fresh mass (0.1187g), root fresh mass (0.0263g), aerial part dry mass (0.0689g) and root dry mass (0.0149g). The lowest averages observed for all characteristics were with the use of black soil substrate.

Keywords: *Anadenanthera macrocarpa*. Germination. Initial development.

1. INTRODUCTION

The tree species *Anadenanthera macrocarpa* (Benth.), commonly known as red angico, belongs to the Mimosaceae family. This tree exhibits significant natural regeneration and can be used for various purposes. It has a remarkably lush flowering and can be used for landscaping in parks, streets, and roads. It is widely utilized for the regeneration and reforestation of degraded areas [1]. Red angico can also be used in civil and naval construction, as well as in the production of firewood and charcoal [2]. It possesses unique characteristics, such as its phytotherapeutic use [3].

Seed germination is considered a crucial phase in plant establishment and development [4]. It is important to emphasize that various factors affect this germination process. Temperature and substrate have a significant influence on this germination process. Knowledge about the suitable conditions for seed germination is of fundamental importance, especially due to the different responses that seeds may exhibit due to factors such as seed viability, light, water, oxygen, temperature, and substrate, among others [5].

When propagating red angico via seeds, it is necessary to use substrates with characteristics favorable to the initial development of seedlings that will later become productive trees. The substrate influences germination based on its structure, aeration, water retention capacity, and susceptibility to pathogen infestation, among other factors, which can either favor or hinder seed germination and initial seedling development. It provides physical support where the seed is placed and maintains the appropriate conditions for germination and seedling development. Moreover, the substrate allows for easy removal of seedlings during transplantation, which is crucial for the plant's optimal development [6, 7, 8].

There is a variety of substrates that can be used for seedling production, such as peat, sand, expanded clay, perlite, vermiculite, rice husk, pine bark, coconut fiber, sawdust, and commercial substrate, among others [9].

According to Silva Júnior & Visconti (1991) [10], substrates are used to produce seedlings of tree species and are intensively studied to achieve excellent results in quality and seedling formation. The use of readily available and low-cost substrates is most relevant for producers aiming to reduce production costs. Each plant species needs experimental verification to determine whether the type of material used allows for the healthy development of plants, making them vigorous [11].

This study aims to evaluate the germination and initial development of red angico based on different substrates.

2. MATERIAL AND METHODS

The experiment was conducted in the Horticulture section of the greenhouse at the Gurupi University Campus (CAUG), Federal University of Tocantins, located at approximately 11°43'45" South latitude and 49°04'07" West longitude, with an average altitude of 280 meters. The region's climate is characterized by a tropical semi-humid climate regime, with an approximate 4-month dry season. With these temperatures and precipitation levels, the climate is classified as AW - Tropical wet summer and winter dry period, according to Köppen & Geiger's classification (1928) [12].

The experimental design adopted was completely randomized, using six different substrates with four replicates. The treatments consisted of S₁ (Commercial Substrate); S₂ (Washed Sand); S₃ (Commercial Substrate 50% + Vermiculite 50%); S₄ (Commercial Substrate 50% + Rice Straw 25% + Pine Bark Powder 25%); S₅ (Black Soil); S₆ (Black Soil 75% + Sawdust 25%). Styrofoam trays with 128 cells were used, divided into four quadrants, with each quadrant representing a replicate, and each replicate consisting of 32 plants.

The red angico seeds were collected at the Federal University of Tocantins on August 30, 2022, and taken to the laboratory for processing, where visually unviable seeds were separated. Subsequently, the vigor and viable seeds were placed in plastic bags and stored in the refrigerator at a temperature of 20°C until the experiment was set up on September 6, 2022. Sowing was done directly in the styrofoam trays, with the standardization of one seed per unit. After sowing, the treatments were placed in the greenhouse and received irrigation using a watering can twice a day.

Seed germination was counted regularly and continued for more than 20 days after first germination. After this period, the following evaluations were made: germination percentage was calculated using the formulas cited by Labouriau and Valadares (1976) [13].

- Germination percentage: $G = (N/A) \times 100$, where: G = germination percentage; N = number of germinated seeds; A = total number of seeds placed for germination.
- Emergence Speed Index: Where $IVE = (E1/N1) + (E2/N2) + \dots + (Ei/Nti)$ IVE = Emergence Speed Index; Ei = the number of seedlings emerged each day; Ti = the number of days between sowing and the first collection, between sowing and the second collection, and so on, up to the last (x) collection;
- Shoot length (cm): evaluated at 20 days after sowing, with the aid of a ruler graduated in centimeters;
- Root length (cm): evaluated at 20 days after sowing, with the aid of a ruler graduated in centimeters;
- Fresh mass (gram): the ground part of the seedlings was detached from the root and weighed using a precision analytical balance;
- Fresh root mass (gram): the root system was detached from the ground part just the substrate and weighed using a precision analytical balance;
- Dry mass (gram): drying the ground part in a constant airflow oven at a temperature of 65°C until a constant weight was reached, the sample was weighed on a precision analytical balance;
- Dry root mass: after drying the root system in a constant airflow oven at a temperature of 65°C until a constant weight was reached, the sample was weighed on a precision analytical balance.

The data were subjected to analysis of variance by the F-test, and means were compared using the Scott-Knott test (1974) [14] at a 1% probability level. The analysis was performed using the "R" software [15].

3. RESULTS AND DISCUSSION

According to the analysis of variance (Table 1), a significant effect was observed for the characteristics of germination (GER) (%), germination speed index (GSI), emergence speed index (ESI), aerial part length (APL) (cm), root length (RL) (cm), fresh mass aerial part (FMAP) (gram), dry mass aerial part (DMAP) (gram), and dry root mass (DRM) (gram) ($F_{5;18} < 0.01$). However, for the characteristic of fresh root mass (FRM), no significance was observed ($F_{5;18} > 0.01$).

Indicators	Sources of Variation (S.V)		Overall average	C.V. (%)
	Treatments	Residue		
	Degree of freedom			
	5	18		
GER	717.37**	132.33	69,79	16.48 %
GSI	214.617**	25.348	26,17	19.24 %
ESI	0.55278**	0.07578	10,51	2.62 %
APL	0.52689*	0.17966	6,08	6.97 %
RL	1.38833**	0.29089	6,15	8.77 %
FMAP	0.00085**	0.000118	0,10	10.8 %
DMAP	0.00040**	0.000061	0,06	13.24 %
FRM	3.5686e ^{-05ns}	2.1100e ⁻⁰⁵	0,02	20.52 %
DRM	2.4881e ^{-05*}	6.1933e ⁻⁰⁶	0,02	14.28 %

Table 1. Analysis of variance for red angico seedling production indicators developed under different substrates. Gurupi - TO, 2022.

For the germination percentage characteristic (Figure 1A), the best result was observed when using T₃, achieving a germination percentage of 78.57%, which did not significantly differ from the use of T₁, T₂, T₄, and T₆, which achieved 75.00%, 72.32%, 76.78%, and 73.21%, respectively. The use of substrate T₅ yielded the lowest germination value, at 42.85%.

Regarding the ESI, the use of T₂ stood out as the treatment with the highest index, obtaining a result of 10.97. However, there was no significant difference from the use of T₅, T₁, and T₆, which achieved 10.72, 10.65, and 10.58, respectively. The lowest ESI was obtained in T₄, with a value of 9.95, differing from the others (Figure 1B).

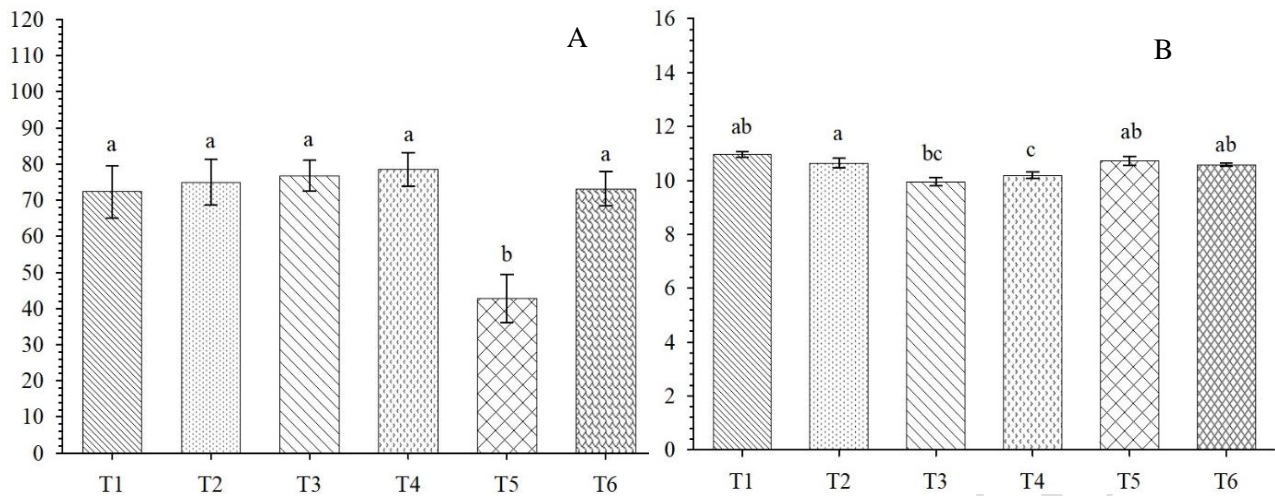


Fig. 1. Averages of germination percentage (A) and emergence speed index (B) of red Angico seedlings developed under different substrates. Gurupi - TO, 2022.

Several studies claim that the use of vermiculite as a substrate, in combination with other types of substrates to produce forest seedlings, provides better germination rates. This is likely because vermiculite can retain water and keep the substrate moist for a longer time, in addition to its high porosity [16]. This often facilitates the emergence of seedlings [17], which explains the better percentage achieved when using vermiculite.

Other substrates, such as washed sand, commercial substrates, or even mixtures of substrates to obtain a composite with suitable characteristics for the development of seedlings, are widely used, especially for reducing production costs. In this study, these treatments achieved satisfactory results, with germination percentages exceeding 70%.

When evaluating the influence of substrates on the germination of *Anadenanthera colubrina* seeds, researchers Oliveira et al. (2012) [18] achieved upper results when using vermiculite as a substrate, reaching nearly 86% germination, which was close to the results obtained in this study. They achieved approximately 73% germination using washed sand, which did not significantly differ from using vermiculite, a result like what was observed in the present study.

Araújo et al. (2020) [19], while studying alternative substrates to produce *Anadenanthera colubrina* seedlings, obtained a germination rate of 50% when testing carbonized rice straw, which was much lower than the almost 77% obtained in this research. This is possibly due to the mixture of commercial substrate and pine bark powder, which allowed the composition of a substrate with physical characteristics conducive to seed germination.

According to Edmond & Drapala (1958) [20], the lower the value obtained by the germination or emergence speed, the higher the physiological potential of seed lots. Considering that the IVE is based on the principle that the faster a seed germinates, the higher its vigor, it can be said that the use of commercial substrate + rice straw + pine bark powder as a substrate (T4) exhibited higher physiological potential for emergence, in contrast to the use of washed sand (T2), which showed lower potential. Considering that the evaluation of seed germination ability is of unquestionable importance, especially in terms of speed and uniformity.

When evaluating the washed sand and different moisture levels of this substrate, ESI values between 3.09 and 3.50 were obtained with 50% humidity [21, 22]. The study at hand achieved a higher ESI result.

Analyzing the results in terms of the aerial part length (cm) of the red Angico seedlings, it can be said that the greatest aerial part length was achieved using T₂ (6.56 cm), while the smallest was achieved with the use of T₅ (5.57 cm), and these values were statistically different from each other (Figure 2A).

Regarding root length (cm), the highest average was obtained with treatment T₄, with a value of 7.06 cm, differing from the averages obtained with T₅ and T₆ (5.40 cm and 5.72 cm respectively), which had the lowest values for this characteristic (Figure 2B).

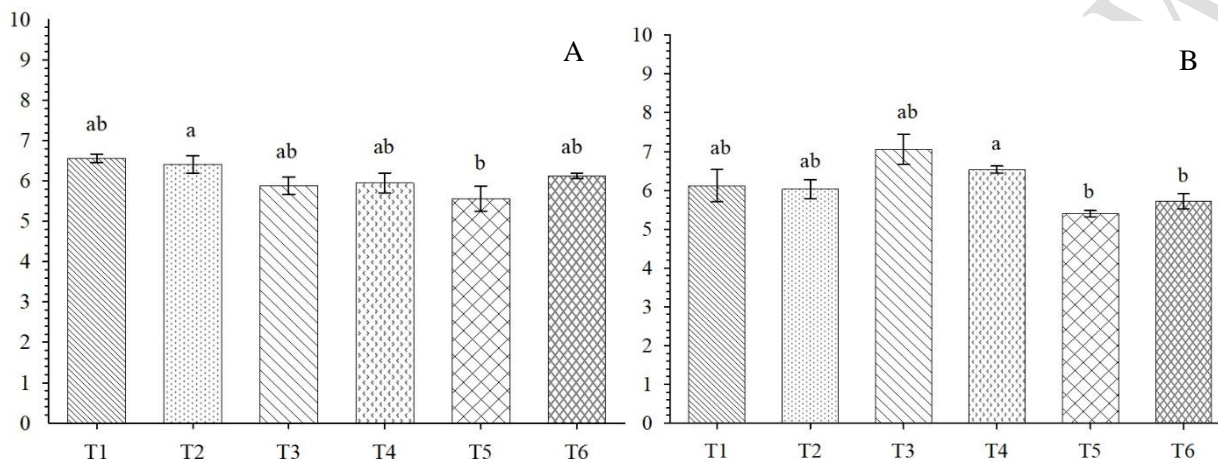


Fig. 2. Means of the aerial part length (A) and root length (B) of red Angico seedlings developed under different substrates. Gurupi - TO, 2022.

In the evaluation of physiological parameters of *Albizia niopoides* (White Angico) seedlings about different substrates, Afonso et al. (2017) [23] observed that the longest aerial part lengths were achieved with 100% washed sand substrate in all four evaluation periods, despite not showing differences about the treatments with 75% washed sand and 25% Tecnomax[®] at 45, 135, and 180 days and with 25% washed sand and 75% Tecnomax[®] at 45 days.

A similar result was observed by Araújo et al. (2020) [19], where they evaluated the effect of using alternative substrates to produce white Angico seedlings and found that when using the soil + washed sand substrate (1:1), the highest average for aerial part length (7.70 cm) was obtained.

The use of washed sand, either individually or as a component in substrate compositions for seedling production, is due to its main advantage, which is its low cost, easy availability, structural stability, good drainage characteristics, chemical inactivity, and ease of cleaning [24].

The use of black soil as a substrate resulted in the lowest average for aerial part length, likely due to the substrate density, which indirectly increased germination time (Figure 1) and affected aerial part length.

The substrate must have physical and chemical characteristics conducive to the proper development of the plant root system. Substrates used in isolation rarely possess all the desired characteristics for seedling production. Therefore, it is necessary to evaluate the best combination to be used for each plant species [25].

Thus, it can be stated that the combination of commercial substrate + rice straw + pine bark powder contributed to the good development of the root system of Angico seedlings, achieving the highest average

root length. In contrast, the combination of black soil + sawdust substrates yielded the opposite result, resulting in the lowest average root length among the treatments.

For the evaluation of freshmassaerial part (gram), a value of 0.1187g was achieved using T_3 , which was the highest average obtained. The lowest averages came from the use of T_4 , T_2 , and T_5 , ranging between 0.100 and 0.078gram (Figure 3A).

As for the characteristic of fresh root mass (gram), there was no significant effect for any of the treatments used, meaning that any of the six substrates used had no significant effect on this characteristic (Figure 3B). However, T_3 had the best average (0.0263 gram), and T_2 had the lowest (0.0175gram).

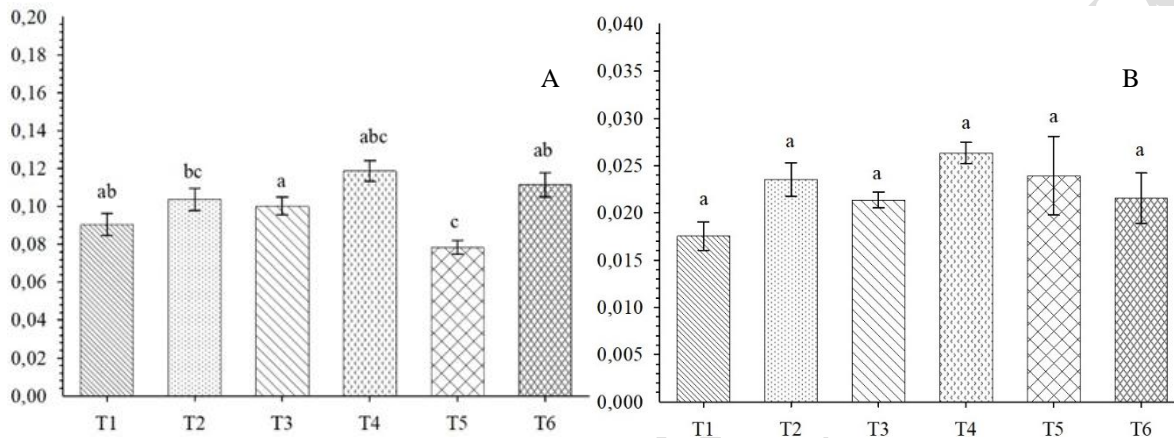


Fig. 3. Averages of fresh mass aerial part (A) and fresh root mass (B) of red Angico seedlings developed under different substrates. Gurupi - TO, 2022.

The use of vermiculite in combination with other substrates provides several advantages, directly affecting the quality of the initial development of seedlings. This compound helps in better aeration and maintenance of humidity in the substrate, enabling greater accumulation of biomass, and giving rise to more vigorous seedlings [26].

The composition of the commercial substrate and vermiculite enabled better average results for both aerial and root fresh mass. Therefore, the quality of the seedlings is directly related to the type of substrate used [27, 28].

The use of the T_3 treatment achieved the highest mean dry mass of the aerial part with 0.0689gram (Figure 4A), not differing from the T_4 , T_2 , and T_1 treatments, which reached respectively the means of 0.0636, 0.0631 and 0.0624gram, differing mainly of T_5 with the lowest average dry mass of the aerial part of 0.04045gram, which in turn did not differ from T_6 with 0.0559gram.

As for the characteristic of average dry root mass (gram), the use of treatment T_3 achieved the highest average with 0.02061gram (Figure 4B), differing mainly from T_2 with the lowest average dry root mass of 0.0149gram. The other treatments did not differ from the highest or lowest average.

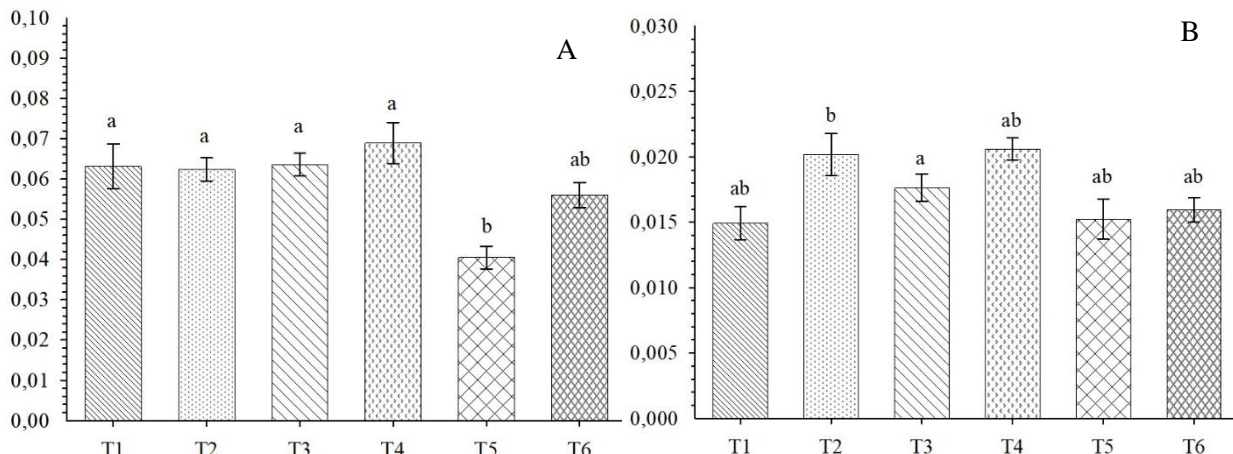


Fig. 4. Averages of dry mass aerial part (A) and dry root mass (B) of red Angico seedlings developed under different substrates. Gurupi - TO, 2022.

The results for dry mass aerial part and dry root mass are like those obtained for fresh mass aerial part and fresh root mass. The use of vermiculite with the commercial substrate provided better averages for both characteristics. This substrate composition creates favorable conditions for the angico seedling to accumulate a greater amount of organic carbon in its biomass.

The use of the commercial substrate is quite common, based on the assumption of easy availability and its reputation for being suitable for seedling development. Carvalho et al. (2021) [29], when evaluating the production of *Cojoba arborea* seedlings in different substrates, also observed positive results for fresh ground and root mass when using the commercial substrate.

However, the choice of substrate should meet the characteristics for proper seedling development and offer a reasonable price. The use of the commercial substrate + vermiculite did not significantly differ from other substrates for this characteristic, such as the commercial substrate alone, washed sand, and the commercial substrate + rice straw + pine bark powder, making them viable options to consider.

CONCLUSION

The use of the commercial substrate + vermiculite yielded the best results, while the lowest averages were obtained when using the black soil substrate.

REFERENCES

1. Meira D, Rubin HM, Bordin R, Dagios RF, Meier C, Aguir ACM, Cantarelli EB. Evaluation of different substrates in the initial development of *Anadenanthera macrocarpa* seedlings. *Revista Cultivando o Saber*, 2017;10(1):87-93.
2. Lorenzi, H. *Brazilian Trees: Manual for Identification and Cultivation of Native Tree Plants of Brazil* (Vol. 2, p. 38). São Paulo, 2010; Instituto Plantarum.
3. Weber CR, Soares CML, Lopes ABD, Silva ST, Nascimento MS, Ximenes ECPA. *Anadenanthera colubrina*: a study of therapeutic potential. *Revista Brasileira de Farmácia*, 2011;92:235–244.
4. Souza FC, Mengarda LHG, Spadeto C, Lopes JC. Substrates and temperatures in the germination of *Gonçalo-alves* (*Astronium concinnum* Schott) seeds. *Revista Tropica: Ciências Agrárias e Biológicas*, 2012;6(3):76-86.
5. Carvalho NM, Nakagawa J. *Seeds: Science, Technology, and Production* (5^a ed.). Jaboticabal: FUNEP; 2010.
6. Wagner JA, Alexandre RS, Negreiros JRDS, Pimentel LD, Silva JODACE, Bruckner CH. Influence of substrate on germination and initial development of yellow passion fruit plants (*Passiflora edulis* Sims f. *flavicarpa* deg). *Revista Ciência e Agrotecnologia*, 2006;30(4):643-647.
7. Baron D, Ferreira G, Boaro CSF, Mischam MM. Evaluation of substrates on the emergence of "araticum-de-terra-fria" (*Annona marginata* (Schltdl.) H. Rainer) seedlings. *Revista Brasileira de Fruticultura*, 2011;33(2):575-586.
8. Silva VF, Brito KSA, Nascimento ECS, Andrade LO, Ferreira AC. Effect of different substrates on sunflower genotype germination. *Revista Verde*, 2014;9(4):16-20.
9. Fernandes C, Corá JE, Braz L. Changes in the physical properties of substrates for cherry tomato cultivation due to their reuse. *Revista Brasileira de Horticultura*, 2006;24:94–98.
10. Silva Júnior AA, Visconti A. Containers and substrates for tomato seedling production. *Agropecuária Catarinense*, 1991;4(4):20-23.
11. Costa LM, Andrade JWS, Rocha AC, Souza LP, Flávio NJ. Evaluation of substrates for cucumber (*Cucumis sativus* L.) cultivation. *Global Science and Technology*, 2009;2(2):21-2.
12. Köppen W, Geiger R; 1928. *Klimate der Erde*. Gotha: Verlag Justus Perthes.
13. Labouriau LG, Valadares MB. On the physiology of *Calotropis procera* seeds. *Anais da Academia Brasileira de Ciência*, 1976;42(2):235-264.
14. Scott A, Knott M. Cluster-analysis method for grouping means in analysis of variance. *Biometrics*, 1974;30(3):507-512.
15. R Core Team. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, 2013; Vienna, Austria.

16. Martins CC, Machado CG, Caldas IGR, Vieira IG. Vermiculite as a substrate for barbatimão seed germination testing. *Ciência Florestal*, 2011;21:421-427.
17. Dousseau S, Alvarenga AA, Arantes LO, Oliveira DM, Nery FC. Germination of Plantain seeds (*Plantago tomentosa Lam.*): influence of temperature, light, and substrate. *Ciência e Agrotecnologia*, 2008;32(2):438-443.
18. Oliveira KSD, Oliveira KSD, Aloufa MAI. Influence of substrates on the germination of *Anadenanthera colubrina* (Vell.) Brenan seeds in greenhouse conditions. *RevistaÁrvore*, 2012;36:1073-1078.
19. Araújo MFP, De Pinho EFM, Da Silva CAP, Ruas MAO. Alternative substrates for the production of White Angico seedlings (*Anadenanthera colubrina (Vell.) Brenan*). *Caderno de Ciências Agrárias*, 2020;12:1-7.
20. Edmond JB, Drapala WJ. The effects of temperature, sand and soil, and acetone on germination of okra seeds. *Proceedings of American Society of Horticultural Science*, 1958;71(2):428-434.
21. Moreira FL, Portella MBS, Moraes CE, Matheus MT. Germination and vigor of red angico seeds under water stress conditions. *Enciclopédia Biosfera*, 2014;10(18):1845-1853.
22. Silva GO, Aguiar BAC, Terra DCV, Sousa RM, Fonseca EF, Souza PB. Substrate moisture and performance of *Anadenanthera peregrina* (L) sp. emergence. *Magistra*, 2019;30:336-341.
23. Afonso MV, Martinazzo EG, Aumonde TZ, Villela FA. Physiological parameters of *Albizia niopoides* seedlings produced in different substrate compositions. *Ciência Florestal*, 2017;27:1395-1402.
24. Krzyzanowski FC, França-Neto JB, Gomes-Júnior FG, Nakagawa J. Seed vigor tests based on seedling performance. In: Krzyzanowski FC, Vieira RD, França-Neto JB, Marcos-Filho J. (Eds.); 2020: Vigor of seeds: concepts and tests (pp. 79-140). Londrina: ABRATES.
25. Biasi LA, Bilia DAC, São José AR, Fornasieri JL, Minami K. Effect of mixtures of peat and sugarcane bagasse on passion fruit and tomato seedling production. *Scientia Agricola*, 1995;52:239-243.
26. Bassaco AC, Júnior BDSB, Ferrera TS, Bassaco GP, Santana NA, Antonioli ZI. Alternative substrates in lettuce seedling production. *Caderno de Pesquisa*, 2019;31(2).
27. Fraga RA, Costa AC, Chagas MAO, Oliveira Carvalho AH, Lima WL. Morphological performance of lettuce from seedlings developed in different alternative substrates. *Cadernos de Agroecologia*, Rio de Janeiro, 2016;11(2):1-5.
28. Antunes LFS, Da Silva DG, Correia, MEF, De Almeida Leal, MA. Chemical evaluation of stored organic substrates and their efficiency in lettuce seedling production. *Revista Científica Rural*, Bagé, 2019;21(2):139-155.
29. Carvalho CA, Da Silva ORF, Ribeiro ÍFN, Andrade RA, De Brito RS, Junior DLT, Do Nascimento MM. Production of *Cojoba arborea* (L. Britton & Rose) seedlings in different substrates and shading levels. *Scientia Naturalis*, 2021;3(1):124-132.

Attachments



Fig. 5. 128 cell styrofoam tray, Gurupi – TO, 2022



Fig. 6. Red Angico seeds collected at the Federal University of Tocantins, Gurupi
– TO, 2022



Fig. 7. Sowing red Angico seeds in Styrofoam trays, Gurupi – TO, 2022

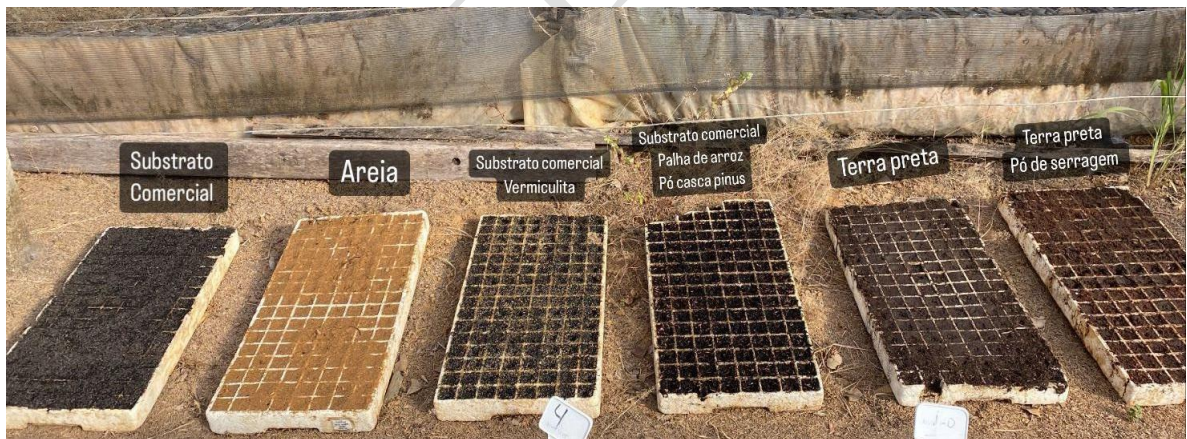


Fig. 8. Trays with the 6 substrates, Gurupi – TO, 2022



Fig. 9. Initial development of red Angico seedlings, Gurupi – TO, 2022



Fig. 10. Red Angico seedling length, Gurupi – TO, 2022

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