

Substrate effect on leaf area development in *Moringa oleifera* Lam. and *Cedrela fissilis* Vell.¹

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Abstract

Objective: To evaluate the effect of various substrates composed of different soil combinations on leaf area development in *Moringa oleifera* Lam. and *Cedrela fissilis* Vell seedlings.

Materials and Methods: The research was carried out at the Institute of Agricultural Sciences of the Federal University of Minas Gerais, in Montes Claros, Brazil, under tropical savanna climate (Aw). *M. oleifera* seeds were sown without pretreatment; while *C. fissilis* seeds were hydrated 12 hours before. The experimental design included 12 treatments with different proportions of soil, organic matter and fertilizers, plus a commercial control, distributed in randomized blocks with four replications. Plants were grown in 170 cm³ conical containers, with controlled irrigation and initial shading. Physical and chemical indicators of the substrates were measured and leaf area was evaluated by digital image analysis with Image J.

Results: The results showed significant differences among treatments for both species. In *C. fissilis*, substrates with cattle manure and combined fertilization (superphosphate + KCl) produced the largest leaf area (129 cm² in T10); while sewage sludge generated lower values (67-70 cm²). The commercial substrate (Bioplant Plus) had the lowest performance (14,3 cm²). On the other hand, *M. oleifera* showed its largest leaf area (8,2 cm²) in substrates with manure and simple superphosphate (T2), but did not respond favorably to the addition of KCl. The commercial substrate showed moderate results in this species (52,8 cm²).

Conclusion: Substrate composition significantly influenced leaf development of the evaluated seedlings, with differentiated responses among species. *C. fissilis* benefited more from the use of cattle manure and mineral fertilization combined; while *M. oleifera* performed better with manure and superphosphate without KCl. Sewage sludge was less effective for *C. fissilis*, and locally formulated mixtures outperformed commercial substrate for both species, suggesting more effective and sustainable alternatives for forest nurseries under similar conditions.

Keywords: *Moringa oleifera*, *Cedrela fissilis*, substrata, plant nurseries

Introduction

The integration of trees and shrubs into agricultural agroecosystems is a viable practice in the face of the effects of the green revolution and climate change. For their establishment and generalization, nursery production is an alternative that ensures better development at early growth ages and guarantees success in reforestation programs, recovery of degraded areas and biodiversity conservation.

The species *Moringa oleifera* Lam. and *Cedrela fissilis* Vell, although with different purposes, can be integrated into systems for agricultural production. García *et al.* (2017) proved the potential of

M. oleifera for ruminant production as it has acceptable nutritional values, good palatability, adaptability to adverse conditions and high biomass production. In turn, *C. fissilis* contributes to improving the quality of life of farmers by providing timber, windbreaks, fuels, biodiversity conservation and promoting other environmental services such as reducing erosion and nutrient loss (Luna *et al.*, 2020).

The substrate used for seedling production significantly influences seedling development during the nursery stage (Abanto-Rodríguez *et al.*, 2016), as it directly influences root development, aerial biomass formation and, consequently, the

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successful establishment of plants in the field. However, studies aimed at defining procedures and technical recommendations for its elaboration show diverse results. The use of organic sources in the preparation of substrates is an environmentally sound practice and reduces production costs (Monsalve-Camacho *et al.*, 2021). However, research addressing plant physiology in response to different concentrations of these organic sources is still incipient.

In this sense, the objective of this work was to evaluate the effect of various substrates composed of different soil combinations on leaf area development in *M. oleifera* and *C. fissilis* seedlings.

Materials and Methods

Location. The research was conducted in areas of the laboratory of Biology and Management of Harmful Plants of the Institute of Agricultural Sciences of the Federal University of Minas Gerais in the Montes Claros municipality, Brazil; referenced at 16,72 ° South latitude and 43,87 ° West longitude at 647,2 m altitude. The climate, tropical savanna with dry winters, is classified as Aw according to Köppen (1936).

Studied species. The species *M. oleifera* and *C. fissilis* were studied. The *M. oleifera* seeds were

donated by the Harmful Plants group and were not subjected to any treatment before sowing; while those of *C. fissilis* were obtained through the State Forest Institute (IEF) and were soaked in water for 12 hours before sowing to favor the imbibition process.

Treatments and experimental design. Table 1 describes the materials, proportions and amount of fertilizer used in each of the evaluated treatments (substrates). The treatments corresponded to different combinations of soil (clayey or sandy), organic matter (cattle manure and sewage sludge) and mineral fertilizers (simple superphosphate and potassium chloride). In addition, as a control (treatment T13), the commercial substrate Bioplant Plus was evaluated, which is made using *Sphagnum* peat, coconut fiber, rice husk, pine husk and vermiculite. In separate experiments, for both species, a randomized block design with four replicas was used and each experimental unit consisted of five plants.

Experimental procedure. The collection of dried cattle manure was done directly in the pen of the experimental farm “Profesor Hamilton de Abreu Navarro” (FEHAN) of the Institute of Agricultural Sciences of the Federal University of Minas Gerais. The dehydrated sewage sludge, approved for use in different agricultural activities by CONAMA reso-

Table 1. Used materials, their proportion in relation to the volume and quantity of chemical fertilizers in the preparation of the substrates.

Treatment	Soil type	Sand	Cattle manure	Sewage sludge	Simple super-phosphate	KCl
T ₁ (3:1:1:0+SS)	Clayey	x	x	-	1 kg/m ³	-
T ₂ (3:0:1:0+SS)	Sandy	-	x	-	1 kg/m ³	-
T ₃ (3:1:0:1+SS)	Clayey	x	-	x	1 kg/m ³	-
T ₄ (3:0:0:1+SS)	Sandy	-	-	x	1 kg/m ³	-
T ₅ (3:1:1:0+KCl)	Clayey	x	x	-	-	1 kg/m ³
T ₆ (3:0:1:0+KCl)	Sandy	-	x	-	-	1 kg/m ³
T ₇ (3:1:0:1+KCl)	Clayey	x	-	x	-	1 kg/m ³
T ₈ (3:0:0:1+KCl)	Sandy	-	-	x	-	1 kg/m ³
T ₉ (3:1:1:0+SS+KCl)	Clayey	x	x	-	1 kg/m ³	1 kg/m ³
T ₁₀ (3:0:1:0+SS+KCl)	Sandy	-	x	-	1 kg/m ³	1 kg/m ³
T ₁₁ (3:1:0:1+SS+KCl)	Clayey	x	-	x	1 kg/m ³	1 kg/m ³
T ₁₂ (3:0:0:1+SS+KCl)	Sandy	-	-	x	1 kg/m ³	1 kg/m ³
T ₁₃ (control)	All the volume with commercial substrate (Bioplant Plus)					

SS: simple superphosphate; KCl: potassium chloride.

lution 375, was collected at the Sludge Treatment Station (ETE) in Montes Claros after preliminary treatment in an anaerobic UASB reactor and drying in a dehydrator to 5 % moisture.

Both species were planted in 170-cm³ rigid conical containers, arranged under shade cloths with the capacity to retain 30 % of sunlight. To guarantee the emergence of a plant in each container, four seeds were sown in each one. In the containers where more than one plant emerged, a thinning was carried out 15 days after sowing, leaving the most vigorous plant. All plants were placed under full sunlight 20 days after thinning and irrigation was carried out three times a day up to field capacity throughout the experiment.

The physical and chemical characterization of the substrates (table 2) was carried out according to EMBRAPA methodology. Centro Nacional de Pesquisa de Solos (1997) at the ICA/UFMG solid waste laboratory and pH, electrical conductivity (EC), moisture holding capacity (MHC), bulk density (BD) and estimated total porosity (TP), were evaluated.

Leaf area of seedlings in both species was evaluated at transplanting, 60 and 120 days after planting for *M. oleifera* and *C. fissilis*, respectively. In each experimental unit, the least developed plant was removed and all leaves of the other four were

scanned. Image processing for leaf area calculation was performed through the Image J 1.48s program (Schneider *et al.*, 2012).

Statistical processing. The data were subjected to the normality test (*Shapiro-Wilk*, 1965) and homogeneity of variance (O'Neill and Mathews, 2002). Statistical processing was performed in R environment (R Core Team, 2020) using the "Exp-Des.pt" library for analysis of variance (ANOVA), followed by Tukey's test at 5 % probability for mean comparison and the "Extended Boxplot Graphics" application for elaborating the graphs (Denis-Ávila and Ramírez-Arrieta, 2020).

Results and Discussion

The analysis of variance showed significant differences among treatments for the leaf area index of the seedlings in the two studied species (table 3). The substrates under study had different incidences in this indicator for the species *C. fissilis* and *M. oleifera* (figures 1 and 2). These results indicate that substrate composition directly influenced leaf development during the nursery stage.

Leaf area for *C. fissilis* was higher in all treatments. The treatment where sandy soil was combined with cattle manure plus the application of simple superphosphate and KCl (T10) achieved the largest leaf area (129 cm²) and did not differ from

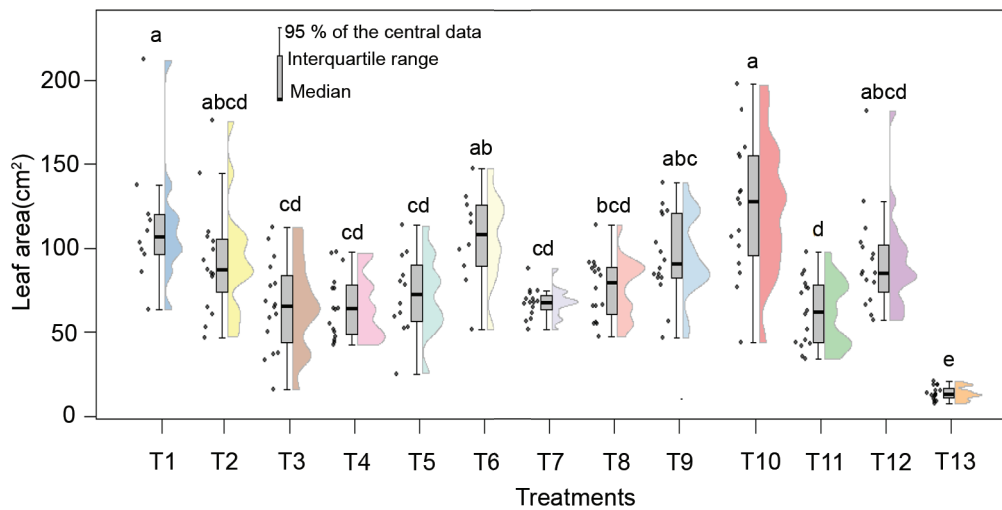
Table 2. Physical and chemical attributes of substrates made from different soil types and organic sources.

Treatment	Substrate attributes				
	Chemical		Physical		
	pH	EC, $\mu\text{s}/\text{cm}$	MHC, ml/g	BD, g/cm ³	TP, %
T ₁ (3:1:1:0+SS)	6,1	335,03	0,57	1,35	44,16
T ₂ (3:0:1:0+SS)	6,8	313,23	0,50	1,41	39,61
T ₃ (3:1:0:1+SS)	5,3	465,07	0,53	1,31	44,61
T ₄ (3:0:0:1+SS)	5,2	457,80	0,45	1,39	44,12
T ₅ (3:1:1:0+KCl)	6,1	501,53	0,53	1,27	48,74
T ₆ (3:0:1:0+KCl)	7,0	513,47	0,50	1,39	42,05
T ₇ (3:1:0:1+KCl)	5,7	456,30	0,50	1,36	44,36
T ₈ (3:0:0:1+KCl)	5,6	431,13	0,48	1,44	38,46
T ₉ (3:1:1:0+SS+KCl)	6,9	545,93	0,55	1,29	47,15
T ₁₀ (3:0:1:0+SS+KCl)	7,1	519,10	0,50	1,43	42,07
T ₁₁ (3:1:0:1+SS+KCl)	5,8	571,43	0,50	1,32	46,37
T ₁₂ (3:0:0:1+SS+KCl)	5,5	558,40	0,45	1,42	42,77
T ₁₃ (control)	5,7	500,00	0,77	0,36	69,37

SS: simple superphosphate; KCl: potassium chloride.

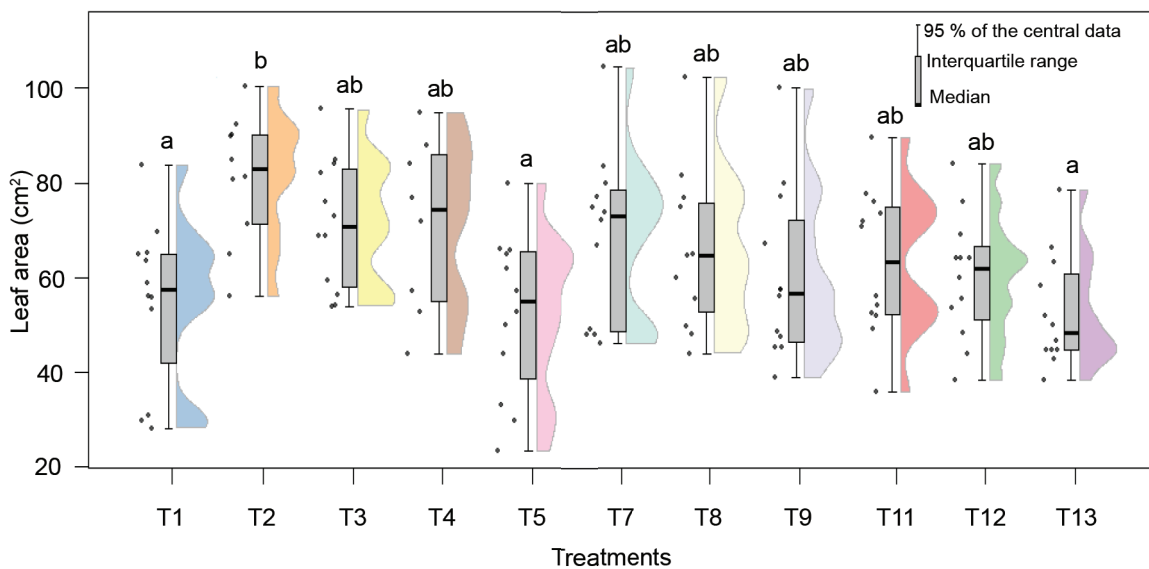
Table 3. Analysis of variance for leaf area index in tree seedlings growing on different substrates.

Source of variation	Species									
	<i>M. oleifera</i>					<i>C. fissilis</i>				
	GL	SC	CM	FC	P - value	GL	SC	CM	FC	P-value
Treatment	10	2 120,6	212,063	2,81695	0,02326	12	41 838	3 486,5	13,1008	0,00000
Blocks	2	82,1	41,058	0,54579	0,58777	3	207	69	0,2593	0,85418
Residues	20	1 504,6	75,228			36	9 581	266.1		
Total	32	3 707,3				51	51 626			
VC %			13,2					19,6		



Letters indicate significant differences among treatments according to *Tukey's test* ($p < 0,05$).

Figure 1. Leaf area in *C. fissilis* seedlings produced on different substrates



Letters indicate significant differences among treatments according to *Tukey's test* ($p < 0,05$).

Figure 2. Leaf area in *M. oleifera* seedlings produced on different substrates.

the treatments that used cattle manure as a source of organic matter (T1, T2, T6, T9 and T12). This suggests that cattle manure, in combination with mineral fertilizers, creates a favorable nutritional environment for this species. This response coincides with that observed by Gomes *et al.* (2002) in native species, where the addition of organic matter significantly improved the morphological characteristics of the seedlings.

In contrast, the treatments containing sewage sludge (T3, T4, T7 and T11) showed lower leaf area values (69,1; 67,3; 69,6 and 67,1 cm²). Despite its value as a soil conditioner, sewage sludge may show limitations associated with its chemical composition, such as high salinity, heavy metals or nutritional imbalances, which could negatively affect the initial development of plants (Singh and Agrawal, 2008).

It is worth noting that treatment T13, which used the commercial substrate Bioplant Plus, produced the lowest leaf area value (14,3 cm²), well below the locally formulated treatments. This calls into question the efficiency of some generic commercial substrates for specific native species, and supports the idea that substrates formulated with local materials may offer better performance, as also suggested by Benito *et al.* (2005).

Substrates combining clay soil, sand, cattle manure plus KCl (T6) and sandy soil, cattle manure, simple superphosphate plus KCl (T10) did not favor the growth and development of *M. oleifera* (figure 2). This may have been due to NPK ratio imbalances or to the sensitivity of this species to high chloride concentrations. This phenomenon agrees with that reported by Yao *et al.* (2010), who for other plant species found a reduction in growth when exposed to high doses of KCl. This suggests a possible ionic toxicity.

The substrate where sandy soil, cattle manure plus simple superphosphate were combined (T2) achieved the largest leaf area (8 224 cm²) as opposed to the substrate that combined clay soil, sand, cattle manure, simple superphosphate plus KCl (T9) (figure 2). This difference can be ascribed to the improvement in soil structure provided by cattle manure, which increases porosity and water retention in sandy soils, thus facilitating root development and nutrient uptake (Loehwing, 1931; Rayne and Aula, 2020). In addition, simple superphosphate (without additional KCl) provides bioavailable phosphorus without risk of negative ionic synergies (Khan *et al.*, 2023).

On the other hand, treatments using the commercial substrate Bioplant Plus showed the smallest leaf areas (54,2 and 52,8 cm²). These results are consistent with previous studies indicating that some commercial substrates may have physical and chemical properties that limit seedling development, possibly due to lower water holding capacity or suboptimal nutritional balance.

Conclusions

Substrate composition significantly influenced leaf development of *C. fissilis* and *M. oleifera* during the nursery stage, with differentiated responses observed according to the used combinations of soil, organic matter and mineral fertilizers. In *C. fissilis*, substrates incorporating cattle manure and mineral fertilization promoted greater leaf area development; while those containing sewage sludge showed limitations possibly associated with their chemical composition.

In *M. oleifera*, the best results were obtained with mixtures of sandy soil, cattle manure and simple superphosphate, with no significant improvement when KCl was added. Commercial substrates were less efficient than locally formulated substrates, suggesting that the use of regionally available materials represents a viable, economical and environmentally sustainable alternative for seedling production. These findings support the formulation of substrates adapted to native or multipurpose species and promote their application in forest nurseries, especially in low-resource contexts, where the use of local resources can optimize the quality of the produced plants.

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Conflict of interests

The authors declare that there is no conflict of interests among them.

Authors' contribution

- Jatnel Alonso-Lazo. Conceptualized, formulated and designed the research. Conducted the field research, compiled and elaborated the databases for statistical analysis and interpreted the results.

Prepared the original manuscript and final writing of the paper.

- Lázaro Castro-Hernández. Collected the data, processed and interpreted the results and final writing of the paper.
- Gustavo Amaral Costa. Intervened in the field activities and evaluations, as well as in the revision of the original manuscript.
- Maria Cecília Mota-Docha. Participated in the field activities and evaluations, as well as in the revision of the original manuscript.
- Iago Thomaz do Rosario Vieira. Involved in the field activities and evaluations, as well as in the revision of the original manuscript.
- Leonardo David Tuffi-Santos. Contributed to the conception of the experiment, participated in the statistical analyses and suggested ideas throughout the experiment execution process. He intervened in the revision of the manuscript and recommended modifications.
- Reginaldo Arruda-Sampaio. Led the project from which the research is derived. Participated in the design of the research, as well as in the interpretation of the obtained results. Intervened in the revision of the manuscript and recommended modifications.

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