SEASONALITY OF THE BARK TANNINS CONTENT OF FIVE-YEAR-OLD *ACACIA MANGIUM* TREES GROWN IN NORTHEAST BRAZIL

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ABSTRACT – This study aimed to assess the influence of the seasonality on the bark tannins content of *Acacia mangium* trees grown in the Northeastern Brazilian Region and the effect of soil preparation on the results. Two experimental plots of 1.0 ha each were submitted to different soil preparation methods, with two different intensities. The experimental design consisted of four treatments, two types of soil preparation and, two different bark collection seasons (end of the rainy and dry seasons). The bark of the trees was collected in each treatment and the contents of condensed tannins were determined. For each experimental treatment, 15 trees were harvested and debarked. Bark material was submitted to extraction with hot water, obtaining the total solids content (TSC), Stiasny index (I), and the condensed tannins content (CTC). There was no influence of the soil preparation method on the TSC, I, and CTC. However, there was a significant difference in these parameters for tree bark collected in the rainy season, with higher values. The less intensive soil preparation method is recommended due to its lower cost, and bark should be collected at the end of the rainy season for the best yield of condensed tannins.

Keywords: Condensed tannins; Rainy and dry season; Soil preparation methods

SAZONALIDADE DO TEOR DE TANINOS CONDENSADOS DA CASCA DE ÁRVORES DE CINCO ANOS DE IDADE DE ACACIA MANGIUM CULTIVADAS NO NORDESTE BRASILEIRO

RESUMO –O presente trabalho objetivou avaliar a sazonalidade do teor de taninos condensados na casca de árvores de ***Acacia mangium*** *cultivadas na Região Nordeste do Brasil e o efeito do preparo do solo nos resultados. Dois campos experimentais de 1,0 hectare cada foram submetidos a diferentes métodos de preparo de solo, com duas diferentes intensidades. O desenho experimental consistiu de quatro tratamentos, dois tipos de preparo de solo e duas épocas diferentes do ano (seca e chuvosa). As cascas das árvores foram coletadas em cada um dos tratamentos e os teores de taninos condensados foram determinados. Para cada tratamento, 15 árvores foram colhidas e descascadas. As cascas foram moídas e extraídas com água quente, obtendo-se o teor de sólidos totais (TST) extraídos, o índice de Stiasny (I) e o teor de taninos condensados (TTC). Não houve diferença estatística entre o TTC de árvores cultivadas com os dois tipos de manejo do solo. Entretanto, houve diferença estatística entre o TST, o I e o TTC, com valores maiores para as árvores colhidas no final da* *estação chuvosa. Para a produção de taninos, recomenda-se o manejo menos intensivo do solo em função do menor custo, com a coleta das cascas no final da estação chuvosa.*

Palavras-Chave: Taninos condensados; Estação seca e chuvosa; Métodos de preparo de solo

1. INTRODUCTION

Tannins have several industrial applications, such as plastic production, oil well drilling, water treatment (Nepomuceno et al., 2018), paint manufacture, wood adhesive production (Hoong et al., 2011; Zhou and Pizzi, 2014; Souza et al. 2020) and manufacture of pharmaceuticals (Carvalho et al., 2018), including fungicides and products to kill cariogenic bacteria (Araújo et al., 2018). In Northeastern Brazilian Region, tannins are used mainly for leather tanning (Paes et al., 2006).

According to Paes et al. (2006), other native tree species are also potential sources of tannins in Brazil, such as *Stryphnodendron adstringens* (Mart.) Coville (barbatimão), *Mimosa tenuiflora* (Mart.) Benth. (jurema-preta), *Mimosa arenosa* (Willd.) Poir. (jurema-vermelha), *Mimosa caesalpiniifolia* Benth. (sabiá) and *Anadenanthera colubrina* var. *cebil* (angico-vermelho). The last species is exclusively used to obtain tannins for leather tanning, but is threatened with extinction. Since the mentioned species grow slowly when planted in pure plantations and some of them are becoming scarce in nature due to deforestation, the need for species adapted to the region's climate and soil is pressing to provide tannins in industrial amounts for the uses listed above.

Acacia mangium is native to Malaysia. It and *Acacia auriculiformis* are the two most planted species of this genus. *A. mangium* is grown in more than 600,000 hectares of planted forests in the world, providing wood as raw material for pulp and paper, low-cost furniture, civil construction, plywood, firewood and charcoal (Souza et al., 2010). The species has fast growth, low nutritional requirements, is tolerant of acidic soils and compaction, and also has high nitrogen fixation rate.

The characteristics cited above result in high production of biomass per hectare (Matsumara, 2011) and high input of nutrients via litter where the trees are grown (Hedge et al., 2013). The species' fast growth occurs when submitted to unfavorable conditions, such as prolonged dry seasons and low-fertility soils, with pH around 4.0 (Souza et al.,2010; Broich et al., 2013). *A. mangium* is recommended to planting in agroforestry systems and also for honey production (Oliveira, 2017).

For these reasons, the species is also recommended for restoration of degraded areas and use for windbreak and shading in agroforestry and other forest management systems (Silva et al., 2018). In Brazil, planting of *A. mangium* is increased due to its good adaptability to the variable edaphoclimatic conditions of the country. However, despite producing good quality wood, *A. mangium* is mainly used as firewood, although it can be employed to produce wood-cement boards. The species is well adapted to edaphoclimatic conditions in many places Northeastern Brazilian Region (Silva et al., 2018).

A. mangium bark has tannins content that makes it viable for production of adhesives and leather tanning, though the content can change from one planting site to another (Paes et al., 2010). So, research is needed to ascertain the tannins content in the species' bark according to the growing site and silvicultural management strategy, as well as different soil preparation methods and harvest seasons.

This study aimed to assess the influence of seasonality on the bark tannins content of Acacia mangium trees grown in Northeastern Brazilian Region and the effect of soil preparation on the results.

2. MATERIAL AND METHODS

2.1. Description of the experimental plot

The study was conducted in a five-year-old forest stand of *A. mangium* located in the municipality of Macaíba, Rio Grande do Norte State, Northeastern Brazilian coastal region. The local climate has Köppen classification of transition from tropical to dry savannah (transition from As to BSw), with average temperature of 27 °C, maximum of 32 and minimum of 21 °C, average relative humidity of 76% and annual rainfall varying from 864 to 1,071 mm.

The local soil is classified as sandy yellow latosol with flat relief (Beltrão et al., 1975) with pH varying from 5.06 to 5.32 (Silva, 2018). The total area was divided into two plots of 1.0 ha each. Two soil preparation methods were applied (T1 and T2), each in one of the areas, as reported in Table 1.The first method (less intensive) involved only opening pits to plant *A. mangium* seedlings with 3.0 m x 3.0 m spacing. The second method (more intensive) consisted in the opening of furrows with 40 cm depth x 70 cm width. The furrows were fertilized with cattle manure (4.0 t ha^{-1}) and triple superphosphate (2.0 t ha^{-1}).

Seedlings were planted with the same spacing, and two months after planting, 2.0 t ha^{-1} of limestone was applied. In both experimental plots, 100 g of NPK (6-30-6) was applied in two lateral holes (5 cm diameter x 10 cm depth) positioned diametrically opposite about 15 cm from the seedling. From the first year of planting until the fifth year, the diameter at breast height (DBH), total height and trunk height were measured of all trees of the two experimental plots, to determine the influence of the soil preparation method on the silvicultural performance, according to the volume of wood and bark per hectare.

2.2. Tannin extraction and qualification

Thirty trees were harvested from each parcel and debarked, corresponding to 15 trees per experimental treatment. The collected material was stored in plastic bags. Samples were used to determine the moisture content of the bark samples. Then the material was placed in a climate-controlled room at 25 ± 2 °C and relative humidity of 65± 5% for drying until reaching moisture equilibrium, around 12 – 15%. After 25 days, the bark was ground in a forage crusher equipped with a 2 mm sieve. For tannin extraction, a proportion (mass : volume) of distilled water and bark equal to 1:10 was employed.

Table 1 – Description of the soil management methods applied in the experimental plots.
Tabela 1 – *Métodos de preparo de solo utilizados nas parcelas do experimento.*

Description	Soil Management Method	
	Less Intensive	More Intensive
Cross Harrowing	x	x
Furrows (40 cm x 70 cm)		x
Cattle Manure (4.0 t ha^{-1})		x
Triple Superphosphate (146 kg ha^{-1})		x
Planting Pits (20 cm x 20 cm x 15 cm)	x	x
6-30-6 NPK (100 g $plant^{-1}$)	x	x
Limestone (2.0 t ha^{-1})		x

The collected material was placed in a 12 L stain less steel pan inside a laboratory autoclave at a temperature of 120 °C for 1 hour. The extraction was carried out two times for each of 10 bark batches. For all batches, the extract was filtered to eliminate fine particles. The extracts were put together to form a composite sample and then placed in stainless steel trays, which were left in a solar oven until complete evaporation of water.

After that, the powder was ground with a porcelain mortar and pestle and sieved to granulometry of 60 mesh. This material was used for later formulation of the adhesive. Before evaporation, 50 mL aliquots of the crude extract were taken to determine the total solids content (TSC), Stiasny number (I) and condensed tannins content (CTC), with four replicates for each parameter. To measure the CTC, the 50 mL aliquots of the crude extract were oven dried at 60 ± 2 °C and their total solids content (TSC) was calculated (Equation 1).

$$TSC=(M1-M2/M1)x\ 100 \qquad \text{Eq. 1}$$

Where: TSC - Total solids content in 50 mL of crude extract (%); M1 - initial mass of crude extract (g); M1 - final mass of solids after evaporation (g).

To obtain the total condensed tannins content (TTC), first the Stiasny number (I) was determined in the extracts by the method described by Guangcheng et al. (1991), with four replications. For this, 4 mL of formaldehyde (37% volume : volume) and 1mL of concentrated hydrochloric acid were added to 50 mL of crude extract. The mixture was kept under reflux for 30 min. After this time, the mixture was cooled and filtered and the solids were oven dried at a temperature of 60 ± 2 °C for 48 h. The dried material was weighed and the Stiasny number was calculated (Equation 2). With the Stiasny number, the condensed tannins content was calculated by using Equation 3 and expressed as percentage of dry mass of bark.

$$I=(M2/M1)x\ 100 \qquad \text{Eq. 2}$$

Where:

I – Stiasny number (%)

M1 - mass of solids in 50 mL of crude extract (g)

M2 - mass of tannin-formaldehyde precipitate (g).

CTC=TSC x I/100 Eq. 3

Where:

CTC – condensed tannins content (%)

TSC - total solids content (%)

I - Stiasny number (%).

2.3. Experimental design and statistical analysis

The experiment design was completely randomized considering four treatments: two types of soil preparation (T1 and T2) and two seasons for bark collection (rainy and dry season, R1 and R2) with the objective of evaluating the influence of these independent variables on the tannins content of the bark of *A. mangium*, with 15 replicates (trees) per experimental treatment.

For statistical analysis, the values of total solids content (TSC), Stiasny number (I) and condensed tannins content (CTC) were converted in arcsine [$\sqrt{x/100}$] to homogenize the variances, as suggested by Steel & Torrie (1980). Experimental means were compared by the T-test ($p < 0.05$). All statistical analyses were carried out with the Infostat software.

3. RESULTS

According to the experimental data listed in Table 2, there were no statistically significant differences of the dependent variables total solids content (TSC), Stiasny number (I) and condensed tannins content (CTC) in function of the type of soil preparation.

As reported in Table 3, the values of total solids content (TSC), Stiasny number (I) and condensed tannins content (CTC) as a function of the season when the bark was collected.

Table 2 – Total solids content (TSC), Stiasny number (I) and condensed tannins content (CTC) of *A. mangium* bark as a function of the type of soil preparation.

Tabela 2 *– Teor de sólidos totais (TSC), índice de Stiasny (I) e teor de taninos condensados (CTC) da casca de* ***A. mangium*** *em função da época de colheita na área com preparo de solo menos intensivo.*

Soil Preparation Method	Parameter (%)		
	TSC	I	CTC
More intensive	14.80 a	83.89 a	12.41 a
Less intensive	15.33 a	95.26 a	14.60 a

Means followed by same letters in columns do not differ by the T-test (p > 0.05)

Médias seguidas por letras iguais não diferem entre si pelo teste T (p < 0.05)

Table 3 – Total solids content (TSC), Stiasny number (I) and condensed tannins content (CTC) of *A. mangium* bark as a function of the season of collection in an area with less intensive soil preparation.

Tabela 3 *– Teor de sólidos totais (TSC), índice de Stiasny (I) e teor de taninos condensados (CTC) da casca de* ***A. mangium*** *em função da época de colheita na área com preparo de solo menos intensivo.*

Season	Parameter (%)		
	TSC	I	CTC
Rainy	14.80 a	83.89 a	12.41 a
Dry	13.84 a	49.26 b	6.81 b

Means followed by same letters in columns do not differ by the T-test (p > 0.05)

Médias seguidas por letras iguais nas colunas não diferem entre si pelo teste T (p < 0.05).

The statistical comparison of diameter at breast height (DBH) and tree and trunk height as a function of the soil preparation method is illustrated in Figure 1. There was a significant difference only between the means of DBH, with the highest value observed for the less-intensive method.

The production of biomass (wood and bark) and tannins in tons ha-1 is displayed in Figure 2. No statistically significant differences between the

Means in each pair of bars followed by different letters differ by the T-test (p < 0.05).

Médias em cada par de barras seguidas por letras diferentes diferem entre si pelo test T (p < 0.05)

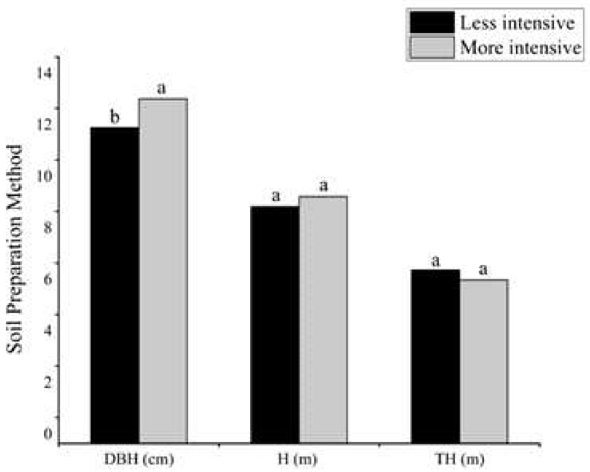

Figure 1 – Statistical comparison of diameter at breast height (DBH), tree height (H) and trunk height (TH) of 5-year-old trees of *A. mangium* as a function of the soil preparation method.

Figura 1 *– Comparação de médias de diâmetro na altura do peito (DBH), altura da árvore (H) e altura do tronco comercial (TH) das árvores de* ***A. mangium*** *em função do método de preparo de solo.*

Means in each pair of bars followed by similar letters do not differ by the T-test ($p > 0.05$).
*Tannins' quantity determined at the end of the rainy season.
Médias em cada par de barras seguidas por letras iguais não diferem entre si pelo test T ($p < 0.05$).
**Quantidade de taninos determinada no fim da estação chuvosa*

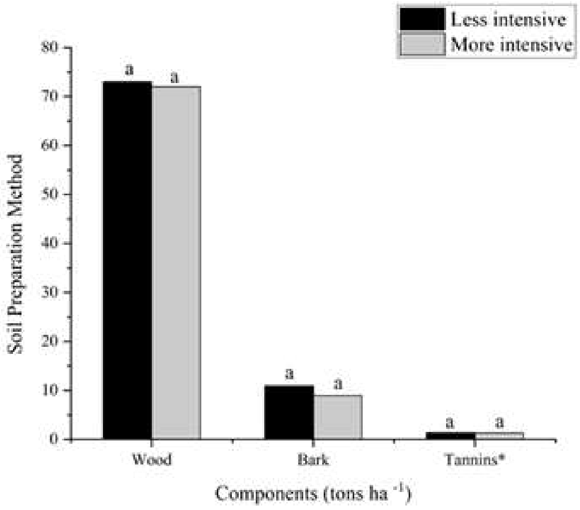

Figure 2 – Statistical comparison of wood, bark and tannins production of 5-year-old trees of *A. mangium* as a function of the soil preparation method.
Figura 2 – *Comparação de médias de produção de madeira, casca e taninos de árvores de* ***A. mangium*** *em função do método de preparo de solo.*

parameters were observed, although higher amounts of wood and condensed tannins,with values of 73.0, 10.95 and,1.36 tons ha^{-1}, were determined respectively in the experimental plot submitted to the less-intensive soil preparation method.

4. DISCUSSION

So, for economic reasons the soil preparation with lower cost should be chosen (Table 2). According to Santos et al. (2012), soil acidity decreases the availability of nutrients to plants and it consequently can result in lower biomass production along with a higher content of bark extractives, which are responsible for tree defense (Rossel, 2019).

However, the less intensive soil preparation did not lead to the expected behavior regarding higher tannins content in the bark. There are virtually no reports in the literature establishing clear correlations between soil pH and either extractives or tannins content in tree bark. Nevertheless, some studies have related soil type and tannins content in plants or their parts of it. For example, Sturion et al. (2004) assessed the contents of total polyphenols, tannins and caffeine in progenies of yerba mate (*Ilex paraguariensis*) grown in three soil classes (haplic nitisol, brown ferralsol and humic cambisol) and observed that the quantity of tannins was not influenced by the variation of soils.

Judging by the soil nomenclature solely, only inferences can be made, but according to the Food and Agriculture Organization - FAO (2015), there is a difference in the natural pH of soils, with nitisols, acrisols and ferralsols usually having higher acidity while cambisols, planosols and regosols have neutral or alkaline pH. Sturion et al. (2004) cultivated the plants in two naturally acidic soils (haplic nitisol and brown ferralsol) and in one with neutral or alkaline pH (humic cambisol), and did not observe differences in the tannins content, indicating that soil pH was not a determinant of that parameter in yerba mate.

On the other hand, Chavarria et al. (2011), to grapevines grown in three types of soil (grayish brown acrisol, planosol and regosol), observed higher amounts of tannins in the seeds and peel of the grapes grown in the last soil type, which usually has neutral or alkaline pH. However, the planosol and regosol had similar pH, so and once again this property was not a determinant of a higher content of tannins in the fruits. As shown in Table 2, the correction of the ferralsol pH from acid to alkaline by liming made no difference in the tannins content in the bark of *A. mangium*.

There was no statistically significant difference in the total solids content (TSC), with values of 14.80 and 13.84% for rainy and dry season, respectively (Table 3). Although statistically the same, their composition was very different from one season to another, since the Stiasny number was 83.89% at the end of the rainy season, double the value one obtained at the end of the dry season (49.26%). Likewise, the total condensed tannins content in the bark of *A. mangium* was 12.41% at the end of the rainy season and only 6.81% at the end of the dry season. Similar behavior was found by Azevedo et al. (2017), who studied the best time of the year (rainy or dry season) to collecting bark of *Mimosa tenuiflora* for extracting tannins.

In their study, Azevedo et al. (2017) observed that the total solids content did not differ statistically from one season to the other but the condensed tannins contents were significantly higher in the bark collected at the end of the rainy season. Jacobson et al. (2005) also observed higher tannins contents in the bark of two species of *barbatimão* (*Stryphnodendron adstringens* and *S. polyphyllum*) collected in the rainy season compared to material sampled in the dry season. According to Vital et al. (2001) and Paes et al. (2010), this behavior may be related to a strategy to protect the fruits, so that at the time of fruiting, the tree directs all tannins to them. Flowering and fruiting of *A. mangium* can vary along the year as a function of geographical location. In some places, the trees produce flowers and fruits throughout the year (Sedgley et al., 1992).

Coincidentally, flowering and fruiting of *A. mangium* occurred in the dry season at the same time when the forest stand was sampled. This timing of flowering and fruiting is usual for this species in the coastal region of Northeastern Brazil, where the experiment was conducted. As pointed out by Paes et al. (2010), tree species with condensed tannins content higher than 10% in the bark have potential for commercial exploitation for both tannins and firewood. The trees of *A. mangium* presented CTC of 12.41% in the bark, but this value was only achieved for the plants harvested at the end of the rainy season. Therefore, the collection of the bark from this species should be carried out at that time of the year for the best industrial yields of condensed tannins.

In an experiment assessing the production of biomass of *A. mangium* as a function of planting space, Tonini et al. (2018) found a value of 26.4 tons ha^{-1} of wood produced with spacing of 3.0 x 2.0 m at the age of four years. That is the same spacing used in the present study, but the trees were one year younger. Those authors varied only the spacing in their experiment and applied fertilization on all the experimental plots. Nevertheless, even with heavy fertilization, the wood production observed by those authors reached less than half the total in the present experiment (73.0 tons ha^{-1} as Figure 2).

This difference can possibly be attributed to variations in origin and edaphoclimatic conditions of the planting site. Other references can be cited to establish a parallel of differences concerning productivity of *A. mangium* as a function of differences of growing sites. For example, Tsai (1986) determined a total biomass value of 82.1 tons ha-1 for 4.5-year-old trees grown in a forest stand established in Sarawak (Malaysia) with planting density of 1,084 trees ha^{-1}. This total biomass value was close to that determined here (82.5 tons ha^{-1}). However, we used a higher planting density (1,667 trees ha^{-1}) and found greater biomass of wood plus bark.

Since more intensive soil preparation costs more, in the case of *A. mangium* cultivated in the Northeastern Brazilian coastal region, the best option is the less-intensive soil management, as demonstrated here. There were no differences in both wood and tannins production when the more-intensive method was applied. So, an acceptable amount of condensed tannins can be collected from bark of trees cultivated with less-intensive soil preparation.

5. CONCLUSIONS

For *A. mangium* trees grown in the Northeastern Brazilian coastal region, the wood and tannin production were not influenced by the soil preparation method. However, the tannins content was significantly affected by harvest timing, with higher production observed at the end of the rainy season. So, the best option for cultivation of *A. mangium* in that region is the less-intensive soil preparation method associated with a bark collection conducted in the rainy season.

6. AUTHOR CONTRIBUTIONS

TKB de Azevedo and JB de Souza conceptualized and supervised the experiment; JB de Souza was in charge of experiment the organization, data collection, statistical analysis and wrote the initial draft of the manuscript; JPS Gomes, JGU Meza Filho, and BR da Silva assisted the experiment organization and data collection; and AS Pimenta revised the statistical analysis, finalized the manuscript and translated it from Portuguese to English. An American native speaker proofread the final version of the manuscript. All authors approved the final version of the manuscript.

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