EFFECT OF THINNING ON VOLUMES OF BIOMASS AND BARK TANNINS CONTENT OF *Mimosa caesalpiniifolia* BENTH. TREES

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ABSTRACT – This research aimed to evaluate the effect of thinning on the wood volumes per hectare on the tannin content in the bark of *Mimosa caesalpiniifolia*. The planting was subdivided into two plots, one thinned at 12 and 55 months of age (T1), while the other plot was kept intact (T2). The dendrometric variables were measured, and ten trees were felled, five in each plot. Subsequently, wood and bark's volume, mass, and moisture content were determined. Then, the total solids content (TST), the Stiasny index (I), and the condensed tannin content (TTC) were quantified. Dry wood productivity was statistically different between the two treatments, with values of 26.7 and 22.8 t ha^{-1} for T1 and T2, respectively. The percentage of dry bark corresponded to 16% of the total biomass for both treatments. The only variable that showed a significant difference was I, with values with and without thinning, 59.83 and 79.31%, respectively. Therefore, it was verified that the way the thinning was used changed the I and how they were conducted, favoring the emission of boles and increasing the frequency in the lower diametric classes instead of favoring the increase in DBH. It is concluded that thinning interferes with the biomass volumes and the Stiasny index of *M. caesalpiniifolia*. However, it does not alter the concentration of tannins present in the species' bark.

Keywords: Secondary metabolites; Dry forests; Silvicultural practices.

EFEITO DO DESBASTE SOBRE OS VOLUMES DE BIOMASSA E O TEOR DE TANINOS CONDENSADOS DA CASCA DE Mimosa caesalpiniifolia *BENTH.*

RESUMO – Este trabalho teve por objetivo avaliar o efeito do desbaste sobre os volumes de madeira por hectare e no teor de taninos na casca de ***Mimosa caesalpiniifolia****. O plantio foi subdividido em duas parcelas, uma que sofreu desbaste aos 12 e aos 55 meses de idade (T1), enquanto a outra foi mantida intacta (T2). Foram realizadas medições das variáveis dendrométricas e, em seguida, abatidas dez árvores, sendo cinco em cada uma das parcelas. Posteriormente, determinou-se o volume, a massa e o teor de umidade, da madeira e da casca. Em seguida, foi quantificado o teor de sólidos totais (TST), o índice de Stiasny (I) e o teor de taninos condensados (TTC) na casca. A produtividade de madeira seca foi estatisticamente diferente entre os dois tratamentos com valores de 26,7 e 22,8 t* ha^{-1} *para T1 e T2, respectivamente. A porcentagem de casca seca correspondeu a 16% da biomassa total para ambos os tratamentos. A única variável que apresentou diferença significativa foi o I, sendo os valores com e sem desbaste de 59,83 e 79,31%, respectivamente. Portanto, verificou-se que a forma em que o desbaste foi empregado altera o I e a massa de madeira seca, porém não alterou a concentração de taninos presentes na casca da espécie.*

Palavras-Chave: Metabólitos secundários; Floresta secas; Práticas silviculturais.

1. INTRODUCTION

Plants' metabolism produces various metabolites, classified as primary and secondary. Primary metabolites are the essential compounds for plants growth and development, such as sugars, lipids, nucleotides, amino acids, and fatty acids, as well as larger molecules synthesized from them, such as proteins, polysaccharides, DNA, and RNA (Taiz et al., 2017). The secondary metabolites, called specialized, are particular and generally belong to the classes of terpenes, phenolic compounds, or alkaloids (Thakur et al., 2019). These metabolites are active in the interface between the primary metabolism and the environment (Taiz et al., 2017).

Condensed tannins, for instance, are phenolic compounds responsible for the defense of plants and are widely distributed in the plant kingdom. They are present as components in wood, bark, leaves, and fruits, protecting them against bacteria, fungi, viruses, and herbivorous attacks, mainly due to the astringent flavor, repulsive odor, and ability to intoxicate animals (Poyer et al., 2015; Suvanto et al., 2017). Condensed tannins are best known for their use in leather tanning, but they have many other uses, such as medicinal formulations (Araújo et al., 2018; Ucella-Filho et al., 2022); adhesives for wood bonding (Souza et al., 2020), and treatment of water and effluents (Bello et al., 2020), among others.

In the Brazilian Northeast, the forest species *Mimosa caesalpiniifolia* has been intensively cultivated in recent decades for multiple uses, especially for firewood, fence posts, forage, and agroforestry systems (Silva et al., 2021; Primo et al., 2021). According to Rebouças et al. (2018), it is the most promising native species for reforestation in the region, mainly due to its fast growth, high biomass productivity, good drought resistance, and carbon fixation ability. Other properties, such as high wood density, higher calorific value, and contents of volatile matter and ash, allow the species to be used for energy generation (Santos et al., 2013; Batista et al., 2020). Besides these features, *M. caesalpiniifolia* has a good capacity for regeneration after harvesting (Podadera et al., 2015), and its bark has a high content of condensed tannins (Marques et al., 2021). Gonçalves et al. (2010) determined that, on average, bark corresponds to 7.5% of the harvested trunk. According to the same authors, the species can provide around 4.2 t ha 1 of condensed tannins.

In the cultivation of *M. caesalpiniifolia*, one of the most common management methods is thinning, which consists of reducing the number of shoots or trunks per hectare so the remaining ones can develop more vigorously due to the increased availability of light, growing space, and nutrients per plant (Saarinen et al., 2020; Rio et al., 2017). However, thinning must be carried out judiciously since it affects the growth rate of the tree due to the decrease of the foliar area (Tonini and Halfeld-Vieira, 2006), thus negatively impacting the production of metabolites. The production and concentration of plants' metabolites can also be affected by altitude, soil nutrients and water availability, seasonality, mechanical induction, and pathogen attack. Under stressful conditions, plants can modify their biosynthetic pathways and produce compounds according to changes in environmental conditions (Li et al., 2020).

Therefore, silvicultural practices can influence, for example, the concentration of tannins in trees. However, only objective studies comparing the concentration of these compounds before and after thinning can indicate the extent of these changes. Based on these existing gaps in the literature regarding the effect of thinning on the production of wood and chemical products from the bark of trees, this research aimed to determine the influence of thinning on biomass productivity and tannin concentration in the bark of *M. caesalpiniifolia*.

2. MATERIAL AND METHODS

2.1. Description of the experimental plots

Trees were harvested in a Forest Experiment Area from a 7-year-old stand of *M. caesalpiniifolia* (sabiá), located in the municipality of Macaíba, Rio Grande do Norte, Brazil at 5°53'52.55" S e 35°21'31.80" W. The local climate is tropical rainy according to the Köppen classification, transitioning between As and BSw types. The rainy season lasts from February to May, and annual rainfall ranges from 864 to 1,071 mm. The average yearly temperature is 27.1°C, with a minimum of 21°C and a maximum of 32 °C (Alvares et al., 2014). In the study forest stand's central point, the height above sea level is 54 m, and the relief is flat. Local soil is classified as sandy-textured quartzarenic neosol having low natural fertility.

The *M. caesalpiniifolia* trees were planted with a 3.0 m x 2.0 m spacing. The plantation was subdivided into two plots of 200 trees each. The plots were subjected to different silvicultural practices. The trees were thinned at 12 and 55 months in one of them. All stems were removed except for the main stem in the plot, where there was thinning. Only one stem was kept (per tree), and the others were removed. *M. caesalpiniifolia* is characterized by the intense production of several trunks from a single root system, and the thinning was carried out to leave only one trunk for each root system. In the other plot, no thinning was conducted, and all trunks remained unaltered. Five trees were selected and harvested from each plot in June 2018, based on selection criteria of vigor and absence of pest attacks and diseases.

2.2. Experimental parameters

Tree diameters were measured at the base (BD) and breast height (DBH). The total height was measured of all individuals in the two plots of 500 m^2. In the non-thinned plot, all forks were considered trunks of the same tree at harvesting time, and their diameters were added. Five trees were collected from each plot and cut into 1-m sections. The Smalian method was employed to calculate the solid wood volume. After sectioning, the logs were piled to determine the stacked volume. Each log was weighed to determine the green moisture content. Then, the logs were debarked, and discs with a thickness of 10 cm were cut to determine the percentage of bark using the Smalian method. Likewise, the dry basis moisture content of both bark and debarked wood was determined for all logs. To measure the green moisture content, the disks were weighed and oven-dried at 103 ± 2 °C until reaching constant weight, when they were weighed again to determine their dry weight. The same procedure was adopted to determine the green moisture content of the bark.

2.3. Extraction and quantification of tannins

The bark volume from both experimental plots was reduced by sequential quartering until ten samples weighing 5 kg came from each plot. The bark samples were then shredded until they reached a sizeable particle to pass through a 2.0 cm mesh sieve. After this, the material was ground in a Wiley mill and sieved to obtain two granulometries, 1.0 mm and 0.25 mm, respectively. For tannin extraction, a proportion (mass/mass) of distilled water and bark equal to 1:10 was employed. The material was placed in a 12 L stainless steel pan inside a laboratory autoclave at a temperature of 100 °C for 2 hours. The extraction was carried out two times for each bark batch. For all batches, the extract was filtered to eliminate fine particles. The extracts were pooled to form a composite sample and then placed in stainless steel trays and left in a solar oven until complete evaporation of the water. After that, the powder was ground with a porcelain mortar and pestle and sieved to a granulometry of 60 mesh. Before evaporation, 50 mL aliquots of the raw extract were taken to determine the total solids content (TSC), Stiasny index (I), and condensed tannins content (CTC), with four replicates for each parameter. The raw extract was oven-dried at 60 ± 2 °C, and their total solids content (TSC) was calculated using Equation 1.

$$TSC = \left(\frac{M1 - M2}{M2}\right) x\ 100 \quad \text{Eq.1}$$

Where:

TSC = Total solids content in 50 mL of raw extract (% on dry basis)

M1 = initial mass of crude extract (g)

M2 = final mass of solids after evaporation (g)

First, to ascertain the total condensed tannins content (TTC), the Stiasny number was determined in the extracts by the method described by Guangcheng et al. (1991), with four replications. Four mL of formaldehyde (37% m/m) and 1 mL of concentrated hydrochloric acid were added to 50 mL of raw extract. The mixture was kept under reflux for 30 min. After that, the mixture was cooled and filtered, and the solids were oven-dried at 60 ± 2 °C for 48 h. The dried material was weighed, and the Stiasny number was calculated by Equation 2.

$$I = \left(\frac{M2}{M1}\right) x\ 100 \quad \text{Eq. 2}$$

Where:

I = Stiasny number (%)

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

M2 = mass of tannin-formaldehyde precipitate (g)

With the Stiasny index, the condensed tannins content was calculated using Equation 3 and is reported as a percentage of the initial dry bark weight.

$$CTC = \frac{TSC \times I}{100} \quad \text{Eq. 3}$$

Where:

CTC = condensed tannins content (%)

TSC = total solids content (%)

I = Stiasny index (%)

2.4. Experimental data analysis

A completely randomized design was applied to evaluate the effect of thinning on dendrometric characteristics, wood production, and condensed tannins in the bark of *M. caesalpiniifolia* trees. For the statistical analysis of the CTC, experimental data were transformed into arcsine $\sqrt{CTC/100}$. This transformation is recommended by Steel and Torrie (1980) and is required to homogenize the variances and permit data analysis. The collected data were tabulated and statistically analyzed using the InfoStat software, subjected to analysis of variance (ANOVA), and, when different from each other, the Tukey test was applied at 5% significance. Graphs were generated in Origin® 2018 analysis software, Massachusetts, USA. Amplitude of diameter classes in the frequency distribution of trunks was defined by the Sturges methodology (Sturges, 1926).

3. RESULTS

Figure 1 displays the statistical comparison between original and thinned plots of *M. caesalpiniifolia*.

*Means followed by different letters are statistically dissimilar by the T-test at a 95% probability.
**Médias seguidas por letras diferentes são estatisticamente diferentes pelo Teste de Tukey a 95% de probabilidade.*

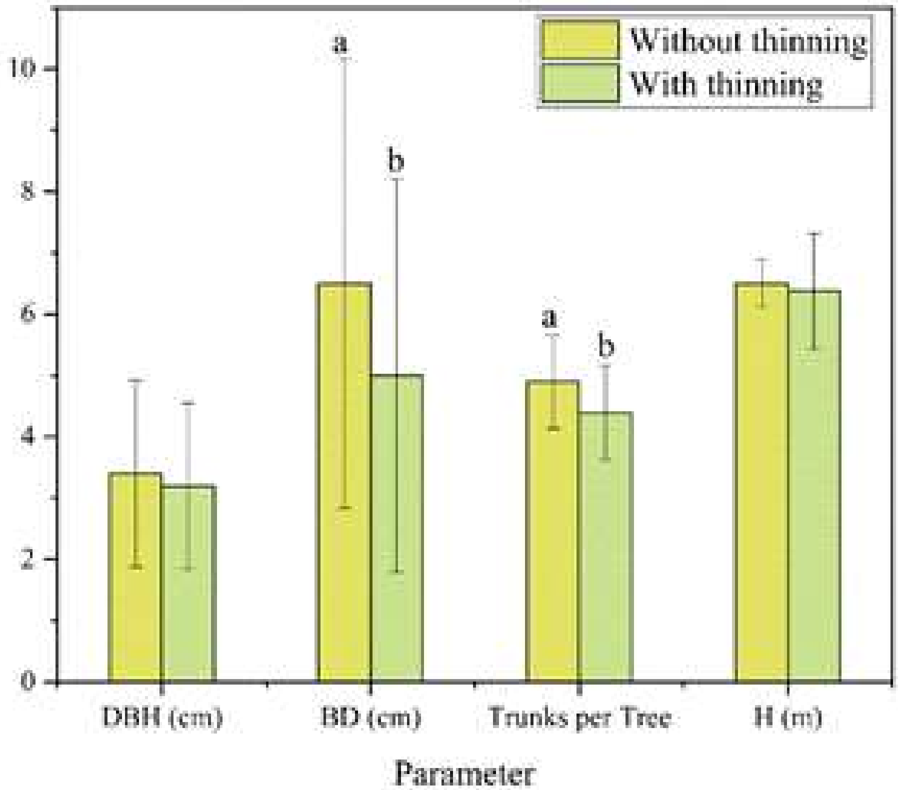

Figure 1 – Mean values of diameter at breast height (DBH), basal diameter (BD), number of trunks per tree, and total height (H) for 85-month-old *Mimosa caesalpiniifolia* subjected to two types of management.
Figura 1 – *Valores médios de diâmetro à altura do peito (DAP), diâmetro basal (BD), número de troncos por árvore e altura total (H) de* ***Mimosa caesalpiniifolia****, aos 85 meses de idade, submetida a dois tipos de manejo.*

Table 1 – Distribution of trunks per diametric class for *M. caesalpiniifolia* subjected to two types of management.
Tabela 1 *– Distribuição de troncos por classe diamétrica para* ***M. caesalpiniifolia*** *submetida a dois tipos de manejo.*

DBH class (cm)	With thinning		Without thinning	
	Trunks ha^{-1}	%	Trunks ha^{-1}	%
<2	1,666.7	16.4	1,966.7	16.6
2-3	3,433.3	33.9	3,700.0	31.3
3-4	2,266.7	22.4	2,400,0	20.3
4-5	1,933.3	19.1	2,033.3	17.2
5-6	366.7	3.6	900.0	7.6
6-7	400.0	3.9	466.7	3.9
7-8	0.0	0.0	200.0	1.7
8-9	33.3	0.3	133.3	1.1
9-10	0.0	0.0	33.3	0.3
>10	33.3	0.3	0.0	0.0
Total	10,133.0	100.0	11,833.0	100.0

Table 2 – Tree volume with (V_{cc}) and without bark (V_{sc}), form factor, cubed volume with (V_{cc}) and without bark (V_{sc}), bark volume (V_c), and bark percentage of *M. caesalpiniifolia* subjected to two types of management.
Tabela 2 *– Volume de árvores com (V_{cc}) e sem casca (V_{sc}), fator de forma, volume cubado com (V_{ccc}) e sem casca (V_{csc}), volume de casca (V_c) e porcentagem de casca de* ***M. caesalpiniifolia*** *submetida a dois tipos de manejo.*

Experimental Plot	Tree Volume			Cubed Volume			
	V_{cc}(m^3 ha^{-1})	V_{sc}(m^3 ha^{-1})	Formfactor	V_{ccc} (m^3) ha^{-1})	V_{csc}(m^3 ha^{-1})	V_c (m^3 ha)	Bark %
With thinning	62.6 b	49.8 a	0.58 ± 0,28	36.3 b	28.9 a	7.4 a	20.4 a
Without thinning	84.9 a	67.7 a	0.50 ± 0,08	42.5 a	34.0 a	8.5 a	20.1 a

*Means followed by the same letters in the columns are statistically dissimilar by the T-test at a 95% probability.
**Médias seguidas pelas mesmas letras nas colunas são estatisticamente iguais pelo Teste T a 95% de probabilidade.*

Table 3 – Mean values of green wood mass, moisture content of green wood, dry wood mass, green bark mass, moisture content of green bark, and dry bark mass for *M. caesalpiniifoli*a subjected to two types of management.
Tabela 3 *– Valores médios de massa de madeira verde, teor de umidade de madeira verde, massa de madeira seca, massa de casca verde, teor de umidade de casca verde e massa de casca seca para* ***M. caesalpiniifolia*** *submetida a dois tipos de manejo.*

Experimental Plot	Green Wood (t ha^{-1})	TU%	Dry Wood (t ha^{-1})	Total(%)	Green Bark (t ha^{-1})	TU (%)	Dry bark (t ha^{-1})	Total (%)
With Thinning	38.4	40.4	22.8	83.8	18.8	76.6	4.4	16.2
Without Thinning	44.8	40.4	26.7	83.8	22.1	76.6	5.2	16.3

Table 1 presents the absolute and relative frequencies of the trunks of *M. caesalpiniifolia* as a function of the diametric class (DBH).

Table 2 reports the tree volume (with and without bark), form factor, tree cubed volume (with and without bark), and bark volume for the experimental plots of *M. caesalpiniifolia* managed without and with thinning.

Table 3 reports the mean values per hectare of wood mass with and without bark.

Figure 2 presents the total solids content, Stiasny number, and condensed tannins content.

4. DISCUSSION

As observed in Figure 1, the basal diameter (BD) was statistically different between the two plots. The values observed for the DBH and H of trees from the thinned plot are lower than those determined by Barros et al. (2010), which were 2.53 and 5.87 cm for the first parameter and 7.10 to 9.25 m for the second in a 6.5-year-old forest stand established in a Brazilian dry tropical forest. Regarding the BD, the results were other than expected with the non-thinned trees, with higher values than the thinned ones. This behavior was probably related to the emission of secondary trunks after the thinning operation since the species presents a strong regrowth ability. Therefore, since only two thinning operations were performed, the thinned trees may have allocated resources to produce more trunks rather than to increase the diameter of existing trunks. According to Maia (2012), it is common for the species to grow several trunks from a single root system. Also, Moura et al. (2006) reported this behavior for *M. caesalpiniifolia* when submitted

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Médias seguidas por letras diferentes são estatisticamente diferentes pelo Teste de Tukey a 95% de probabilidade.

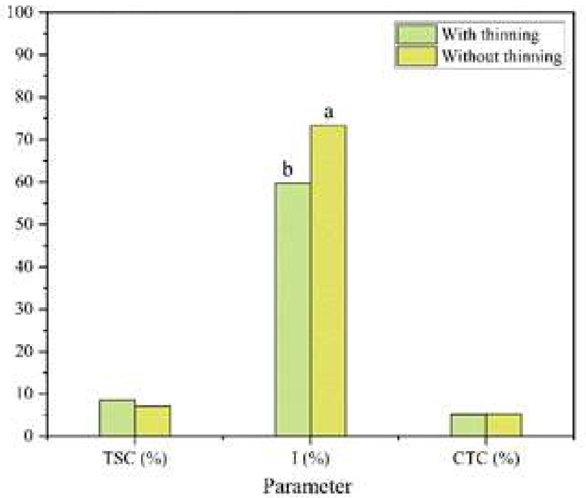

Figure 2 – Statistical comparison of total solids content (TSC), Stiasny index (I), and condensed tannins content (CTC) for the bark of *M. caesalpiniifolia* subjected to two types of management.
Figura 2 – *Comparação estatística do teor de sólidos totais (CST), índice de Stiasny (I) e teor de taninos condensados (CTC) da casca de* ***M. caesalpiniifolia*** *submetida a dois tipos de manejo.*

to thinning. So, the mean number of trunks differed between the two experimental treatments.

For native species from Brazilian dry forests whose wood is used to make fence posts, a 7 to 14-cm diameter is recommended. For charcoal, the suggested diameter is above 8 cm. This way, the trees in both plots studied were better suited for energy when having DBH lower than 7cm, with percentages of 99.3 and 96.9% for plots with and without thinning (Table 1). Since more trunks were in the plot without thinning, more intensive thinning is recommended if the objective enhances the increased diameter. This follows the results of Carvalho et al. (2004), who suggested constant control of the lateral regrowth of *M. caesalpiniifolia* to obtain trunks with larger diameters.

As shown in Table 3, the tree volume (84.9 m^3 ha^{-1}) estimated for the experimental plot without thinning was lower than the value found by Gonçalves et al. (2010) of 90 m3 ha-1 for a forest stand of the same age (7 years) and similar plant spacing (1.5 m x 3.5 m). The difference is most likely due to the dissimilarity of edaphoclimatic conditions from one site to another, as Santos et al. (2012) commented. There was no statistical difference between the form factor and the experimental plots. The form factor usually employed for species from the Brazilian dry forests in natural stands is 0.90 (RMFC 2005). A value of 0.88 was determined by Souza et al. (2016) for the same forest typology. However, since in the present work, the trees were cultivated in a homogenous plantation, a lower form factor was expected due to less intense competition among trees. Working with other species from the same genus, Machado et al. (2008) carried out volumetric modeling of forest stands of *M. scabrella* with 5 to 16 years and determined form factors in the range of 0.6 to 0.7. The trunk form varies with the species and genus, as well as with the growth rate and genetic traits. Differences between form factors can also be explained by site quality, plant spacing, thinning intensity, and age (Ferraz-Filho et al., 2018; Kohler et al., 2016).

The bark thickness average was 1.8 mm (for trunks in which DBH was lower than 5 cm), 3.6 mm (for trunks with DBH between 5 and 10 cm), and 5.3 cm (for trunks with DBH upper than 10 cm). The cubed volumes with bark for T1 and T2 were 36.3 and 42.5 m^3 ha^{-1}, respectively (Table 3). Assessing commercial plantations of *M. caesalpiniifolia* with plant spacing and age similar to those of the present study, Barros et al. (2010) found a cubed volume of 41.76 m^3 ha^{-1}, a value very close to what we observed in T2 (non-thinned plot). Assessing the wood volume available for harvesting in Brazilian dry forest fragments, Santos et al. (2016) estimated a mean value of 15.5 m^3 ha^{-1}. A comparison of this volume with the values in Table 3 indicates that plantations established with *M. caesalpiniifolia* are a viable solution to supply wood for firewood and fence posts. The conversion factors presented in Table 4 can be compared with values found in the literature. For example, Araújo et al. (2004) determined 631.3 kg of green wood mass with bark for thirty sampled trees of *M. caesalpiniifolia* at five years of age. Also, they reported that 1 m^3 corresponds to 1,054 kg of green wood mass with bark, and 1 st equals 392 kilograms. A stacking factor of 2.55 was measured by Barros et al. (2010), a similar value to found in this work since the species usually assessed present tortuous trunks that form piles with a high percentage of voids.

The wood mass represented, on average, more than 83% of the overall mass, and the bark was around

16% of the total. At the time of harvesting, the region of the experimental plots was in the rainy season, which may have affected the trees' moisture content. The value for the percentage of wood mass found here was higher than that measured by Azevêdo et al. (2014) for the same species, which was 68.2%. The sums of the greenwood with bark were 66.9 and 57.2 t ha^{-1} for the non-thinned plot (T2) and the thinned plot, respectively. For the same species at eight years of age cultivated with a plant spacing of 3.0 m x 3.0 m, Moura et al. (2006) determined total biomass of 65.49 t ha^{-1}, similar values to the non-thinned plot of this experiment. The wood moisture content (40.4%) found here was lower than the value of 44.8% determined by Azevêdo et al. (2014). However, the moisture content of the green bark of 87.1% was significantly higher than the value found by the same authors, which was 76.6%. Also, studying the same species, Gonçalves et al. (2010) determined a mean % wood moisture content of 36%. The difference may be related to morphophysiological changes among the trees assessed caused by local climate, the nutritional state of plants, and other factors.

There was no statistical difference between the experimental treatments T1 and T2 for TSC and CTC (Table 6). Assessing the TSC in *M. caesalpiniifolia* bark from 5-year-old trees, Marques et al. (2021) determined a value for TSC of 9.18%, close to the values found here for T1 and T2 of 8.57 and 7.12% respectively. TSC corresponds to the hot-water-soluble extractives from the bark, while CTC indicates the content of extractable tannins in each material. For this variable, the value was lower than those found by the authors cited above, which was 8.18%. For the same species with ages from 8 to 12 years, Gonçalves and Lelis (2001) found values of CTC as low as 2%. Based on the values presented in the literature, it seems that as trees get older, the CTC in the bark declines, which means that the contents of the metabolite decrease with time. The stage of development and age can influence the intensity of the production of metabolites and their concentration in the plant tissues (Taiz et al., 2017; Souza et al., 2021).

The Stiasny number refers to the percentage of phenolic compounds in each plant extract that can react with formaldehyde in acid media. We found a statistical difference between the experimental treatments T1 and T2, with the highest value found for the extracts from the bark of the non-thinned plot (T2), 73.31%. However, this value is lower than that of Marques et al. (2021), 91.27%. When subjected to stressful situations, plants show alteration in the production of secondary metabolites as a defense mechanism (Isah, 2019). Also, Wasternack and Strnad (2016 and 2019) confirmed the increase in the production of secondary metabolites when the plant was subjected to mechanical injuries. The *M. caesalpiniifolia* trees presented different behaviors in the work since their tannins decreased due to thinning. The literature does not report the effect of mechanical injuries in producing secondary metabolites in higher plants. However, thinning might have induced the increase in the concentration of other secondary metabolites, but since this was not analyzed, we cannot positively state this. Since the trees from the plot submitted to thinning emitted more shoots than the others, they invested their resources in producing primary metabolites and directed them to biomass production. This strategy is corroborated by the results of Yu et al. (2015), who stated that abiotic stresses in the environment could alter the assimilation, translocation, partition, and storage of biomass in plants as well as the prioritization in the production of essential metabolites for growth and development.

5. CONCLUSION

The thinning influenced the emission of trunks with smaller diametric classes rather than an increase in DBH. However, despite having a negative effect, thinning can be acceptable when the goal is a high number of trunks per hectare to produce fence posts and firewood, for example. On the other hand, since the Stiasny index and the concentration of tannins decreased with the thinning operation, this silvicultural practice is not recommended for planted forests established to produce non-timber products. More studies should be carried out to elucidate how secondary metabolites are produced and allocated in trees subjected to thinning and other types of abiotic stresses.

AUTHOR CONTRIBUTIONS

LL Paiva conducted all the experiments, collected and tabulated the experimental data, interpreted the experimental data, drafted the manuscript, and prepared the final version in Portuguese. TKB Azevêdo was responsible for the general coordination of research activities and fundraising, allocating inputs and reagents, interpreting experimental data; and statistical data. JL do Canto coordinated the conduction of the experiment, interpreted experimental data, interpreted statistical data, and assisted in preparing the final version of the article. AS Pimenta translated the manuscript from Portuguese to English, sent the document for English reviewing English, and followed all the steps of the submission version. JU Meza Filho contributed to interpreting experimental and statistical data and writing the Portuguese version of the article. MJC de Souza assisted with the experiments and collecting samples in the field.

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