

## Association of edaphoclimatic characteristics and variability of condensed tannin content in species from Caatinga<sup>1</sup>

### Associação das características edafoclimáticas e variabilidade no teor de taninos condensados em espécies da Caatinga

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**ABSTRACT** - Condensed tannin (CT) concentrations in plants are influenced by species, plant part, season, growth phase, environmental conditions, disease and defoliation. The objectives of this study were to determine the effects of edaphoclimatic factors on the concentration of CT stored in leaves and bark from *Mimosa tenuiflora* (Wild.) Poiret and *Anadenanthera macrocarpa* (Benth.) Brenan., collected at three distinct locations in northeastern Brazil. At each study site, sampling was carried out by randomly selecting five trees, each tree represented one replication and samples of leaves and bark of each plant species was collected. Soil samples were collected near the base of each plant for moisture, physical and chemical analysis. Meteorological data from each month and site were obtained of the INMET automatic station. We conducted a principal components analysis using a multivariate technique to observe the influence of soil and weather on condensed tannin concentration. Overall, *Anadenanthera macrocarpa* has the least total CT concentration in both plant fraction and site. Our results suggest that soil chemical characteristics, soil moisture and weather explain 76.27% and 75.92% of the variation in CT produced by *Mimosa tenuiflora* and *Anadenanthera macrocarpa*, respectively. Condensed tannin concentration in these two species is negatively associated with soil moisture and rainfall, and positively associated with temperature and soil chemical elements.

**Key words:** Legume. Polyphenol. Specialized metabolite.

**RESUMO** - As concentrações de tanino condensado (TC) nas plantas são influenciadas por espécies, parte da planta, estação do ano, fase de crescimento, condições ambientais, doenças e desfolhação. O objetivo deste estudo foi determinar o efeito dos fatores edafoclimáticos na concentração de TC armazenados em folhas e casca de *Mimosa tenuiflora* (Wild.) Poiret e *Anadenanthera macrocarpa* (Benth.) Brenan., coletados em três locais distintos no Nordeste do Brasil. Em cada local de estudo, a amostragem foi realizada selecionando-se aleatoriamente cinco árvores, cada árvore representou uma repetição e amostras de folhas e casca de cada espécie de planta foram coletadas. Amostras de solo foram coletadas próximo da base de cada planta para análises físicas, químicas e de umidade. Os dados meteorológicos de cada mês e local foram obtidos em estação automática do INMET. Realizou-se uma análise de componentes principais usando uma técnica multivariada para observar a influência do solo e do clima na concentração de tanino condensado. No geral, *Anadenanthera macrocarpa* apresentou a menor concentração total de TC em todas as frações e locais de coleta. Estes resultados sugerem que as características químicas do solo, a umidade do solo e as variáveis meteorológicas explicam 76.27% and 75.92% da variação do TC produzido por *Mimosa tenuiflora* e *Anadenanthera macrocarpa*, respectivamente. A concentração de tanino condensado nestas duas espécies está negativamente associada à umidade do solo e à precipitação, e positivamente associada à temperatura e aos elementos químicos do solo.

**Palavras-chave:** Leguminosa. Polifenol. Metabólito especializado.

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## INTRODUCTION

The semiarid climate of northern Brazil has a marked influence on the native Caatinga vegetation, which is classified according to Köppen as BSh, dry, tropical climate with high incident radiation, evapotranspiration that exceeds rainfall, an irregular rainfall distribution concentrated over a short period of time (ALVARES *et al.*, 2013) and soils that tend to be medium to high fertility. The irregular rainfall distribution decreases the period of time that plants have access soil-water pools in this ecosystem, which is a major factor affecting physiological processes including secondary metabolism of plant species in the Caatinga (ANDRADE *et al.*, 2010; MUIR *et al.*, 2019).

The edaphoclimatic characteristics linked to the Caatinga ecosystem stimulate the plants to produce secondary metabolites such as condensed tannins (CT), polyphenolic compounds produced by many perennial legumes (NAUMANN *et al.*, 2013), that can be important for plant protection. The concentration and chemical composition may be highly variable and are affected by species (MALACARNE *et al.*, 2018; SCOGINGS *et al.*, 2015), plant fractions, growth stage (GARCÍA *et al.*, 2017; LI *et al.*, 2014), climatic conditions (NAUMANN; COOPER; MUIR, 2016; SCOGINGS *et al.*, 2015), defoliation (COOPER *et al.*, 2014), as well as soil characteristics (JOANISSE; BRADLEY; PRESTON, 2018).

Condensed tannins may also be synthesized with different degrees of polymerization and hydroxylation, which can impact digestion of plant materials by animals (NAUMANN *et al.*, 2013). Diets with high concentrations of CT are typically less palatable due to bitterness and astringency caused by their characteristic ability to bind with salivary and dietary proteins (NAUMANN *et al.*, 2017). However, the protein binding ability of CT may also provide a nutritional benefit to livestock by contributing to increased rumen undegradable protein (MEZZOMO *et al.*, 2015), and gastrointestinal anthelmintic effects (NAUMANN *et al.*, 2014).

Among Caatinga species, *Mimosa tenuiflora* and *Anadenanthera macrocarpa* are considered to have the greatest potential to provide a source of CT for livestock (PAES *et al.*, 2006). These species are important due to being xerophytic arboreal species that provide nutritious forage and a source of herbal medicines that play an important role among Caatinga legumes (MUIR *et al.*, 2019).

However, despite the fact that there are many data in the literature about CT concentrations in several species of livestock interest, specific data on the main variables that influence the production of these metabolites are lacking.

The objective this work was to evaluate the relationship between edaphoclimatic factors

and the production of CT from leaves and bark of *Mimosa tenuiflora* (Wild.) Poiret and *Anadenanthera macrocarpa* (Benth.) Brenan., collected at three sites of the semiarid Brazilian Northeast.

## MATERIAL AND METHODS

This study was conducted in the Caatinga region of northeastern Brazil at three distinctly different sites, with two plant species and two plant parts. Each site was characterized by different edaphoclimatic conditions. In Arcoverde, Pernambuco (PE), the sample collection was performed in October, 2014 in Caatinga located at Moxotó backwoods (36°57'5'' W and 8°19'00'' S) at 663-m altitude, characterized by a rainy season that lasts from February to September and vegetation consisting of sub-deciduous and deciduous forest, typical of semiarid lands.

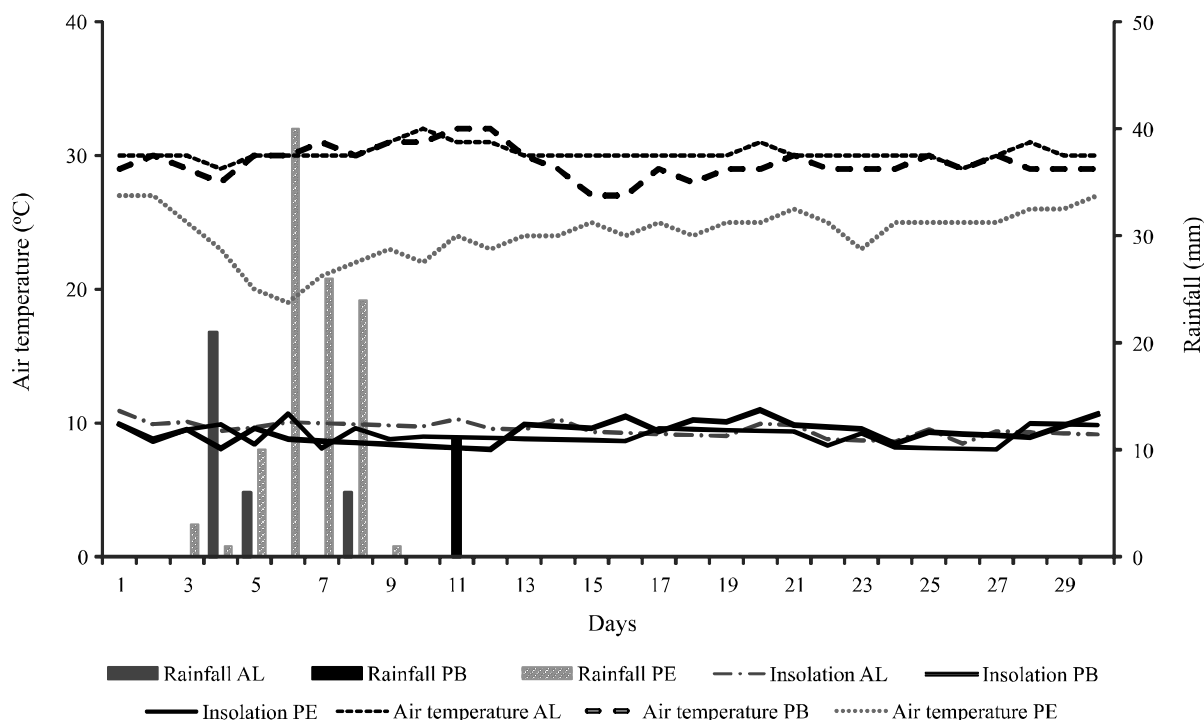
In Patos, Paraíba (PB), sample collection was performed in November, 2014 at 37°16'30'' W and 7°01'30'' S, 242-m altitude, and characterized by a shrub-arboreal layer composed mostly of *Mimosa tenuiflora* (Wild.) Poiret.

In Delmiro Gouveia, Alagoas (AL), collection was performed in December, 2014 at 37°57'18'' W and 9°23'42'' S, 256-m altitude, and characterized by 432-mm per year rainfall average, a rainy season from November to April, and vegetation composed mostly of hyper-xerophytic Caatinga with deciduous forest.

Meteorological data (Figure 1) including average air temperature (T; °C) and rainfall (Rain; mm) for each month of collection were recorded of an automatic station of the National Institute of Meteorology (INMET) located near each study site. The total rainfall amounts and, minimum average and maximum air temperatures recorded during collections were 105-mm; 19 °C, 24 °C and 27 °C for Arcoverde, 33-mm; 27 °C, 29 °C and 32 °C for Delmiro Gouveia, 11-mm; 29 °C, 30 °C and 32 °C for Patos, respectively.

Soil samples were collected at 0-20 cm depth, at five distinct points within one meter radius around each tree sampled, and analyzed for physical, chemical and moisture characteristics according to guidelines described by Embrapa (1997) (Tables 1 and 2). Soil samples were subsampled and analyzed for moisture immediately following collection. Soil samples were then smashed, homogenized, dried at room temperature to a constant weight, and sifted through a 2-mm sieve in preparation for further chemical and physical analyses.

**Figure 1** - Rainfall and air temperature recorded during each month of collection



**Table 1** - Chemical, fertility and physical characteristics of soil conform to specie and site

Specie/site		pH	P	K <sup>+</sup>	Al <sup>3+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	SB	t	V	m	OM	Um	N
		mg dm <sup>3</sup>	cmol <sub>c</sub> dm <sup>3</sup>				%							
<i>M. tenuiflora</i>	PE	4.7	8.2	0.3	0.6	2.3	0.5	3.3	9.1	37.5	16.5	3.6	19.0	2.4
<i>M. tenuiflora</i>	AL	5.8	24.8	0.5	0.1	4.6	1.0	6.2	8.7	70.4	2.2	1.5	1.4	1.1
<i>M. tenuiflora</i>	PB	6.4	16.8	0.5	0.0	9.4	4.0	14.0	16.1	87.0	0.0	2.4	2.3	1.5
<i>A. macrocarpa</i>	PE	6.1	6.6	0.5	0.0	7.2	1.1	8.9	12.8	69.2	0.0	5.2	6.5	2.3
<i>A. macrocarpa</i>	AL	5.7	16.8	0.4	0.1	3.5	0.6	4.5	6.5	60.0	7.3	1.6	1.4	0.9
<i>A. macrocarpa</i>	PB	6.4	20.0	0.6	0.0	9.0	2.4	12.1	14.2	85.1	0.0	2.1	1.8	1.8

Soil samples collected at 0 - 20 cm depth; P = Phosphorus; K<sup>+</sup> = Potassium; Al<sup>3+</sup> = Exchangeable Acidity; Ca<sup>2+</sup> = Calcium; Mg<sup>2+</sup> = Magnesium; SB = Sum of Bases; t = Effective Cation Exchange Capacity; V = Base Saturation; m = Aluminum Saturation; OM = Organic Matter; Um = Soil Moisture; N = Nitrogen

**Table 2** - Soil physical characteristics according to site

Specie/Site		Sand	Silt	Clay	Texture class
		g kg <sup>-1</sup>			
<i>M. tenuiflora</i>	PE	713.89	230.11	56.00	Sandy loam
<i>M. tenuiflora</i>	AL	808.71	157.29	34.00	Loamy sand
<i>M. tenuiflora</i>	PB	675.21	238.79	86.00	Sandy loam
<i>A. macrocarpa</i>	PE	572.47	337.53	90.00	Sandy loam
<i>A. macrocarpa</i>	AL	779.47	178.53	42.00	Loamy sand
<i>A. macrocarpa</i>	PB	726.96	203.04	70.00	Sandy loam

At each study site sampling was carried out by randomly selecting five trees, each tree representing one replication, and samples of leaves and bark from each replication of the *Mimosa tenuiflora* (Wild.) Poiret and *Anadenanthera macrocarpa* (Benth.) Brenan. were collected, resulting in 30 samples from each specie including the plant parts. Leaf samples were collected in an intercalated pattern around the perimeter of the plant. For bark samples, outer fractions of the bark were cut from stem. Following sample collection, samples were stored in plastic bags and transported in thermic boxes with ice in an effort to preserve the CT in leaves and bark (TERRILL *et al.*, 1990).

Samples were then processed and oven-dried at 40 °C (TERRILL *et al.*, 1994) until reaching a constant weight, ground to pass a 1-mm sieve in a Wiley mill, and stored in a freezer until final analysis for CT according method butanol-HCl described by Terrill *et al.* (1992). Condensed tannin concentration was estimated according to a specific standard curve equation developed for each species, plant fraction, and site in order to accurately quantify CT.

The amount of CT estimated was set by a dendrogram and performed a cutting at a 35% level of agglomeration, which were split into a specific number of groups representing the high-, medium-, and low-CT concentration. A multivariate technique by principal components analysis was used to analyze the association of soil moisture, soil chemicals, and meteorological data with CT concentration from species, plant fraction, and site, based on a correlation matrix among variables, processed on Genes computer program version 2015.5.0. Normality and homoscedasticity of data were checked using Shapiro Wilk and Levene tests, respectively, and processed on SAS Learning Edition 2.0 software.

## RESULT AND DISCUSSION

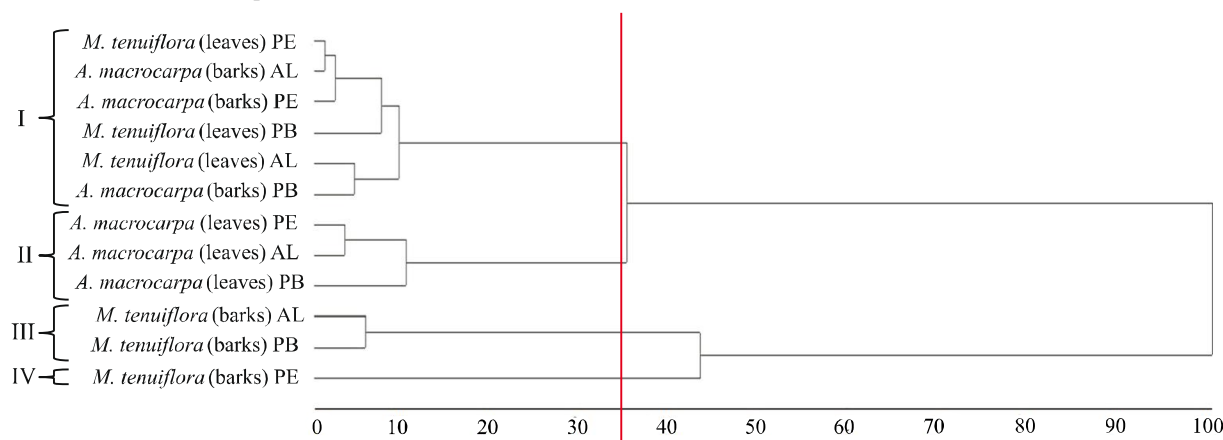
The groups obtained by dendrogram (Figure 2) represent the medium group (138 - 194 g CT kg DM<sup>-1</sup>), low (36 - 74 g CT kg DM<sup>-1</sup>) and two high-CT groups (400 - 550 g CT kg DM<sup>-1</sup>), respectively.

Overall, the total CT concentrations from bark and leaves of *Mimosa tenuiflora* (Wild.) Poiret feature greater than those from *Anadenanthera macrocarpa* (Benth.) Brenan. in our study. Paes *et al.* (2006) report similar results related to the technical viability of CT in species from semiarid Brazilian. For example, *Mimosa tenuiflora* (Wild.) Poiret synthesizes a greater amount of CT than *Anadenanthera macrocarpa* (Benth.) Brenan., which is traditionally exploited for tannin extraction in northeastern Brazil. Similarly, Guimarães-Beelen *et al.* (2006) evaluated CT using the HCl-butanol method from *Mimosa caesalpinifolia* Benth., *Bauhinia cheilantha* (Bong.) Steud. and *M. tenuiflora* (Willd) Poiret samples. The authors report that *Mimosa tenuiflora* produces greater total CT (309.8 g kg<sup>-1</sup> DM).

Of the fractions evaluated in our study, *Anadenanthera macrocarpa* leaves has the lowest total CT and *Mimosa tenuiflora* bark has the greatest, independent of site, while *Mimosa tenuiflora* leaves and *Anadenanthera macrocarpa* bark are similar. Thus, *Mimosa tenuiflora* is a tanniferous species with potential to support the forage-livestock industry, possibly contributing to farmholders income, in addition to possible use as a product additive for rumen fermentation management, either as part of complete diet or to identify plant compounds associated with ruminal bioactivity.

Eigenvalues by covariance matrix were estimated for soil characteristics, CT concentration and

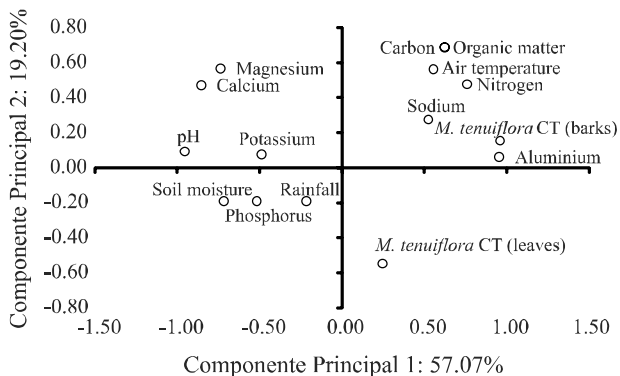
**Figure 2** - Similarity dendrogram of the total condensed tannins obtained by UPGMA algorithm, from *Mimosa tenuiflora* and *Anadenanthera macrocarpa* plants fraction collected in three sites of Northeast semiarid



meteorological data from each species, which show 76.27% and 75.92% of total variability in relation to CT concentrations from samples collected from leaves and bark of *Mimosa tenuiflora* and *Anadenanthera macrocarpa*, respectively. These results can be explained by the first two principal components.

For *Mimosa tenuiflora*, variables related to chemical characteristics of soil, magnesium ( $r = -0.5267$ ), phosphorus ( $r = -0.7770$ ) carbon ( $r = 0.5130$ ), organic matter ( $r = 0.5138$ ) and nitrogen ( $r = 0.7430$ ) have greater influence on total CT concentration of leaves. For bark CT, the most related soil chemical characteristics are pH ( $r = -0.8890$ ), aluminium ( $r = 0.9209$ ), calcium ( $r = -0.7527$ ), magnesium ( $r = -0.6384$ ), carbon ( $r = 0.7037$ ), organic matter ( $r = 0.7030$ ), nitrogen ( $r = 0.8505$ ) and soil moisture ( $r = -0.9448$ ). Additionally, air temperature and rainfall influence total CT variability observed in leaves ( $r = 0.5384$  and  $r = -0.622$ , respectively) and bark ( $r = 0.7250$  and  $r = -0.7194$ ), respectively (Figure 3).

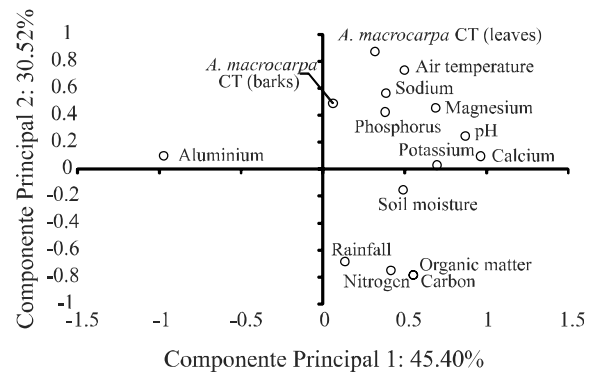
**Figure 3** - Principal components of condensed tannins (CT) from leaves and bark of *Mimosa tenuiflora* and edaphoclimatic characteristics of Arcoverde PE, Delmiro Gouveia AL and Patos PB



Soil sodium ( $r = 0.5535$ ), magnesium ( $r = 0.6963$ ), air temperature ( $r = 0.9093$ ) and soil moisture ( $r = -0.5889$ ) are directly related to total CT from *Anadenanthera macrocarpa* leaves. For this species' bark however, soil phosphorus ( $r = 0.7115$ ), soil moisture ( $r = -0.7738$ ), rainfall ( $r = -0.5692$ ) and air temperature ( $r = 0.6443$ ) are variables that most influenced the total CT (Figure 4).

Based on our results, it is possible to infer that there is a positive relationship among CT synthesis and factors of soil fertility and air temperature, whereas an inverse relationship to the available water supply exists for the species and sites evaluated.

**Figure 4** - Principal components of condensed tannins (CT) from leaves and bark of *Anadenanthera macrocarpa* and edaphoclimatic characteristics of Arcoverde PE, Delmiro Gouveia AL and Patos PB



Low soil moisture in addition to high temperatures drive CT synthesis was observed with species from different physiographic zones, that explains due to the limited growth of these species under low soil moisture and high temperature conditions, it is possible that a large investment in protection of limited growth (i.e. CT) can improve the plant's ability to persist, as compared to those species which are not limited by rainfall and high temperatures (OLIVEIRA *et al.*, 2015).

Our results suggest that high temperatures and low soil water availability for plants lead to higher CT synthesis. This is why environmental factors regulate the main pathway of synthesis of secondary metabolites (shikimic acid) (PAVARINI *et al.*, 2012; TAIZ; ZEIGER, 2013), the flavonoids synthesized by this metabolic pathway play an important ecophysiological role as protection of vegetative tissues against harmful effects of high temperature and UV radiation.

The induction of CT polymers due to high UV radiation is synergistically increased by drought. Hydric stress and high temperatures (from 30 to 45 °C) correlate to flavonoid concentration from *Cistus ladanifer* (CHAVES; ESCUDERO; GUTIERREZ-MERINO, 1997).

Vegetation growth is limited by climatic factors. It is possible that greater investment in protection of what little growth occurs favor plant survival compared to regions where browse regrowth is not as limited by rainfall and hot temperatures (OLIVEIRA *et al.*, 2015).

Plant strategies for stress response are explained by Bryant, Chapin and Klein (1983), who propose there is a tradeoff between carbon accumulation and biomass production for secondary compounds synthesis, which is induced when plants experience stress at the cellular

level. Thus, secondary metabolite production varies, but is inversely related to plant growth, where the carbon demand increases during tissue differentiation and cell growth processes, reducing secondary metabolite production.

The decrease in leaf area, induction of leaf abscission, and change to cell osmoregulation due to water movement from cytoplasm to vacuole, induces biosynthesis of secondary compounds (RAMAKRISHNA; RAVISHANKAR, 2011). Scogings *et al.* (2015) suggest that CT in woody species from a semiarid savanna do not increase during the rainy season, whereas during the dry season there was a significant increase in these compounds for the species evaluated.

Severe drought drives cellular oxidation, which causes lipid peroxidation in cell membranes, and consequently the production of reactive oxygen species (ROS) as hydroxyl radicals (HO·) (NXELE; KLEIN; NDIMBA, 2017; OUZOUNIDOU *et al.*, 2014). This increases the CT and phenolic acid concentrations, which have an antioxidant effect due to the presence of the hydroxyl groups on the aromatic rings in the CT structure. The presence of these compounds ultimately cause ROS stabilization, conferring a pro-oxidant function (HOSU; CRISTEA; CIMPOIU, 2014; LÓPEZ-ANDRÉS *et al.*, 2013).

In addition to the factors cited, the soil chemical composition also influences the accumulation and expression of genes related to secondary metabolite biosynthesis (JOANISSE; BRADLEY; PRESTON, 2018; PAVARINI *et al.*, 2012), specifically the soil nutrients nitrogen, phosphorus, potassium, and calcium (AKULA; RAVISHANKAR, 2011). Thus, secondary metabolites can be synthesized as an alternative source of energy and as nitrogen storage for plant use in N-limited situations. This can be explained by synthesis of intermediates generated by the pentose-phosphate pathway and chalcone metabolism, which are regulated by nutritional status and temperature (TAIZ; ZEIGER, 2013).

## CONCLUSIONS

The survey demonstrates the potential of the species sampled to produce total CT under different edaphoclimatic conditions, which may increase the possibility for using these species in animal diets to benefit dietary protein utilization. Low rainfall and soil moisture, combined with greater air temperature, favor the accumulation of CT, possibly limiting plant growth and favoring plant survival. Soil characteristics such as nitrogen, carbon, and organic matter increase the production of CT in *M. tenuiflora* and *A. macrocarpa*,

possibly as a means of storage as a stress response.

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