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METHYLXANTHINS AND TANNINS CONTENT IN YOUNG LEAVES OF GUARANAZE CULTIVARS UNDER DIFFERENT PRODUCTION SYSTEMS

Lucio Pereira Santo
Researcher; Embrapa Amazônia Ocidental
Manaus, Amazonas

Flávia Camila Schimpl
Professor, EBTT of: ``Instituto de Educação Ciência e Tecnologia do Amazonas`` – Campus Presidente Figueiredo (IFAM-CPRF)

Enilson de Barros Silva
Professor; Universidade Federal dos Vales do Jequitinhonha e do Mucurí
Diamantina, Minas Gerais

Géssica Aline Nogueira dos Santos
PhD student; Postgraduate Program in Tropical Agronomy– Universidade Federal do Amazonas

José Ferreira da Silva
Professor, Universidade Federal do Amazonas
Manaus, Amazonas

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Abstract: Young leaves of seven guarana cultivars were collected (April/2019), in the Amazonian municipalities of Maués, Presidente Figueiredo and Manaus, from ten plants/cultivar, in each location, in three different production systems (Embrapa production system; production system adopted by Jayoro and integrated production system). The samples were dried in a forced ventilation oven and the extraction and quantification of caffeine, theobromine, catechin and epicatechin followed the methodology proposed by Schimpl et al. (2014) and Machado et al. (2018). A two-factor ANOVA without repetition was performed. The factors were guarana tree cultivars and production system, with averages of cultivar repetition and production system of the municipalities where guarana tree is cultivated. There were no significant differences between cultivars for caffeine, theobromine and epicatechin content. In turn, catechin discriminated the cultivars into three groups; the cultivar BRS CG Amazonas presented the highest content (2.67%), followed by BRS CG Andirá (1.60%) and the group consisting of the cultivars BRS CG Maués, BRS CG Cereçaporanga, BRS CG Luzeia, BRS CG Mundurucânia and, BRS CG Saterê, the last five not having differed from each other. Caffeine and epicatechin showed no differences between the Production Systems, whereas, for theobromine, the Integrated Production System presented the highest content (5.71%), with the Jayoro and Embrapa Production Systems in second place, not differing between yes. Catechin revealed a higher content in the Embrapa Production System, with the Integrated Production and Jayoro systems in second place, not differing from each other. This information can contribute to the understanding of preference and non-preference, as mechanisms of susceptibility and resistance, respectively, of guarana genotypes in the relationship with insect pests, in the process of herbivory. Likewise, it can help to elucidate the fungus' preference: *Colletotrichum guaranicola* (Anthracnose, main disease) by the leaflets of young leaves.

Keywords: Caffeine, Theobromine, Epicatechin, Catechin, Fertilization, *Paullinia cupana*.

INTRODUCTION

The guarana tree: *Paullinia cupana* var. sorbilis is an endemic species of the Amazon, where it has great economic and social importance. Presenting several ways of using its stratum, the one that stands out most among all is its destination for the soft drink industry, since this segment accounts for more than 70% of all dry seeds that are produced in this State, where guaranaiculture prevails. family-based, with few representatives of corporate guaranaiculture. The Manaus Industrial Complex (PIM) is the largest demander for guaraná seeds, for the manufacture of soft drinks, the main ones being: Kuat guaraná and Fanta Guaraná, both from Coca-Cola, and Antarctica guaraná, from Ambev.

Analyzing from another point of view, we note that little importance has been given to the other structures of the guarana plant, which also contain considerable levels of nutrients and bioactive compounds. An example can be found in the young leaves of the guarana tree, which are produced in abundance by the plant. And, if managed properly, through special cultural treatments such as pruning, soil and foliar fertilization, irrigation during dry periods, among others, the production of new leaves can be enhanced, as well as the levels of their bioactive compounds and nutrients. can be increased, thus opening up opportunities for the production of new types of raw materials for the animal feed, human food, cosmetics and pharmaceutical industries.

This work was developed with the general objective of evaluating the potential of using young guarana leaves as raw material for the food, cosmetics and pharmaceutical industries, and with specific objectives of assessing the possible existence of differential responses of cultivars and production systems. to the variable methylxanthines (caffeine and theobromine) and condensed tannins (epicatechin and catechin) from new, recently sprouted guarana leaves.

MATERIAL AND METHODS

To evaluate the influences of cultivars and production systems on the variables methylxanthines (caffeine and theobromine), condensed tannins (epicatechin and catechin) of young guarana leaves, and identify the possible potential of this plant organ as raw material for the human food industry, cosmetics and pharmaceuticals, an experiment was conducted with leaf samples collected in three locations (Manaus, Presidente Figueiredo and Maués). Each location was considered a repeat of the treatments. The soils where the experiments were implemented are classified as Very Clayy Yellow Oxisol, which are deep, with high levels of exchangeable aluminum, acidic, with pH varying from 3.5 to 4.7, with low levels of calcium, potassium and phosphorus and high aluminum saturation. The climate, according to Köppen's classification, is tropical rainy type Afi (Antonio, 2005).

The plantings were carried out in 2008 and the differentiated treatments, according to each production system, began to be applied in 2017. The cultural treatments of the three areas, in all phases of the culture, followed the general recommendations of Pereira (2005), however, the differentiated covering fertilizations, according to the Production System adopted, met the specifications: Table 1 (Conventional Production System, recommended by Embrapa); Table 2 (Production System adapted by Agropecuária Jayoro); Table 3 (Integrated Production System, which is in the validation phase by Embrapa). The foliar fertilizers used in two of the systems tested (Integrated Production and Conventional Production-Jayoro) are presented in Table 4. In the analyzes of methylxanthines and tannins, samples of leaves from only one stage of maturation (new leaves just sprouted) were used, in factorial scheme (7 x 3), respectively, seven cultivars x three production systems. The cultivars used were: (BRS CG Andirá, BRS CG Amazonas, BRS CG Maués, BRS CG Cereçaporanga, BRS CG Luzeia, BRS CG Mundurucânia, BRS CG Saterê). And the production systems: (conventional system; system adapted by Agropecuária Jayoro; and integrated production system).

In April 2019, samples of new, recently sprouted leaves were collected in Manaus, Presidente Figueiredo and Maués, in Technological Reference Units – URT containing treatments alluding to the three production systems mentioned above, at the ends of branches of new releases, a sheet composed per plant, of ten plants/cultivar/site.

All leaf structures (rachis and petiole, which unite the leaflets forming the composite leaf) were used and these samples were dried in a forced ventilation oven at 70 oC until they reached a constant weight, ground in a Wiley mill, with a 20-mesh sieve. per inch and homogenized. In the extractions and quantifications of caffeine, theobromine, catechin and epicatechin, methodologies proposed by Schimpl et al. (2014) and Machado et al. (2018). The data relating to the variables caffeine, theobromine, catechin and epicatechin were subjected to analysis of variance (double-factor ANOVA, without repetition). The factors were guarana tree cultivars and production system, with averages

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Production system ▶	Conventional (Embrapa)		
Periods ▶	Early February	Early April	Early May
Inputs ▼	1º Part (g/pl.)	2º Part (g/pl.)	3º Part (g/pl.)
Ammonium sulfate	90	90	180
Simple Super phosphate	300	-	-
Potassium chloride	-	40	80
Magnesium sulfate	50	-	-
Zinc sulfate	-	10	-
Borax	-	05	05

Table 1: Fertilizers used in the Conventional Production System (recommended by Embrapa), after the 3rd year, with the doses of top dressing in grams per plant (g/pl.), and their respective installments

Note: Embrapa's conventional production system (Pereira, 2005), currently used by producers in Amazonas, uses simple fertilizers as sources of macro and micronutrients, does not recommend foliar fertilization or limestone and gypsum inputs.

Production system ▶	Conventional (Adapted by Jayoro)		
Periods ▶	Early February	Early April	Beginning of June
Inputs ▼	1º Part (g/pl.)	2º Part (g/pl.)	3º Part (g/pl.)
17-17-17 + Leader 2	150	-	-
24-00-24 + Leader 2	-	200	250
Limestone	225	-	225
Plaster	25	-	25

Table 2: Fertilizers and correctives used in the Conventional Production System, adapted by Agropecuária Jayoro Ltda (Presidente Figueiredo), after the 3rd year, with the doses of top dressing in grams per plant (g/pl.), and their respective installments

Note: The conventional production system, adapted by Jayoro, uses formulas applied to coverage as sources of macronutrients, associated with Líder 2 as a source of micronutrients. It also uses foliar fertilizers, with macro and micronutrients, as well as limestone and agricultural gypsum inputs, although the gypsum is used in small doses.

Production system ▶	Integrated (Embrapa)		
Periods ▶	Early February	Early April	Beginning of June
Inputs ▼	1º part (g/pl.)	2º part (g/pl.)	3º part (g/pl.)
17-17-17 + Leader 2	150	-	-
24-00-24 + Leader 2	-	200	250
Limestone	(V = 50%)	-	-
Plaster	500	-	-

Table 3.: Fertilizers and correctives used in the Integrated Production System (in the validation phase by Embrapa), after the 3rd year, with the doses of top dressing in grams per plant (g/pl.), and their respective installments

Note: The dose of limestone is calculated according to soil analysis, to obtain (V = 50%) and mixed with agricultural gypsum (500 g/plant) and this mixture applied in a single time, on the soil surface, without incorporation, in an area of 4 m2 around the plant, between February and April of each year. In the literature, there are few and inconclusive studies with limestone in guarana and, for agricultural gypsum, this input was researched for the first time, with the validation of the results in these URTs that generated the data here for this work.

After 3rd year of field	*Integrated and Conventional Production Systems adapted by Jayoro*					
Periods ▶	May	June	July	August	September	October
	Fertilizer dose					
Fertilizer ▼	(g ou mL/pl)	(mL/pl.)	(mL/pl.)	(mL/pl.)	(mL/pl.)	(mL/pl.)
START Mn	6,4 (mL/pl)	-	-	-	-	-
STOLLER Cu	-	3,2	-	-	-	-
SETT	-	-	4,8	4,8	4,8	-
Sodium Molybdate	0,46 (g/pl)	-	-	-	-	-

Table 4: Foliar fertilizers used in two Production Systems (Integrated Production and Conventional Production adapted by Jayoro), with installments and doses.

Notes: - A volume of 800 mL of syrup was applied per plant, containing the dose of each fertilizer specified in Table 4, after having timed the time necessary to reach this volume;

- Sodium Molybdate was applied only in the Integrated Production System plots;

- In the Conventional Production System recommended by Embrapa (Pereira, 2005), there is no recommendation for any of the fertilizers listed in Table 4, nor for any other foliar fertilizer for guarana crops.

FV	GL	Medium square			
		Caffeine	Catechin	Epicatechin	Theobromine
Grow crops	6	0,0003	0,9392*	0,0630	5,1696
Production system	2	0,0002	6,7733*	0,4532	49,5502*
Mistake	23	0,0003	0,3830	0,1326	3,8298
CV (%)		53,24	56,74	90,49	38,93
Overall Average		0,03	1,09	0,40	5,03

Table 5: Mean squares of the variables “Caffeine Content (%)”, “Catechin Content (%)”, “Epicatechin Content (%)” and “Theobromine Content (%)” of young leaves of guaranazeiro cultivars, for the sources of variation “cultivate” and “production system”.

* Significant at 5% by F test.

Cultivars	Caffeine (%)	Catechin (%)	Epicatechin (%)	Theobromine (%)
BRS CG Andirá	0,03 a	1,60 b	0,49 a	4,87 a
BRS CG Amazonas	0,03 a	2,67 a	0,57 a	5,41 a
BRS CG Maués	0,04 a	0,40 c	0,21 a	5,29 a
BRS CG Cereçaporanga	0,03 a	1,07 c	0,19 a	5,04 a
BRS CG Luzeia	0,03 a	0,95 c	0,46 a	4,78 a
BRS CG Mundurucânia	0,03 a	0,85 c	0,51 a	4,77 a
BRS CG Saterê	0,03 a	0,72 c	0,55 a	5,19 a
Production system				
Integrated Production	0,03 a	0,82 b	0,44 a	5,71 a
Jayoro	0,03 a	0,57 b	0,39 a	4,66 b
Embrapa	0,03 a	1,31 a	0,34 a	4,80 b
CV (%)	52,24	56,74	90,49	38,93

Table 6: Estimated average data of the characteristics “Caffeine Content (%)”, “Catechin Content (%)”, “Epicatechin Content (%)” and “Theobromine Content (%)” of young leaves of guarana cultivars, in three Production Systems.

Means followed by equal letters, in the column (comparisons of cultivars among themselves and production systems among themselves), do not differ from each other using the Tukey test at 5%.

of cultivar repetition and production system of the municipalities where guarana tree is cultivated. In other words, the locations were used as repetitions. Then, mean tests were performed (Tukey, $p < 0.05$). The analyzes were carried out using the SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

There was an effect of production system for the variables catechin and theobromine in young guarana leaves ($p < 0.05$) (Table 5)

There were no significant differences between cultivars for caffeine, theobromine and epicatechin contents ($p > 0.05$) (Table 5). In turn, catechin divided the cultivars into three groups, with BRS CG Amazonas presenting the highest content (2.67%), BRS CG Andirá in second place (1.60%) and a third group, consisting of the BRS cultivars CG Maués, BRS CG Cereçaporanga, BRS CG Luzeia, BRS CG Mundurucânia and BRS CG Saterê, the latter five not having differed from each other. (Table 6). It must be noted that the cultivar that presented the highest catechin content, BRS CG Amazonas, among all those tested is the one with the greatest susceptibility to Anthracnose, a disease of guarana trees caused by the fungus: *Colletotrichum guaranicola*, which mainly attacks young leaves. Due to this susceptibility, this cultivar is no longer planted by producers. On the other hand, the cultivar that ranked second in terms of catechin content, BRS CG Andirá, is the genotype, among all the others, that presents the greatest resistance to *Colletotrichum*, displaying its leaves with an evergreen color and free from the symptoms of the disease.

Caffeine and epicatechin showed no differences between the Production Systems, while for theobromine the Integrated Production System presented the highest content (5.71%), with the Jayoro and Embrapa Production Systems in second place, not differing from each other. In turn, catechin revealed a higher content in the Embrapa Production System, with the Integrated Production System and Jayoro in second place, not differing from each other.

CONCLUSIONS

- The catechin content in young guarana leaves varies depending on the cultivars, with the BRS CG Amazonas cultivar having the highest content (2.67%).
- Production systems do not affect the levels of caffeine and epicatechin in young guarana leaves.
- Production systems influence the theobromine content in young guarana leaves, with integrated production presenting the highest content (5.71%).
- Production systems influence the catechin content in young guarana leaves, with the Embrapa production system revealing the highest content (1.31%).

SUGGESTIONS

It is suggested that further studies be carried out aiming at the use of young guarana leaves for various uses, such as energy and antioxidant teas, their use in the cosmetics and various pharmaceutical industries, due to the multiple bioactive compounds present in this plant structure. New investigations into the high levels of nutrients that these new leaves have, which were found by Santos et al., also deserve attention. (2020), and which may have potential for the composition of animal feed and even human food.

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