Phytochemical composition of yerba mate leaves (*Ilex paraguariensis*) and its relation with cultivation conditions

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ABSTRACT: Yerba mate, *Ilex paraguariensis*, of the family Aquifoliaceae, is a native South American plant species. Its leaves exhibit levels of protein, methylxanthines, and phenolic compounds, which have been considered significant quality parameters that extend its industrial use in several products. In the present study, different management techniques were employed in three apparent levels of luminosity 60%, 45% and 30% for five years to analyze the relation of the phytochemical properties of the leaves. There was an increase in protein content in the mate leaves without fertilization during the management of the greater shading 30%, which was an increase of 8% compared to the luminosity levels of 45% and 60%. The conventional mineral fertilizer of ready solubility increased the protein content of the leaves with 30% of luminosity in up to 5,65%. The shading and fertilization of the herbs did not change the phenolic compound contents in the yerba mate leaves. Shading below 45% luminosity of the herbs increased 12,5% of the methylxanthine contents in the leaves. Conventional mineral fertilization of ready solubility increased 6,29% of the total methylxanthine content while the yerba mate was cultivated at a 45% apparent luminosity level. The use of yerba mate products in different cultivation conditions results in products with heterogeneous phytochemical properties, a relevant finding regarding herb quality for medicinal purposes.

Key words: *Ilex paraguariensis*, Silvicultural management, Methylxanthines, Phenolic compounds, Proteins, Yerba mate

RESUMO: Composição fitoquímica de folhas de erva-mate (*Ilex paraguariensis*) e sua relação com as condições de cultivo. A erva-mate *Ilex paraguariensis*, Aquifoliaceae é espécie vegetal nativa da América do Sul. Suas folhas apresentam teores de proteína, metilxantinas e os compostos fenólicos que têm sido considerado um parâmetro de qualidade importante para ampliar o seu uso industrial em diversos produtos. Neste trabalho diferentes condições de cultivo foram aplicadas por 5 anos para analisar a relação direta nas propriedades fitoquímica das folhas. Houve um aumento de 8% no teor de proteína nas folhas de mate sem adubação durante o manejo de maior sombreamento 30%, em comparação com os níveis de luminosidade aparente de 45% e 60%. O fertilizante mineral convencional de pronta solubilidade aumentou o teor de proteína das folhas em 5,65% na luminosidade aparente de 30%. O sombreamento e a fertilização da erva mate não alteraram os teores de compostos fenólicos nas folhas. No sombreamento de 45% houve um aumento de 12,5% nos teores de metilxantina nas folhas. A fertilização mineral convencional da solubilidade pronta aumentou 6,29% do teor total de metilxantina, enquanto a erva-mate foi cultivada a um nível de luminosidade aparente de 45%. Pesquisadores devem estar atentos ao utilizar produtos a base de erva-mate, ao aplicar diferentes condições de cultivo tem-se como resultado produtos heterogêneos fitoquimicamente, sendo relevante na qualidade da erva-mate para fins medicinais.

Palavras-chave: *Ilex paraguariensis*, Manejo Silvicultural, Metilxantinas, Compostos fenólicos, Proteínas, Erva-mate

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INTRODUCTION

Yerba mate (*Ilex paraguariensis* St. Hil.), of the botanical family Aquifoliaceae, was classified in 1822 by the French botanist, Auguste de Saint-Hilaire. The cultivation of yerba mate, applying agroforestry systems, is a production initiative that increases income security of weed producers, who also benefit from the high interaction of the tree component in the shading function. The yerba mate is a native plant species from South America, which is broadly used for its industrial potential in the preparation of beverages, teas, and dermo cosmetic products (Dartora et al. 2011).

Teas have attracted significant attention in recent years due to their antioxidant capacity and abundance in the diet of thousands of people around the world. They are characterized by containing compounds with antioxidant and stimulant properties (Gugliucci 1996; Schinella et al. 2000).

Yerba leaves contain functional chemical substances, such as methylxanthines and phenolic compounds, which enable their industrial use in several products, representing important food supplements with therapeutic functions, in addition to the traditional *chimarrão*. Also, a variety of vitamins are found in yerba mate leaves, such as vitamin C, E, beta-carotene, and those of the B complex, among other compounds (Berté et al. 2011).

The characteristic flavor and slight bitterness of yerba mate products is related to the presence of phenolic compounds and methylxanthines, as well as other compounds present in the leaves, and the employed agronomic cultivation techniques, such as the fertilization and light conditions to which the plants are subjected (Schubert et al. 2006; Rossa et al. 2017).

Yerba mate contains alkaloids (caffeine, theophylline, and theobromine), phenolic compounds, vitamins (A, B1, B2, C, and E), minerals (aluminum, calcium, phosphorus, iron, magnesium, manganese, and potassium), proteins (essential amino acids), glycides (fructose, glucose, raffinose, and sucrose), lipids (essential oils and wax substances), as well as cellulose, dextrin and starches in its composition (Barboza 2006; Junior 2006; Frizon 2011).

Methylxanthines and phenolic compounds are natural products, known as secondary metabolites produced by plants, which display significant ecological functions such as protection against herbivores and pathogens, allelopathic activity, and act as attractive agents for pollinating animals. Preliminary data from previous studies concluded that the difference in contents of flavor-bound chemicals is primarily due to luminosity (Rachwal et al. 2002).

Factors such as development and seasonality; rainfall index and seasonality; temperature and altitude, and luminosity and fertilization may together influence in secondary metabolism (Spring et al. 1987; Heck et al. 2008).

The chemical composition of yerba mate may vary according to several factors, such as the type of cultivation, climate, agronomic conditions, plant age, and genetic variability (Da Croce et al. 1994). This variation also involves the different methods of transportation of the crop to the field, which can modify its qualitative and quantitative composition, and, consequently, the activity of bioactive compounds such as methylxanthines and phenolic compounds.

Nutrients and luminosity are important factors that influence the content of secondary plant metabolites. The addition of nutrients and the intensity of luminosity influence the production of different secondary metabolites, and the impact of changes in their availability may affect the properties of the yerba mate product (Gobbo-Neto and Lopes 2007).

Despite the recognized influence of these factors on plant development, few studies explain their relationships. The acquisition of new products derived from yerba mate stimulates agricultural herb productivity, increasing the demand for the product and also the profitability of the entire production chain (Berté et al. 2011). The product would be able to aggregate nutritional and antioxidant elements of great relevance to the human diet, increasing the supply of new yerba mate products and flavors, popularizing its consumption.

In view of this context, the phytochemical study of protein, total phenolic compound, and methylxanthine content was carried out using extracts of yerba mate leaves submitted to the different silvicultural treatments, such as luminosity and fertilization.

MATERIALS AND METHODS

The present study was carried out in the city of Guarapuava-PR, in an agroforestry system containing yerba mate (*Ilex paraguariensis* A. St.-Hil.) and eucalyptus (*Eucalipto grandis* Hill ex Maiden), located at 25º23’36” south latitude, 51º27’19” west longitude, at an altitude of 1,110 m a.s.l., and at a distance of 286 km from Curitiba. The soil of the area is classified as a shallow, haplic cambisol, with a prominent type A loamy texture. The basic saturation of the soil is low, since it is epidystrophic, displaying high acidity and low fertility, with elevated aluminum content. According to Köppen’s climatic classification, the experimental area is characterized as a humid mesothermal subtropical climate, with an average temperature of the warmest month below 22 ºC, and of the coldest month, below 18 ºC. The area lacks dry season, has a mild summer, and severe frosts occur frequently.

Illuminance determinations (NBR ISO/CIE 8995-ABNT) were performed using a portable digital luxmeter, with a range of 0 to 200,000 Lux. In order to calculate the apparent luminosity index, the light intensity was measured in the open field, maintaining the luxmeter in the horizontal plane at a height of approximately 2.0 m. The measurements, relating to the luminous intensity of each plot, were taken in order to define the apparent luminosity levels of 60%, 45%, and 30%, corresponding to the illuminance observed in the open field. To establish the light intensity levels of each plot, the luxmeter was employed registering the north face of the top of the canopy of the 10 usable plants of yerba mate at 9 am, 12 pm, and 3 pm, during three consecutive days.

Following the observation of maximum illuminance in the plots without eucalyptus - average level of 60% - the lower light intensity levels of 45% and 30% were determined in the plots with eucalyptus. Eucalyptus pruning was carried out during the experimental period to maintain pre-established levels of 45% and 30% apparent luminosity. Light intensity readings were performed during the first week immediately after the beginning of each season (summer, fall, winter, spring).

The collected illuminance data refer to the average diffuse light intensity in each plot, establishing the apparent luminance levels of 60%, 45%, and 30%, by measuring the Illuminance in Lux (Lx) - which is equivalent to 1 lumen per square meter - considering the mean of the detected illuminance during the spring, summer, autumn, and winter seasons, in the years from 2011 to 2015.

Conventional ready solubility fertilization, containing 15.0% of N; 5.0% of P_2O_5, and 30.0% of K_2O, was applied twice a year (September and December), in the period from 2011 to 2015, totaling 10 applications. The dosage consisted of 100 g per yerba mate tree per application, following the manufacturer's technical recommendations, using the circular routing methodology of 20 cm according to canopy projection. The controlled release fertilization treatment, containing 15.0% N; 8.0% P_2O_5, and 12.0% K_2O, was applied once a year (September), totaling 5 applications. The dosage consisted of 30 g per yerba mate tree per application, using the methodology of three 20 cm-deep pits, opened according to canopy projection.

The treatments corresponding to the different levels of luminosity were combined with distinct types of fertilization: without fertilization in the NF herbs; with conventional ready solubility mineral fertilization in CF herbs, and controlled release mineral fertilization in CRF herbs; under different levels of luminosity: 60%, 45%, and 30%. The employed experimental design consisted of randomized blocks with 9 treatments and 3 repetitions each, totaling 27 plots of 180 m^2 on average. Each block contained 10 usable yerba mate plants and double border strips of approximately 20 m. The selection of the usable plants for each plot was defined using the Steel formula (Steel and Torrie 1960), and resulted in 10 dominant plants per plot, among the 20 plants that could have been used.

The plants in the net area were labeled and individually considered for data collection in the blocks, according to the treatments. The research was carried out over the course of five years, counting from 2011 to 2015. In order to analyze the yerba mate leaves, samplings were performed during the pruning season, that is, in the winter, before new budding occurred. The leaves were ripe, and the herb trees, in a physiological state of rest.

During the five stages of growth, from 2011 to 2015, 100 leaves from the middle third of the 10 usable plants were collected, limiting sampling to the middle third of the branch, in a northern geographic orientation (Oliveira et al. 2001).

Following sample collection, the leaves were washed three times with deionized running water and dried in a microwave oven (maximum net power of 1100 W; operational frequency of 2450 MHz; consumption of 1.6 kW/hour; turntable speed of 3 rpm) in 2 cycles of 2 minutes and 1 cycle of 1 minute, until constant weight. The rapid drying of yerba mate leaves in a microwave oven is necessary to avoid degradation and chemical changes caused by tissue oxidation, which may occur in a slow forced-air drying method, modifying the chemical composition of the dry material (Hansel et al. 2008).

After leaf drying, the samples were ground in a stainless steel Willey chamber, equipped with a 0.5 mm sieve, in order to obtain fine, homogeneous material. Following milling, the samples were packed in polypropylene bags and stored in a freezer at -4 °C until analyses.

Extraction and isolation

Protein quantification was performed according to the micro-Kjeldahl method (Brasil 2005), which consists of sample heating with sulfuric acid and a catalyst in order for digestion until carbon and hydrogen oxidation. The nitrogen from the protein is reduced and transformed into ammonium sulfate. Concentrated NaOH is added, and the mixture heated, releasing the ammonia into a known volume of boric acid solution, forming ammonium borate. The formed ammonium borate is then dosed with a solution of hydrochloric acid (0.1N HCl).

The extraction of phenolic compounds was carried out according to the methodology consisting of the addition of 100 ml of a 1:1 (v/v) water: ethanol solution in 2 g of yerba mate, which was maintained for 12 h at room temperature. Next, three extractions were performed using 25 ml of 50% hydroethanolic solution under reflux for 30 min each and then filtered (Dutra et al. 2010). The TPC content was determined by spectrophotometry following the Folin-Ciocalteau method, according to the methodology (Singleton 1999). A total of 500 µl of the extracts was mixed briefly with 2.5 ml of Folin-Ciocalteau reagent (1:10) and 2.0 ml of sodium carbonate solution (Na_2CO_3) (4% w/v). After 120 min of reaction, in the absence of light and at room temperature, the absorbance was measured at 740 nm. As a standard, 5-caffeoylquinic acid (5CQA), was employed, and the results expressed as mg of equivalent 5CQA/g of sample.

The methylxanthines were extracted from 2 g of yerba mate samples using sulfuric acid in a water bath, followed by neutralization with 40% sodium hydroxide, according to the methodology described by Dutra et al. (2010). The extracts were then filtered using 0.45 µm Millipore® polytetrafluoroethylene (PTFE) filter membranes, and subsequently analyzed.

Following the extractions, 5.0 µl aliquots of the samples were injected into a High-Performance Liquid Chromatograph (HELC - Agilent) using an automatic injection system (ALS) equipped with a diode arrangement detector (DAD - 1200 series model, controlled by EZ Chrom Elite Software). A Zorbax Eclipse XDB-C18 column (4.6 mm x 150 mm; 5.0 µm) was employed. The applied mobile phase was a water/methanol solvent (80:20 v/v), with an isocratic flow rate of 1.0 ml/min. The total methylxanthine content was calculated by summing the concentration relative to the area of each peak identified as caffeine, theobromine, and theophylline, characterized by the retention time and the absorption spectrum at 272 nm.

Statistical analysis

The data were analyzed in an entirely randomized block design, using mixed models. The types of fertilization (NF, CF, and CRF), levels of luminosity (60%, 45%, and 30%), and their interactions were considered as fixed effects, and the years, blocks, and residues, as random effects, according to the following statistical model: Yijkl = µ + Si + Tj + bk + pl + (ST)ij + eijkl, in which Yijkl = value observed in the plot that received the i type fertilizer, the j level of luminosity, in block k, during year l; µ = general effect of the mean; Si = fixed effect of fertilizer type i; Tj = fixed effect of the j luminosity level; bk = random effect of block k; pl = random effect of year l; (ST)ij = interaction between fertilizer type i and the j luminosity level, and eijkl = random error of the plot that received the type i fertilizer, the j luminosity level, in block k, during year l. The covariance structure was estimated by the restricted maximum likelihood method. When a significant effect of the model factors on the dependent variables was observed, the means were compared by the Student's test at a 5% significance level.

RESULTS AND DISCUSSION

Total protein content was identified and quantified in the yerba mate leaves submitted to different levels of luminosity and fertilization. During data analyses, the applied statistics showed that there was a significant interaction (P < 0.05) between both variables. The results are shown in (Table 1).

The leaf samples submitted to the NF, CF, and CRF treatments, at different luminosities (60%, 45%, and 30%), exhibited mean values between 16.67% and 18.12% of total protein. These results are considered high when compared to previous studies (Esmelindro et al. 2002; Barboza 2006), in which values of 11.59% and 14.49% were obtained, respectively.

The protein content was higher, at 60% luminosity, in the treatments with CRF (17.67%) and CF (17.14%), when compared with the non-fertilized (16.67%) treatment. The CRF treatment resulted in a greater percentage of protein in the leaves in

TABLE 1. Protein content in yerba mate leaves submitted to different levels of luminosity and fertilization.

Protein[3] (% Dry base)				
Treatment	**[2]level of luminosity**			**F[1] test**
	60%	45%	30%	0.0001*
NF	16.67 cC	17.29 bB	18.12 aA	VC %[4]
CF	17.14 bC	18.06 aA	17.60 bB	8.59
CRF	17.67 aA	17.77 aA	17.74 bA	

Table Legend: Mean values in a row followed by the same capital letter do not differ between each other using the Student's testat 5% probability. Mean values in a column followed by the same lower case letter do not differ between each other using the Student's test at 5% probability. (1) F Test: *significant at 5%;**significant at 1%; ^{ns} not significant. (2) NF non-fertilized, CF conventional fertilization, CRF controlled release fertilization. (3) Data from 2011 to 2015. (4) VC% variation coefficient.

the 60% light range, differing statistically from CF and NF. At 45% luminosity, the protein content in the leaves increased in up to 5.65%, due to the improvement in soil fertility (Xavier et al. 2003).

Regarding the 30% luminosity environment (Table 1), leaf response considering protein content was higher (18.12%) in the non-fertilized treatment. This result infers that the lower apparent luminosity range in the yerba mate leaves affected the behavior of the mechanism of protein aggregate formation, thus characterizing the shade as beneficial, where they state that shaded plants display better quality, particularly higher protein content in the leaf blade. Both conventional and controlled release fertilization treatments did not contribute to the increase in protein content in the leaves in the more shaded environment (Paciullo et al. 2007).

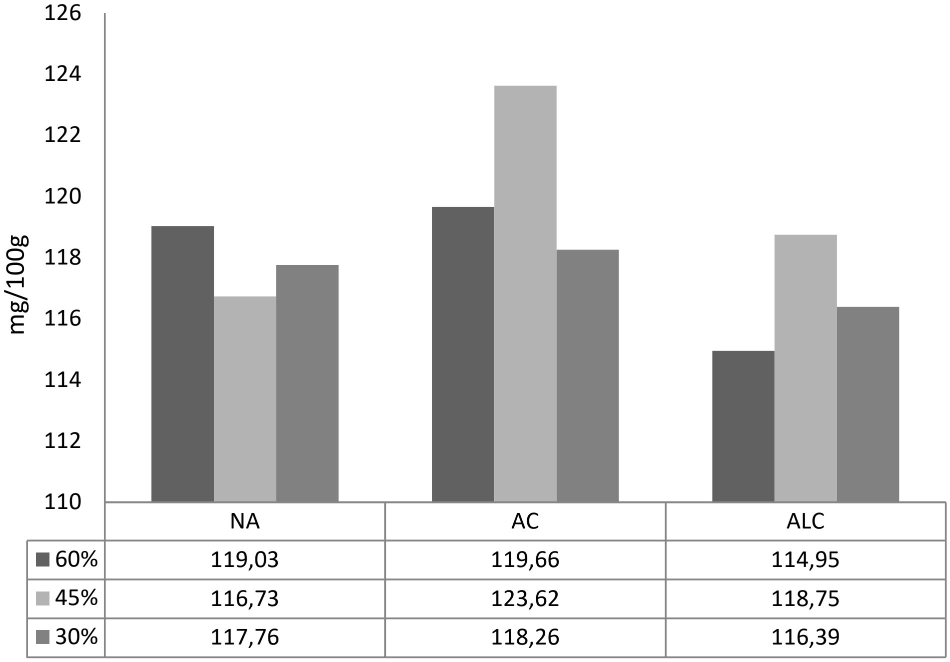
The protein content in yerba mate leaves was greater in the 30%, 45%, and 60% shadow managements, respectively, reaching an 8.0% increment in the environment with lower luminosity. The data shown in the present study can be applied as a reference for producers and in studies employing yerba mate, contributing with information on protein characteristics of the species in continuous agroforestry systems, especially in order to create products containing higher levels of protein.

The contents of total phenolic compounds were identified and quantified in the yerba mate leaves submitted to different levels of luminosity and fertilization. The mean obtained data (123 mg/g) (Figure 1) are slightly below what is considered in literature, 145 mg/g on average. Previous research results show that phenolic compound contents may vary from 12.8 to 287.58 mg/g (Bertè et al. 2001; Meurer 2012).

When compared to species such as *Rosmarinus officinalis* L. (rosemary), *Achyrocline satureioides* (Lam.) DC. (macela), *Cynara cardunculus* subsp. scolymus (L.) Fiori (artichoke), *Salvia officinalis* (sage), *Matricaria chamomilla* L. (chamomile), *Cymbopogon citratus* (DC.) Stapf (lemon grass), and other species of the same genus, the obtained results from the yerba mate leaves were below average (Barboza 2001).

Considering the analyses of the obtained data, interaction between luminosity and fertilization was not observed, which does not allow inferring that the intensity of luminosity exerts significant influence ($P < 0.05$) on phenolic compound contents. However, in (Figure 1), an increase in content regarding phenolic compounds is observed at the 45% luminosity level, which can be justified by the effect that fertilization exerted on the contents (Table 2).

Statistical differences between treatments subjected to different levels of luminosity were not observed, thus disagreeing with results described by other authors, who reported that plants submitted to greater solar radiation exposure display higher concentrations of phenolic compounds (Rachwal et al. 2002; Dartora 2010). Plants produce antioxidant compounds in order to protect themselves. Therefore,



	NA	AC	ALC
■ 60%	119,03	119,66	114,95
■ 45%	116,73	123,62	118,75
■ 30%	117,76	118,26	116,39

FIGURE 1. Contents of phenolic compounds in yerba mate leaves submitted to different levels of luminosity (60%, 45%, and 30%) and fertilization (nf-non-fertilized, cf-conventional fertilization, and crf-controlled release fertilization).

TABLE 2. Effect of fertilization on phenolic compound contents in yerba mate leaves in an agroforestry system.

Treatment[2]	Phenolic compounds[3] (mg/100 g)	F[1] test
NF	117.84 ab	0.0319*
CF	120.51 a	VC %[4]
CRF	116.70 b	13.78

Table Legend:Mean values in a column followed by the same lower case letter do not differ between each other using the Student's test at 5% probability. (1) F Test: *significant at 5%;**significant at 1%; ns not significant. (2) NF non-fertilized, CF conventional fertilization, CRF controlled release fertilization. (3) Data from 2011 to 2015. (4) VC% variation coefficient.

when directly exposed to the sun or attacked by pests, plants display higher levels of chlorogenic acids, which are phenolic compounds that are widely found in *Ilex paraguariensis* (Heck et al. 2008).

All types of shading (forest shading and self-shading, within the canopy of monoculture plants) resulted in a characteristic bitter taste of processed yerba mate leaves when compared to the leaves grown under direct sun exposure (Rakocevic et al. 2011).

The data obtained in the present research may be related to other factors, such as progeny adaptability influencing the content of these phenolic compounds, and/or that changes may occur in total polyphenol concentration of yerba mate plants from distinct origins and localities (Donaduzzi et al. 2003).

The yerba mate leaves employed in the present study were sampled during the winter period, thus being possibly influenced regarding contents regardless of the amount of received luminosity. When analyzing the physical-chemical composition of adult yerba mate plants, an increase in phenolic compound concentration was verified during the summer in agroforestry systems, where shading is greater than in monocultures (Vieira et al. 2003).

The sex of the plants may also affect variable contents. In her work, the author verified that sun-grown female herbs exhibited higher concentrations of phenolic compounds than those grown in shaded areas. However, this difference was not observed in male plants (Serafim 2013).

The significant effect ($P < 0.05$) of fertilization on phenolic compound content is shown in (Table 2). Statistical difference between treatments was observed, in which conventional fertilization (CF) obtained higher contents of phenolic compounds (120.51 mg/100 g), when compared to controlled release fertilization (CRF; 116.70 mg/100 g). However, statistical difference was not observed regarding the non-fertilized (NF) treatment.

Phenolic compounds contain nitrogen atoms in their structure and correlate with stress factors, such as low availability of N in the plant. The nutritional contribution of this element increases the concentration of alkaloids in most of the studied plant species (Cerovic et al. 1999), which may justify the results obtained in the treatment using CF (Table 2).

Phenolic compounds make up for approximately 12% of the dry weight in yerba mate plants (Schneider et al. 2006), indicating their importance in the possible determination of flavor of the final product. Additionally, the perception of astringency in the yerba mate beverage has been positively correlated with total polyphenol content (Schneider et al. 2006). Results that beverage bitterness correlated negatively with the content of theobromine and caffeine, and positively with that of glucose and sucrose (Rakocevic et al. 2011).

Regarding the data obtained in the present study, it is possible to infer that the 60%, 45%, and 30% luminosity levels did not alter phenolic compound content in the leaves. On the other hand, conventional ready solubility fertilization, at 45% luminosity, exhibited a positive effect on the increase of phenolic content but did not result in a significant difference when compared to the non-fertilized treatment.

With respect to the analyses of total methylxanthine content data, the applied statistical method showed that there was a significant interaction ($P < 0.05$) between luminosity and fertilization. The results are shown in (Table 3). The mean content of total methylxanthines exhibited values of up to 1.60 mg/g, and a minimum of 1.40 mg/g during the analyzed period. The obtained values are inferior to those reported in literature, which are between 1.77 and 10.37 mg/g (Coelho et al. 1987; Bertè et al. 2001).

Suggest that the harvesting period influences on the concentration of methylxanthines in the species, varying from 1 to 10 mg per gram of total methylxanthines, depending on the time of year (Schubert et al. 2006). This corroborates with the theory that high levels of methylxanthines identified during the summer can be attributed to younger, developing leaves. In the mentioned study, the results refer to the end of autumn and winter, which may indicate older, more mature leaves, with low biosynthetic activity.

The CF and CRF treatments did not differ statistically from the NF treatment at 30% luminosity (Table 3). When at 45% light intensity, the behavior of the CF treatment differed statistically when compared to the NF and CRF treatments. The conventional

TABLE 3. Methylxanthine content in yerba mate leaves submitted to different levels of luminosity and fertilization.

Methylxanthines[3] (mg/g)				
Treatment[2]		**level of luminosity**		
	60%	45%	30%	Teste F[1]
NF	1.40 aB	1.49 bB	1.60 aA	0.0037*
CF	1.46 aB	1.59 aA	1.51 aAB	VC%[4]
CRF	1.45 aB	1.42 bB	1.55 aA	42.77

Mean values in a row followed by the same capital letter do not differ between each other using the Student's test at 5% probability. Mean values in a column followed by the same lower case letter do not differ between each other using the Student's test at 5% probability. (1) F Test: *significant at 5%; **significant at 1%; ^{ns}not significant. (2) NF non-fertilized, CF conventional fertilization, CRF controlled release fertilization. (3) Data from 2011 to 2015. (4) VC% variation coefficient.

fertilization treatment was significant regarding the increase in methylxanthine contents, which, after successive fertilizations, were incremented during the course of the study. This result corroborates with other researches, in which was identified at 45% luminosity, conventional ready solubility fertilization resulted in an increase in total methylxanthine content (Rossa et al. 2017).

Methylxanthine content may also be associated with genetic and environmental factors (Schubert et al. 2006). With respect to the environmental factors, sunlight is one of the most significant, since it directly or indirectly alters the synthesis of secondary compounds (Zervoudakis et al. 2012). This confirms the data obtained in the present study (Table 3), in which differences were observed regarding contents and luminosity levels. A higher content of methylxanthines was described in the environment with more shade (30%; 1.60), followed by 45% (1.59) and 60% luminosity (1.46).

The results obtained point out that yerba mate leaves that receive more shade display greater amounts of defense chemicals (Coelho et al. 2007). Howeve, Rachwal et al. (2002) and Silva (2012) did not observe changes in methylxanthine concentration in shaded yerba mate cultures until up to 60% relative luminosity. Reported that sharp increases in caffeine and theobromine content occur only when applying a certain level of shade (Coelho et al. 1987).

Nevertheless, there is yet no consensus as to how radiation conditions affect the production of secondary compounds. Described those plants, grown directly under the sun, displayed lower caffeine content, detected higher concentrations of caffeine in partially shaded leaves (Medrado et al. 2004; Jacques et al. 2007).

The methylxanthine contents of leaves that receive low luminosity are higher than those that receive higher light intensity (Esmelindro et al. 2002). However, in a recent study, concluded that leaves grown directly under the sun retain the highest content of biologically active principles (cafeoil derivatives, caffeine, theobromine, and rutin) when compared to those grown in shaded areas (Dartora et al. 2011).

Shade-tolerant species exhibit a number of adaptations to understory conditions, including defenses against defoliating insects (Coley 1987). Considering the obtained results, it can be inferred that up to 45% of apparent luminosity, shading tends to increase the total methylxanthine content in yerba mate leaves. Shading of the herb trees below 45% luminosity increased methylxanthine content in up to 12.5% in the yerba mate leaves. In addition, conventional ready solubility fertilization enhanced total methylxanthine content in the leaves by 6.29%, when cultivated in 45% apparent luminosity.

Considering the obtained results in the present study, a direct relationship was observed between cultivation conditions and the phytochemical composition of yerba mate leaves. The yerba mate characteristics enable is cultivation in both direct sunlight and in the shade, and the phytochemical composition of the plant may be influenced by the type of herbarium (Santin et al. 2015). Fertilization management should be considered for the production of specific raw material, with higher or lower content, and allow the production of differentiated raw material, according to consumer market requirements (Pires et al. 2016).

Researchers should be aware during the use of yerba mate products since the application of different cultivation conditions results in products with heterogeneous phytochemical properties, a relevant finding regarding yerba mate quality.

CONFLICT OF INTERESTS

The authors declare no competing interests.

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