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SHORT COMMUNICATION

Cultivation conditions and their impact on yerba mate ecophysiology and antioxidant potential

Gabriel Scherner Zanotto[1], André Luiz Montes[2], Luciana Bavaresco Andrade Touguinha[2], Wendel Paulo Silvestre[3,*], Cátia dos Santos Branco[4], Joséli Schwambach[5]

Abstract - This work aimed to evaluate the effect of light and shading on yerba mate's ecophysiology and antioxidant potential. To this end, an experiment was carried out in an entirely randomized design, with three treatments, being cultivated in full sun (Ts), in agroforestry (Ta), and in native forest (Tm). Photosynthesis activity and chlorophyll, phenolic compounds, flavonoids, and tannins, and antioxidant activity were analyzed to determine the best cultivation environment that influences the ecophysiology and antioxidant potential of Yerba mate. Ts environment yielded higher concentrations of phenolic compounds, flavonoids, and tannins. Shaded environments showed greater antioxidant activity. Chlorophyll and photosynthesis indices differed in shaded environments, with Tm having higher concentration and photosynthetic activity and Ta having lower concentration and photosynthetic activity. The results show that PAR positively influenced the biochemical parameters. Shading was more efficient for antioxidant activity, and agroforestry contributed to greater antioxidant activity. The highest concentrations of chlorophyll and highest photosynthetic activity were found in yerba mate in native forests.

Keywords: Agroforestry. Bioactive compounds. Photosynthetic active radiation. Physiology. *Ilex paraguariensis*.

Condições de cultivo e seu impacto na ecofisiologia e no potencial antioxidante da erva-mate

Resumo - Este trabalho teve como objetivo avaliar o efeito da luz e do sombreamento na ecofisiologia e no potencial antioxidante da erva-mate. Para tanto, foi realizado um experimento, em delineamento totalmente casualizado, com três tratamentos, sendo cultivado a pleno sol (Ts), em agrofloresta (Ta) e mata nativa (Tm). Análises de fotossíntese e clorofila, compostos fenólicos, flavonoides, taninos e atividade antioxidante foram realizadas para determinar o melhor ambiente de cultivo que influencia a ecofisiologia e o potencial antioxidante. O ambiente de pleno sol apresentou maior concentração de compostos fenólicos, flavonoides e taninos. Ambientes sombreados apresentaram maior atividade antioxidante. Os índices de clorofila e fotossíntese diferiram em ambientes sombreados, sendo que Tm apresentou maior concentração e atividade fotossintética e Ta apresentou menor concentração e atividade fotossintética. Os resultados mostram que a RFA influenciou positivamente os parâmetros bioquímicos. O sombreamento foi mais eficiente para atividade antioxidante e a agrofloresta contribuiu para maior atividade antioxidante. As maiores concentrações de clorofila e maior atividade fotossintética foram encontradas na erva-mate em florestas nativas.

Palavras-chave: Agrofloresta. Compostos bioativos. Radiação fotossintenticamente ativa. Fisiologia. *Ilex paraguariensis*.

[1]Curso de Agronomia, Universidade de Caxias do Sul, RS, Brasil.
[2]Laboratório de Biotecnologia Vegetal (LBV) e Programa de Pós-Graduação em Biotecnologia (PPGBIO), Universidade de Caxias do Sul, RS, Brasil.
[3]Curso de Agronomia, Laboratório de Estudos do Sistema Planta-Ambiente (LESPA) e Programa de Pós-Graduação em Engenharia de Processos e Tecnologias (PGEPROTEC), Universidade de Caxias do Sul, RS, Brasil. [*]Corresponding author. E-mail: wpsilvestre@ucs.br
[4]Laboratório de Estresse Oxidativo e Antioxidantes (LEOA) e Programa de Pós-Graduação em Biotencologia (PPGBIO), Universidade de Caxias do Sul, Caxias do Sul, RS, Brasil.
[5]Curso de Agronomia, Laboratório de Biotecnologia Vegetal (LBV) e Programa de Pós-Graduação em Biotecnologia (PPGBIO), Universidade de Caxias do Sul, RS, Brasil.

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Yerba mate (*Ilex paraguariensis* St. Hil.) is a species of the Aquifoliaceae family, present in the phytophysiognomy of the Mixed Ombrophilous Forest, adapted to the understory and native forests, and is also found in monoculture environments. As it tolerates shading, it presents excellent productivity when shaded by other species (Borges *et al*., 2019).

Yerba mate is the leading Non-Timber Forest Product (NTFP) in the southern region of Brazil. The strength of the sector and the yerba mate industries guarantee a source of income for several small farmers (Chechi; Schultz, 2016). The IBGE (2022) showed that 19,003 establishments produce yerba mate in Brazil, corresponding to a cultivated area of 76,843 ha. These establishments produce 618,601 t of yerba mate, generating a revenue of R$ 846 million. Also, there is excellent potential for expansion of this market, mainly due to the increased use of yerba mate in soft drinks, cosmetics, medicines, and sweets, in addition to infusion drinks, such as *chimarrão* and *tereré*, which use yerba mate the most (Oliveira; Waquil, 2015).

Climatic factors impact plant traits such as genetic variability, adaptability, and natural selection (Wrege *et al*., 2020). These factors can extend the yerba mate's flowering period, influencing its height, leaf mass, branch diameter, and trunks (Fritzsons *et al*., 2020). Light, a key abiotic factor, affects plant physiology. Shaded environments generate greater biomass and leaf area. In contrast, environments with greater luminosity prioritize root growth to compensate for water losses in transpiration, as this type of environment generates higher photosynthesis activity (Caron *et al*., 2014a).

Yerba mate production systems include harvesting in natural forests (on-site harvesting), cultivation and mechanical harvesting (*mate* plantations), and a mixed method combining harvesting and cultivation in natural forests, including re-plantation or agroforestry (Iommi, 2021). The agroforestry system is still essential, combined with conserving forests and native yerba mate (Marques; Reis; Denardin, 2019; Sangalli *et al*., 2022). Bringing the benefits of agroforestry, two agroforestry systems with yerba mate are standard, the first being yerba mate and crops, and the second yerba mate and tree species. Single or full sun cultivation is characterized by yerba mate in monoculture (Penteado Junior; Goulart, 2019).

The ecophysiological profile of yerba mate is influenced by biotic and abiotic factors like light and shading, which alter the plant's metabolite concentrations. For example, chlorophyll, responsible for the plant's green color, increases with shading (Tang *et al*., 2020), and phenolic compounds, which affect color, flavor, and astringency, are higher in yerba mate in brighter environments (Pires *et al*., 2016). Anthocyanins and flavonoids also increase with more light, though not under full light (Ferrera *et al*., 2016; Ferreyra; Serra; Cassati, 2021). Yerba mate's phenolic compounds contribute to its potent antioxidant capacity, which is influenced by light (Mesquita *et al*., 2021; Lorini *et al*., 2021).

Considering the health benefits and characteristics relevant to the consumer market, such as the color, flavor, and astringency of yerba mate, this work aimed to evaluate the influence of growing conditions in terms of light and shading on physiological potentials and antioxidant activity in yerba mate from native forests, in an agroforestry system and in full sun, to determine the most efficient form of cultivation relative to biochemical parameters and physiological characteristics of the plants.

The work was carried out in the first half of 2023, in the autumn period (April and May), on cultivated and native plants on the Zanotto Family property. The

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property is in the Nossa Senhora de Lourdes community, in the interior of the municipality of Ipê, RS. The property is characterized by the organic production of vegetables and yerba mate, and it has a family agroindustry that processes yerba mate. The place has an average altitude of 750 m and coordinates 28°48'0.19" S and 51°17'14.77" W. According to the Köppen climate classification, the characteristic climate is Cfb (humid temperate climate with mild summer) (Kuinchtner; Buriol, 2001). Based on the climatological normal of the National Institute of Meteorology (INMET) for the region, it has an average annual rainfall of 1,830 mm, and the average maximum and minimum yearly temperatures are 20.1 ºC and 14.5 ºC, respectively. According to Embrapa (2023), in Vacaria – RS, the average temperature was 15.2 °C and 13.7 °C in April and May 2023, respectively, and the rainfall was 152.4 mm and 104.6 mm in April and May 2023, respectively.

The yerba mate samples analyzed were collected from plants grown in three types of management: native forest (Tm), shaded in agroforestry (Ta), and cultivation in full sun (Ts). The plants' phenological stage was mature during the sample and leaf collection period. The interval between the last harvest and the time of collecting leaf samples for analysis was two years.

The individuals of native forest yerba mate presented an undefined age range, ranging from 15 to 20 m in height, with harvesting every four years. This climax forest with these yerba mate individuals hosts diverse flora and fauna, with minimal human interference beyond extractive activities like firewood, yerba mate, and fruit collection. The agroforestry area, established in 2006 in a mixed area of grass, bushes, and larger native trees and deciduous species, involved planting yerba mate seedlings in 2007 at 2.0 m x 1.5 m spacing. Current agroforestry practices include mowing and occasional tree pruning and cutting for sunlight. Yerba mate in full sun was planted in 2007 in a single row, with plants 1.5 m apart. The individuals from the agroforestry and full sun area are 16 years old, both from the Cambona 4 cultivar. The criteria of similar plant size, crown size, and diameter at breast height (DBH) were used to select the individuals for sample collection.

The photosynthesis rate was determined in the field, and five plants from each treatment were analyzed. The analysis was conducted between 8:00 a.m. and 11:00 a.m. on May 22, 2023. Each plant canopy was divided into four parts (quadrants), and the leaves located in the west and east quadrants of the plant, e.g., one leaf in each quadrant, were assessed, totaling two leaves per plant and ten measurements per treatment. Gas exchanges were evaluated using an infrared radiation gas analyzer model LCpro-SD (ADC Bioscientific Ltd., United Kingdom), calibrated with CO_2 and water vapor standards, being zeroed using free CO_2 and H_2O in the air. The variables studied were the assimilation of CO_2 (A), stomatal conductance (gs), transpiration rate (E), and water use efficiency (WUE). The evaluations were conducted with a constant photosynthetic photon flux density (PPFD) of 1,500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ controlled by the light source coupled to the device and ambient CO_2 concentration (Savacinski *et al.*, 2022). Assessments were recorded when the total coefficient of variation was less than 0.5 % and under temporal stability.

A completely randomized design was used, with three treatments and five samples per treatment, whereas each sample was composed of a pool of plant material (leaves) collected from three different plants. A total of 15 individuals (plants) had their leaves collected for each treatment. One-hundred grams (100 g) of fully expanded leaves from the annual shoot were collected

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for each plant at the canopy's tip between 6:00 a.m. and 8:00 a.m. on April 10, 2023, and taken immediately for analysis. The leaves from each sample were homogenized, and 300 g of each sample was reserved for the tests.

In the laboratory, 20 g of each sample was separated, chopped, and infused with boiling water for 90 s at a concentration of 5 % (w/v), adapted from Veber *et al.* (2014). Afterward, the aqueous extract was strained and placed in test tubes to analyze antioxidant activity and evaluate the levels of phenolic compounds, flavonoids, and tannins. The assays were performed in triplicate using a spectrophotometer (B542, Micronal, Brazil). Antioxidant activity was evaluated using the $DPPH^{\bullet}$ radical method, as described by Yamaguchi *et al.* (1998). Absorbance reading was performed at 517 nm. The absorbance measurement of the blank corresponds to the $DPPH^{\bullet}$ concentration of the solution (250 µM), and the results were expressed as a percentage of $DPPH^{\bullet}$ radical scavenging. Total phenolic compounds were determined by the Folin-Ciocalteau method, as described by Pereira, Arruda and Pastore (2018). The total phenolic content of the extract was calculated from a gallic acid standard curve, with the results expressed in milligrams of gallic acid equivalent per milliliter of extract ($mg_{EAG}\cdot mL^{-1}$). For the quantification of flavonoids, the colorimetric aluminum complexation method was used, according to Matic, Sabljic and Jakobek (2017). Quercetin was used as standard, so the results were expressed in milligrams of quercetin equivalent per 100 g of plant material ($mg_{EQ}\cdot 100\ g^{-1}$). Hydrolysable tannins were extracted using a hydroalcoholic solution (ethanol 70 vol. %), where 5 g of plant material was kept in contact with 50 mL of the solution at room temperature for 24 h. The hydrolyzable tannin content was determined by mixing a 1.0 mL aliquot of each sample with 5 mL of a 2.5 % w/v solution of potassium iodate. The mixture was kept at rest for 5 min, and the absorbance was read at 550 nm. A curve of tannic acid in 70 vol. % ethanol was prepared as a standard for quantification. The results were expressed in milligrams of tannic acid equivalent per 100 g sample ($mg_{EAT}\cdot 100\ g^{-1}$).

A macerated extract was prepared with 100 mg of plant material and 1.5 mL of acetone for chlorophyll analysis, according to Ross's method (Ross, 1974). The remaining material in the microtube was dried in an oven at 60 ºC for at least five days to determine the dry weight. At least three replications were performed, and chlorophyll concentrations were determined in milligrams per gram of dry plant material ($mg\cdot g^{-1}$).

For data analysis, the normality was assessed using Shapiro-Wilk or Kolmogorov-Smirnov tests. If the data followed a normal distribution ($p > 0.05$), the test used was Analysis of Variance (ANOVA), followed by Tukey's mean comparison test. In non-parametric results ($p < 0.05$), the Kruskal-Wallis test was used, followed by Dunn's mean comparison test. The SPSS 22.0 software (SPSS Inc., Chicago, USA) was used for all analyses performed, and the statistical significance threshold was established at $p \leq 0.05$ ($\alpha = 0.05$).

The results found in this study describe the ecophysiological characteristics and antioxidant potential of yerba mate across different cultivation systems. The photosynthetic parameters of yerba mate plants, evaluated in shaded and full sun cultivation environments, showed a statistical difference between the Ta and Tm treatments for photosynthetic rate (A) and between the Ta treatment and the Ts and Tm treatments for transpiration rate (E). There was no difference among treatments regarding water use efficiency (WUE) and stomatal conductance (gs) (Table 1).

Shade-adapted species grow more slowly and

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struggle to respond to sudden environmental changes. Shade tolerance can be assessed by photosynthetic rate and stomatal conductance in the short term and by growth and resource allocation changes in the long term. Prolonged shading leads to adaptation, causing limited growth when exposed to light (Avalos, 2019). Rakocevic *et al*. (2009) found that yerba mate leaves in forest understory had lower photosynthetic rates than those in monoculture, like the lower rates observed in shaded environments (Ta) in this study (Table 1). Ts had higher results for photosynthetic rate and did not differ from Tm, which also included a shaded environment and had the highest photosynthetic rate evaluated. Lennon *et al.* (2021) also observed a lower photosynthetic rate in shaded cacao trees. Shaded plants look for ways to compensate for the decrease in light so as not to reduce photosynthesis in its absence (Rakocevic; Janssens; Schere, 2012). One of these means is using diffuse radiation to carry out photosynthesis, penetrating environments, and even inside the plant canopy (Caron *et al.*, 2014a).

Studies on *Cupania vernalis* Camb. showed higher stomatal conductance and transpiration rates in full sun, like the results obtained in this study where full sun plants (Ts) had higher stomatal conductance and transpiration rates than agroforestry cultivation (Ta) (Lima Junior *et al*., 2005). Water use efficiency showed no statistical difference among treatments. Yang *et al.* (2021) stated that plants control the water use efficiency by stomatal closure and less transpiration, which happens when plants are under drought stress. Therefore, it would also be necessary to evaluate the amount of water available to plants to understand the high variation presented in this parameter.

A higher photosynthetic rate and respiration rate were noted in Tm, with lower luminosity. This increase may be related to the shade factor tolerance explained by Avalos (2019), where older plants are more adapted and tolerant to shade and more efficient in the photosynthetic process. Excess sunlight can damage photosynthetic machinery, limiting photosynthesis (Walter; Kromdijk, 2022). It could also be related to the results presented by the plants in Ts. Caron *et al*. (2014b) noted increased photosynthetic efficiency in yerba mate when intercropped with pine, attributed to diffuse radiation in the environment caused by the interaction between the pine canopy and global radiation. In this scenario, radiation arrives in various ways, through clearings in the pine canopies, along the edges of the consortium, and from diffuse reflective radiation from pine leaves, the soil surface, and even reflected by the leaves of yerba mate itself. The Tm results can also be related to morphological differences in shaded leaves and higher chlorophyll concentration, which enhances photosynthesis (Caron *et al.*, 2014b; Ribeiro *et al.*, 2020).

The chlorophyll values observed in this study (Table 2) did not differ significantly for chlorophyll *b* and total chlorophyll. However, there was a difference for chlorophyll *a*, where Ta differed statistically from Tm. The highest chlorophyll *a*, *b*, and total contents were found in Tm and the lowest in Ta.

Chlorophyll is a vital pigment for photosynthesis and intercepts light energy in leaves. In shaded environments, leaves typically have higher chlorophyll concentrations to improve light capture, while leaves in bright environments have lower concentrations due to chlorophyll degradation caused by high light stress (Melo Júnior *et al.*, 2017; Hu *et al*., 2021). In this study, leaves in full light (Ts) showed lower chlorophyll concentrations than in the shaded environment Tm, which is consistent with this trend; however, the shaded environment Ta showed a higher concentration, contrary to expected. Avalos (2019) suggests this could

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be related to the leaf's age, as chlorophyll synthesis increases only when leaves are fully developed. Delayed greening, a defense against herbivory, postpones the synthesis of critical compounds until leaves are fully expanded (Lev-Yadun, 2021). The difference found in shaded environments is contrary to the results of Ribeiro (2020), who found no significant differences in chlorophyll a and b concentrations between secondary rainforests and agroforestry systems. However, chlorophyll levels were higher in rainforests during winter, aligning with this study's findings.

Table 1. Photosynthetic parameters in yerba mate plants in shaded systems (native forest and agroforestry) and full sun. Ipe, RS. 2023.

Treatment	A ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)	E ($mmol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)	gs ($mol\ H_2O \cdot m^{-2} \cdot s^{-1}$)	WUE
Ta – agroforestry	2.86 ± 0.35 a	0.174 ± 0.06 a	0.024 ± 0.005 ^{ns}	40.60 ± 26.11 ^{ns}
Ts – full sun	4.61 ± 0.30 ab	0.510 ± 0.11 b	0.043 ± 0.006	8.89 ± 1.36
Tm – native forest	5.12 ± 0.59 b	0.601 ± 0.14 b	0.041 ± 0.008	9.70 ± 1.11

Values are the average of five replications per treatment ± standard error. A – photosynthetic rate; E – transpiration rate; gs – stomatal conductance; WUE – water use efficiency. Means in the column followed by the same letter did not differ by Dunn's test ($p < 0.05$) and followed by ^{ns} indicates a non-significant result.

Table 2. Chlorophyll concentration in yerba mate in shaded systems (agroforest and native forest) and full sun. Ipe, RS. 2023.

Treatment	Chlorophyll *a* ($mg \cdot g^{-1}$)	Chlorophyll *b* ($mg \cdot g^{-1}$)	Total chlorophyll ($mg \cdot g^{-1}$)	Chlorophyll ratio (*a*/*b*)
Ta – agroforestry	1.79 ± 0.77 b	2.43 ± 1.25 ^{ns}	2.14 ± 1.11 ^{ns}	0.7 ^{ns}
Ts – full sun	2.97 ± 0.66 ab	4.48 ± 2.25	3.95 ± 1.99	0.6
Tm – native forest	3.49 ± 0.88 a	4.85 ± 1.08	4.28 ± 0.96	0.7

Values are the mean of five samples per treatment ± standard deviation. Means in column followed by the same letter do not differ by Tukey's test ($p < 0.05$) and followed by ^{ns} indicates a non-significant result ($p < 0.05$).

The chlorophyll *a*/*b* ratio tends to be higher in light environments, with a higher concentration of chlorophyll *a* than *b*, especially in heliophyte species, where the ratio exceeds three. In sciophyte species, this ratio drops closer to one (Melo Júnior *et al.*, 2017). However, this work did not verify this relationship, as the chlorophyll *a*/*b* ratios were below one for all treatments. Such chlorophyll *a*/*b* ratio is related to the lower concentration of chlorophyll *a*, which degrades faster than chlorophyll *b*., as Hu *et al.* (2021) noted.

The antioxidant activity (Table 3) differed statistically for the three treatments, with Ta having the highest percentage (81.9 %) and Ts having the lowest rate (79.4 %). Phenolic compounds (Table 3) also differed statistically between treatments, with a higher concentration in Ts ($0.34\ mg_{EAG} \cdot mL^{-1}$) and a lower concentration in Tm ($0.20\ mg_{EAG} \cdot mL^{-1}$).

Antioxidants inhibit free radicals, agents that

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cause degenerative diseases and aging. Yerba mate's antioxidant properties are preserved in its beverages, offering health benefits (Branco *et al*., 2013; Ferrera *et al.*, 2016; Mesquita *et al.*, 2021). Phenolic compounds are responsible for color, flavor, astringency, and participating in plant defense mechanisms. In yerba mate, it contributes to bitterness, which may interest some consumer markets (Pires *et al*., 2016).

Antioxidant activity was higher in shaded treatments (Ta and Tm) and lower in full sun (Ts), like Serafim's (2013) findings, which reported greater antioxidant capacity in shaded male yerba mate progeny compared to the progeny in the sun. Ferrera *et al.* (2016) also observed greater antioxidant capacity in shaded plants, associating it with higher flavonoid content. However, this study found the opposite, with lower antioxidant activity in treatments with higher flavonoid concentration (Table 4), which suggests that other unstudied compounds may be involved (Serafim, 2013).

Table 3. Concentration of phenolic compounds and antioxidant activity in yerba mate extracts from plants cultivated in shaded systems (agroforest and native forest) and full sun. Ipe, RS. 2023.

Treatments	Antioxidant activity (DPPH$^{\cdot}$ radical sweeping percentage)	Phenolic compounds ($mg_{EAG} \cdot mL^{-1}$)
Ta – agroforestry	81.9 ± 0.78 a	0.27 ± 0.07 b
Ts – full sun	79.4 ± 0.73 c	0.34 ± 0.07 a
Tm – native forest	80.3 ± 1.43 b	0.20 ± 0.03 c

Values are the mean of five samples per treatment ± standard deviation. Means in the column followed by the same letter did not differ by Tukey's test ($p < 0.05$).

Table 4. Concentration of flavonoids and tannins in yerba mate leaves from plants cultivated in shaded systems (agroforestry and native forest) and full sun. Ipe, RS. 2023.

Treatments	Flavonoids ($mg_{EQ} \cdot 100\ g^{-1}$)	Tannins ($mg_{EAT} \cdot 100\ g^{-1}$)
Ta – agroforestry	20.38 ± 6.10 b	34.11 ± 4.82 ab
Tm – native forest	15.94 ± 2.28 b	28.11 ± 2.95 b
Ts – full sun	32.51 ± 7.13 a	42.51 ± 6.69 a

mg_{EQ} – milligrams equivalent of quercetin; mg_{EAT} – milligrams equivalent of tannic acid. Values are the mean of five samples per treatment ± standard deviation. Means in column followed by the same letter do not differ according to Tukey's test ($p < 0.05$).

In this work, yerba mate was infused similarly to chimarrão for antioxidant activity analyses. Mesquita *et al.* (2021) found that consumers best use the antioxidant properties of yerba mate if they ingest it through chimarrão and tereré. Full sun environments (Ts) were more effective at accumulating phenolic compounds than shaded environments (Ta and Tm), which may be due to higher flavonoid and tannin

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concentrations (Table 4). Lorini *et al*. (2021) noted that plants in full sun have higher phenolic concentrations, consistent with Pires *et al*. (2016), who found that light intensity boosts phenolic synthesis. Increased light leads to more phenolic compounds, which protect plants from solar radiation, especially UV light (Rossa *et al*., 2017). Phenolic compound concentrations vary due to factors like isolation, leaf age, defense mechanisms, and light, the last being a key factor in their regulation (Rossa *et al*., 2017; Lorini *et al*., 2021).

The concentration of flavonoids (Table 4) differed statistically between the shaded and full sun treatments, with a higher concentration in Ts (32.51 mgEQ·100 g-1) and a lower concentration in Tm (15.94 mgEQ·100 g-1). Tannins (Table 4) also differed statistically, with a higher concentration for Ts (42.51 mgEAT·100 g-1) and a lower concentration for Tm (28.11 mgEAT·100 g-1). Flavonoids, known for their antioxidant properties, were found in higher concentrations in full sun treatment, contrary to Ferrera *et al*. (2016) and Lorini *et al*. (2021), who reported higher concentrations in shaded environments. Ferreyra, Serra and Cassati (2021) explain that flavonoids accumulate in environments with great luminosity to protect against photo destruction carried out by this compound when absorbing or dissipating solar energy. Thus, like melanin in animals, flavonoids in plants act as UV filters, primarily accumulating in surface tissues for UV-B protection. Tannins are phenolic compounds responsible for the astringency in yerba mate (Mora *et al*., 2022). The treatments also differed statistically for tannin concentration, with Ts having the highest concentration. Rachwal *et al.* (2000) also found an increase in tannin concentration with greater luminosity and positively correlating tannin content and luminosity.

Yerba mate in agroforestry management showed the highest antioxidant activity, followed by native forest and full sun. Therefore, agroforestry management is recommended to stimulate this characteristic. However, productivity must be evaluated, relating it to ecophysiological parameters to better understand the environment and management most suitable for yerba mate. Based on the results, agroforestry is recommended to obtain higher levels of antioxidant activity, and a full sun environment is recommended for accumulating phenolic compounds.

Conflict of Interests

The authors declare that the research was conducted in the absence of any potential conflicts of interest.

Ethical Statements

The authors confirm that the ethical guidelines adopted by the journal were followed by this work, and all authors agree with the submission, content and transfer of the publication rights of the article to the journal. They also declare that the work has not been previously published nor is it being considered for publication in another journal.

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ORCID

Gabriel Scherner Zanotto
https://orcid.org/0000-0001-9411-4233

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André Luiz Montes
https://orcid.org/0000-0003-0673-9749
Luciana Bavaresco Andrade
https://orcid.org/0000-0003-0732-8714
Wendel Paulo Silvestre
https://orcid.org/0000-0002-9376-6405
Cátia dos Santos Branco
https://orcid.org/0000-0003-3709-3004
Joséli Schwambach
https://orcid.org/0000-0001-6785-4164

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