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Pre-and postharvest treatments aimed at the production of secondary metabolites in the essential oil obtained from the leaves of *Cymbopogon nardus*

Tratamentos pré e pós-colheita visando à produção de metabólitos secundários no óleo essencial obtido a partir das folhas de *Cymbopogon nardus*

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Abstract

This work aimed to evaluate the effect of different periods of water restriction supply performed in the preharvest in *Cymbopogon nardus*, evaluating different conditions of storage in the postharvest. The treatments performed were consisted of four periods of water restriction supply in the plants: 5, 10, 15, and 20 days, and two methods of storage (and preserving) the leaves in the postharvest: 10 days in natural environment (32 ± 2 ºC and 75 ± 3% of R.H.) and under cold storage at 0 ± 0.5 ºC and 90 ± 2% of R.H. At the final, 18 chemical components have been identified. The main components verified were citronellol (19.3%), at 10 days stored at room temperature, and citronellol (14.1%) and trans-geraniol (55.8%), at 15 days stored at room temperature. In the essential oil, most of the treatments used proved to be effective in inhibiting the enzyme acetylcholinesterase, and fungicidal and bactericidal effects were observed in the minimum inhibitory concentrations of CMIs. The water restriction supply of citronella plants for 10 and 15 days associated with the cold storage of the leaves at 0 ºC favored the formation of secondary metabolites. The formation of carotenoids and chlorophyll was superior in the plants subjected to water restriction supply for 10 days (room temperature). For phenolic compounds and antioxidant activity, water restriction supply for 10 days and storage at 0 ºC resulted in the highest means, while for flavonoids, water restriction supply for 15 days and storage at 0 ºC promoted the best results.

Additional keywords: Antioxidants; secondary compounds; water stress; phenolic compoumds

Introdução

Citronella (*Cymbopogon nardus* L. Rendle), also known as citronella grass, is a medicinal and aromatic plant originating in southern India that belongs to the Poaceae family, subfamily Panicoideae (Castro et al., 2007). It is a perennial, cespitose plant, and may measure from 0.80 m to 1.20 m in height. Its leaves are flat, narrow, and long, 0.5 m to 1.0 m high, with a bright dark green color and intense aroma (Castro et al., 2007).

Due to its medicinal and aromatic potential, the cultivation of this plant has grown in several countries, mainly because of its essential oil, abundant in citronellal and geraniol, and used in the manufacture of mosquito repellents. Due to these and other characteristics, this species has gained increasing importance in domestic and international markets (Rocha et al., 2012). Some drugs from natural products, especially from plants, are effective in the treatment of several diseases, such as alzheimer (Morais et al., 2013), and many studies have shown that the essential oils found in citronella have an inhibitory activity against acetylcholinesterase (AChE). About AChE, it is important to mention that its inhibition generates an accumulation of ACh, causing a collapse of the central nervous system, such as loss of muscle

control, convulsions, and death from cardiorespiratory arrest (Souza et al., 2013). Another important factor is the use of these essential oils in the food industry, which has proven to be a very viable alternative to act in food preservation and phytosanitary control. In addition, it is seen as an ecologically safe product that can fight bacteria and microorganisms that present resistance to antibiotics and conventional disinfectants (Andrade et al., 2012).

The chemical interface in this kind of species is constantly influenced by environmental factors, which may act alone or interact with each other (Miranda et al., 2013). Therefore, the environment, genetic factors, cultural treatments, postharvest management, drying, refrigeration, storage, and extraction process, among other factors, are determinants in the quality and quantity of the active principles and other secondary metabolites in its biological process (Silva, 2010).

Among the environmental factors that cause changes in metabolism, we have the water deficit, from which the consequences for plants range from physiological irregularities to survival mechanisms in adverse conditions. Therefore, when the water level is below or above what is tolerable in a living organism, the plant is considered to be under water stress (Souza; Souza, 2017). In this sense, plants subjected to water scarcity undergo changes in their physiological and metabolic processes, such as decreased stomatal conductance, inhibition of photosynthesis, decreased transpiration, leaf growth and expansion, and opening and closing of stomata (Scalon et al., 2011).

Plant species that grow in semi-arid regions have considerable concentrations of bioactives when compared to those cultivated in milder climates (Selmar; Kleinwächter, 2013), with a considerable increase in all metabolites, especially flavonoids and terpenes (Marchese et al., 2010). However, there is an unpredictability in relation to the products formed, as they change according to the intensity and time of stress. And more, medicinal plants subjected to water stress demonstrated a significant influence on the production of secondary metabolites (Alvarenga et al., 2011), and when water stress leads to critical physiological changes, interferences occur in the synthesis of metabolites via shikimic acid, as well as in the variation of phenol totals and tannins (Andrade et al., 2012).

Although water stress is considered a negative fact, causing great damage and reduction in agricultural productivity, it may be seen from another angle in regard to medicinal plants, bringing benefits and adding value to its raw materials by intentionally adopting moderate stress in its cultivation (Kleinwächter; Selmar, 2016).

Thus, understanding the effect of water stress on the physiology and composition of essential oil and secondary metabolites of citronella, it is essential to define new agronomic techniques and management that can maximize these products. So, this work aimed to evaluate the effect of different periods of water restriction supply in the plants, performed during the preharvest, as well as to evaluate different storage conditions in the postharvest of citronella in order to maximize the formation of secondary metabolites obtained from the production of essential oil.

Material and Methods

Location

The field experiment was conducted in an experimental area of Embrapa Roraima (Agricultural Research Company), located at BR-174 km 8, Industrial District, Boa Vista county, Roraima State, Brazil (02º42'3" N; 47º38'0'' W). After the field work, the experiment was conducted at the Chemical Research Laboratory of the Federal University of Roraima – UFRR, also in Boa Vista. This work was carried out from 2019 to 2020.

The region's climate is hot and humid, of the A_w type, equatorial and tropical-humid, according to the Koppen classification, being basically defined by two seasons: winter and summer, with an average annual rainfall of 1,678 mm. During the experimental period, the temperature of the region varied between a maximum of 42.8 ºC and a minimum of 24.0 ºC, with an annual average of 33.4 ºC in areas of smooth or flat relief.

Citronella seedlings were produced under nursery conditions and obtained by clump fractionating of adult plants, in a total of 125 seedlings, which were standardized to 20 cm height, and grown in a greenhouse until the day of planting. The substrate used to prepare the seedlings was made in a 1:1:1 ratio of manure, clay, and sand.

The plants were grown in five beds (5 m wide and 25 m long), where holes of 30 cm × 30 cm were made with spacing of 1 m × 1 m between plants. The soil in the area is classified as a yellow latosol with a sandy texture. For soil correction, soil analysis was initially carried out, with fertilization in two stages: the first application was carried out in the planting hole, using limestone and N, P, and K, and the second application was carried out after four months of planting around each seedling using only N, P, and K. Irrigation was carried out by spraying according to the crop needs, using Santeno® spray tapes. The seedlings were transplanted to the field after 60 days, remaining in the field until reaching the desired size and characteristics for the first harvest. Weed control was performed through manual weeding, and the crop did not present problems with pests or diseases.

Experimental design and treatments

The experiment was installed in a randomized block design in a factorial scheme (4×2), with three replications. The treatments consisted of four periods of water restriction supply of the plants:

5, 10, 15, and 20 days; and two methods of storage (and preserving) the leaves in the postharvest: natural (10 days under room temperature at 32 ± 2 ºC and 75 ± 3% of R.H.) and under cold storage (10 days at 0 ± 0.5 ºC and 90 ± 2% of R.H.).

The period of water restriction supply of plants occurred in the summer, one of the driest seasons of the year, from the end of February to the beginning of March. In February, during the experimental time, the average maximum and minimum temperatures were 32.6 ºC and 22.9 ºC, respectively, and the monthly precipitation was 1.4 mm. As of March, the average maximum and minimum temperatures were 32.6 ºC and 22.5 ºC, respectively, and an average monthly rainfall of 57.6 mm in the period (Inmet, 2020).

Samplings

The harvest of citronella leaves from the treatments was carried out in the dry season, 120 days after planting. Leaves were sampled from the central region of the clump of the plants (younger leaves), with an average quantity of 500 g of leaves for each treatment. After harvesting, the leaves were cleaned and selected by the absence of spots, rots, standardized size, and position on the plant, then evaluated after 10 days in a natural state at room temperature (32 ± 2 ºC and 75 ± 3% of R.H.) and under cold storage at 0 ± 0.5 ºC and 90 ± 2% of R.H.). The preparation of samples for the analysis consisted of drying the leaves, followed by their crushing and homogenization (particle size). The following analyzes were performed: total anthocyanin content, antioxidant activity (ORAC and DPPH), total carotenoids, phenolic compounds, total chlorophylls, flavonoids, soluble solids, titratable acidity, and pH. In addition, essential oils were extracted from the samples to determine their yield, identification, quantification, antimicrobial action, and anti-acetylcholinesterase action. All the chemical results were expressed on a dry basis.

Chemical analysis of leaves

For the determination of total anthocyanins, the mass of the samples was corrected to 0.2 g lyophilized, corresponding to each evaluation period, in a beaker. Then, 6 mL of 95% ethanol extraction solution + 1.5 N HCl (85:15) was added. The sample extract was transferred to a 10 mL flask and corrected, completing the volume with the extracting solution. This material was transferred to test tubes, shaken on a tube shaker, wrapped in aluminum foil, and stored in the refrigerator. After 24 h, the sample extracts were filtered and immediately subjected to reading in triplicate on a spectrophotometer at 535 nm. The results were expressed in mg 100 g^{-1} of sample on a dry basis and calculated using the formula: dilution factor × absorbance / 98.2 (AOAC, 2012).

The antioxidant activity by the ORAC method was performed on microplates with fluorescein based on the method of Ou et al. (2001) adapted by Huang et al. (2002). The assay was performed on a 96-well reader (Synergy HT Multi-Mode Microplate Reader, BioTek Industries). For that, 25 µL of the sample was mixed with 150 µL of fluorescein (55.5 nM) and incubated for 15 min at 37 ºC in the microplate before the automatic injection of 25 µL of the AAPH solution (155 mM). Fluorescence was monitored for 50 min by readings (λ excitation = 485 nm; λ emission = 520 nm). Trolox solutions were prepared for the calibration curve (8, 15, 24, 32, and 40 M). All solutions were diluted in phosphate buffer (75 mM, pH 7.4). The samples were analyzed in three dilutions, considering the average as the final ORAC value. The quantification of antioxidant activity was based on the calculation of the area under the fluorescence decay curve as proposed by Prior et al. (2005). The results were expressed in µmol Eq Trolox 100 g^{-1}.

DPPH radical-scavenging antioxidant activity was performed in a 96-well reader (Synergy HT Multi-Mode Microplate Reader, BioTek Industries), and the reduction in absorbance at 517 nm was monitored every 5 min until the reaction reached a plateau. The determinations were made by adding 250 µL of the DPPH solution and 40 µL of methanol to each microplate well for the control or the same volume for the standard solutions (BHA, BHT, ascorbic acid, chlorogenic acid, quercetin, and Trolox) and sample extracts. The DPPH remaining at the end of the reaction was determined and quantified as the DPPH radical scavenging activity using a standard Trolox curve. The antioxidant activity by the DPPH method was expressed in µmol Eq Trolox 100 g^{-1} (Brand-Willians et al., 1995).

For the quantification of total carotenoids, 0.2 g of leaves from each treatment were weighed and placed in test tubes covered with aluminum foil, where 10 mL of the hexane-acetone extracting solution (6:4) was added. The extracts were stirred on a tube shaker for 1 min. After 9 min, the extracts were filtered through cotton and immediately read in triplicate using a spectrophotometer at 450 nm. B-carotene was used as a standard for making the calibration curve. The results were expressed in µg of β-carotene 100 g^{-1} (AOAC, 2012).

The total phenolic compounds were determined by spectrophotometry using the Folin-Ciocalteau reagent, following the methodology proposed by Wettasinghe and Shahidi (1999) with a standard curve of gallic acid. The results were expressed in mg of gallic acid 100 g^{-1}.

The quantification of total chlorophylls was determined by removing 10 cm^2 in the form of a strip 1 cm wide and 10 cm long from each sample. The strips were then placed in airtight glass flasks (220 mL) containing 20 mL of 80% acetone extracting solution. The flasks were coated with aluminum foil

and kept at 4 ± 1 ºC for 72 h, when the absorbance of the solution was read on a spectrophotometer (Shimadzu Model UV-3600-UV-VIS-NIR), at 645 and 663 nm. The total chlorophyll content was obtained by the following formula: total chlorophylls = 8.0 (Absorbance at 663 nm) + 20.2 (Absorbance at 645 nm), being the results expressed in mg (40 cm^2)$^{-1}$.

For the quantification of flavonoids, 0.5 g of leaves from each treatment were weighed in a beaker, where 20 mL of methanol was added and mixed with the aid of a glass stick. Then, the extracts were transferred to test tubes, agitated in a tube shaker for 1 min, packed with aluminum foil and stored in a refrigerator. After 24 h, 3 mL of each extract was removed and placed in test tubes, where 2 mL of 5% aluminum chloride was added and homogenized and left to stand for 30 min. Then, a triplicate reading was performed on a spectrophotometer at 441 nm, where a blank was made for each sample consisting of 3 mL of the extract of each sample plus 2 mL of methanol and without the addition of 5% aluminum chloride. Quercetin was used as a standard for the calibration curve, and the content of total flavonoids was expressed in mg of quercetin 100 g^{-1} (AOAC, 2012).

The content of soluble solids was determined using a portable analog refractometer (RT - 30 ATC), with automatic temperature correction, using a drop of juice from citronella leaf extract in triplicate, and the results were expressed in ºBrix (AOAC, 2012).

For determination of the titratable acidity and pH of citronella leaves, 5 g of leaf extract was used, which was initially mashed and crushed with distilled water. Then, the extracts were placed in an Erlenmeyer flask and diluted in 50 mL of distilled water. The solution was subsequently filtered, and three subsamples of each plot were prepared. The titratable acidity was determined by titrating the filtrate (dilution 1:5) with 0.1 N and 0.1% alcoholic phenolphthalein solution, according to AOAC (2012), and the results were expressed in mg of citric acid 100 g^{-1}. The pH was determined by reading in a pH meter according to the temperature of the standards and samples (AOAC, 2012).

Extraction, yield, identification, and quantification of essential oils

For the extraction of essential oils from citronella leaves, hydrodistillation by steam entrainment was used. For that, 300 g of fresh leaves were used for each extraction, where the material was properly washed and cut into smaller parts, and then it was placed in a round-bottom flask, using distilled water as a solvent. Subsequently, the samples were subjected to a hydrodistillation process for uninterrupted 2 h. The process was carried out in a Clevenger-type device consisting of a heating blanket, a round-bottom volumetric flask with a capacity of 2,000 mL, a condenser with running water at room temperature and an extractor separator. The balloon was adapted to the extractor and taken to the heating blanket. The mass of the essential oil obtained was determined by weighing on an analytical balance. Subsequently, the essential oils were packed in labeled bags and stored at 0 °C. After extraction, the yield of essential oils in each sample was determined using the formula: essential oil yield (%) = $\frac{\text{essential oil mass (g)}}{\text{leaf fresh mass (g)}}$ x 100.

The identification and quantification of essential oils from citronella leaves were performed by gas chromatography with a flame ionization detector (GC-FID) and coupled to mass spectrometry (GC-MS). To perform GC-FID, an HP 7820A gas chromatograph (Agilent), Supelcowax-10, 30 m × 0.2 mm × 0.2 µm column (Supelco), column temperature at 50 °C (3 min), 3 °C min^{-1} to 220 °C, injector 250 °C split (1:20), FID detector 220 °C, carrier gas H_2 at 5 mL min^{-1}, volume injection 1.0 µL, and data acquisition software OPENLAB (Agilent) were used. The essential oils were diluted 2% in ethyl acetate. To perform the GC-MS, the analyses were carried out on a GCMS-QP2010 ULTRA (Shimadzu), Supelcowax-10, 30 m × 0.2 mm × 0.2 µm (Supelco) column, column temperature at 50 °C (3 min), 3 °C min^{-1} to 220 °C, injector 200 °C split (1:50), GC-MS interface at 220 °C, MS detector (electronic impact at 70 eV) at 220 °C, gas drag He at 3.0 mL min^{-1}, injection volume 1.0 µL, GCMS Solution data acquisition software (Shimadzu), Spectral library: NIST11.

Data software used was GC-MS Solution (Shimadzu). Identification of peaks was made by comparison of the mass spectra obtained by GC-MS spectra with the library and NIST11 by comparing the Kovats Indices calculated by GC-FID and literature data. Quantitative analysis was done by standardization areas using the chromatograms obtained by GC-FID.

Bioassay of inhibitory activity of the enzyme acetylcholinesterase (AChE)

The acetylcholinesterase enzyme activity test of citronella leaf extracts was conducted based on Ellman's spectrophotometric method, modified by Rhee et al. (2001), in 96-well microplates. Eserin was used as a standard inhibitor (10 mg mL^{-1}), and as a negative control, the test was performed without the presence of inhibitors. The tests were performed in quintuplicate. In each well, 25 µL of acetylcholine iodide (15 mM), 125 µL of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), 50 µL of tris-HCl buffer pH = 8, 0.1% were dispensed w/v of bovine serum albumin and 25 µL of extract (10 mg mL^{-1}) solubilized in v/v Tween-DMSO. The reading of the plates was performed on a spectrophotometer at 405 nm nine times in a period of 8 min. Right after the first reading, 25 µL of the enzyme acetylcholinesterase (Electrophoruselectricus Sigma Aldrich) (0.222 U mL^{-1}) was added, and nine readings were performed in a period of 8 min at 405 nm. The interference of spontaneous substrate hydrolysis was corrected by

subtracting the mean absorbance measured before adding the enzyme. The percentage of enzyme inhibition was calculated using the following formula: inhibition (%) = [(C-A) □ 100]/C, where C = control containing enzyme and substrate; A = assay containing the extract, enzyme, and substrate.

Bioassay of antimicrobial activity

For determination of the antimicrobial activity of citronella leaf extracts, a pre-inoculum was prepared in which the tested microorganisms were transferred from the culture medium where they were preserved to test tubes containing 3.0 mL of culture medium, BHI for bacteria, and Sabouraud for yeast. Then, the tubes were incubated in an oven at 37.5 ºC for 24-48 h. With the aid of a micropipette, 500 µL of this pre-inoculum was transferred to test tubes containing sterile distilled water. The tubes were homogenized, and the concentration was adjusted to 600 nm for bacteria and 530 nm for yeasts until transmittance between 74-75% for bacteria and 75-76% for yeasts, corresponding to the McFarland 0.5 scale standard turbidity, i.e., 10^5 CFU mL^{-1}, thus obtaining the suspensions of the inoculants used in the bioassay.

For preparation of the working solution, the samples were previously solubilized in dimethyl sulfoxide (DMSO) at a concentration of 12.5 mg mL^{-1}. From this solution, an aliquot of 40 µL was removed, which was added to 960 µL of the culture medium used in the bioassay, obtaining the working solution at a concentration of 500 µg mL^{-1}.

The bioassays were performed in 96-well plates in triplicate, adding 100 µL of the working solution at a concentration of 500 µg mL^{-1} in three wells. Then, 100 µL of the inoculum of the standardized microorganism was added to each well.

Four controls were performed: growth control of the microorganism (to verify cell viability); the blank, which consisted of the sample solution in the same concentrations evaluated, replacing the inoculum with sterile distilled water; positive control (replacing the working solution with a commercial antibiotic); and the sterility control of the culture medium, containing 100 µL of culture medium and 100 µL of sterile distilled water. The microplates were incubated in an oven at 37.5 ºC, and after 24 h, the test was read in a plate reader at 490 nm.

The antibiotics used for the quality control of the assays were ampicillin for bacteria and miconazole for yeasts, whose working solutions were prepared as previously described for the tested samples. The samples were tested against the following microorganisms: *Candida albicans*: ATCC 18804 (yeast); *Staphylococcus aureus*: ATCC 29212 (Gram-positive bacteria); *Bacillus cereus*: ATCC 11778 (Gram-positive bacteria); *Escherichia coli*: ATCC 25922 (Gram-negative bacteria); and *Salmonella typhimurium*: ATCC 14028 (Gram-negative bacteria).

Statistical analysis

The data regarding the chemical characteristics of citronella leaves were subjected to analysis of variance (ANOVA) and the means compared by the Tukey test ($p < 0.05$) and to the regression analysis, selecting the model that best fits as a function of the coefficient of determination (R^2). The statistical program R version 3.4.2 was used, and the regressions were constructed with the aid of SIGMAPLOT v.12 software. For the data obtained regarding the identification and quantification of essential oils and AChE inhibitory activity bioassays, comparative non-parametric tests were performed. Means of antimicrobial activity were compared by Tukey's test ($p < 0.05$).

Results and Discussion

Chemical analysis of leaves

In relation to total anthocyanin content, there was a significant increase after 10 days of water stress on leaves subjected to 0 ºC, which is the best result (Figure 1A). Thus, the restriction of water supply for a period of 10 days and storage at 0 ºC is ideal to obtain the maximum production of anthocyanins in citronella leaves, with quantities close to 5.0 mg 100 g-1.

In this sense, Leão et al. (2019), when evaluating the amounts of citronella anthocyanins on different days and storage temperatures, obtained values of 5.0 and 7.0 mg 100 vg-1 at 30 and 60 days of storage at 0 ºC, values higher than those found in this work, probably due to the long-term exposure time at this temperature. Nevertheless, it was expected that during the processing and manufacture of products from anthocyanins, the favoring of their deterioration and color is greater, especially during storage, as the color of the products degrades even more. Temperature could be considered one of the factors capable of modifying color and speeding up anthocyanin degradation. So, temperature, storage time, and the processing of medicinal products deserve special attention, since anthocyanins are unstable in relation to processing, as one of the main factors involved in their deterioration is temperature. Thus, frozen storage is indicated both for the conservation of plant material, without degrading anthocyanins, and for the optimization of these secondary constituents.

In the antioxidant activity of citronella leaves by the ORAC method, it was found that the best results were those the plants were subjected to periods of 10 and 15 days of water restriction supply with or without cold storage at 0 ºC (Figure 1B).

The highest values of antioxidant activity were obtained at 10 days of water restriction supply with storage at 0 ºC (702.45 µmol Eq.Trolox g-1). Leão et al. (2019), when evaluating the antioxidant activity by the citronella ORAC method, obtained values of 888.75 µmol Eq.Trolox g-1, 1,183.79 µmol Eq.Trolox g-1, and 118.12 µmol Eq. Trolox g-1 when storing citronella leaves at 0 ºC at 30, 60, and 90 days, respectively,

which are values higher than those found in this work with the submission of water stress and cold storage at 0 ºC, however understandable, since the storage period was much longer.

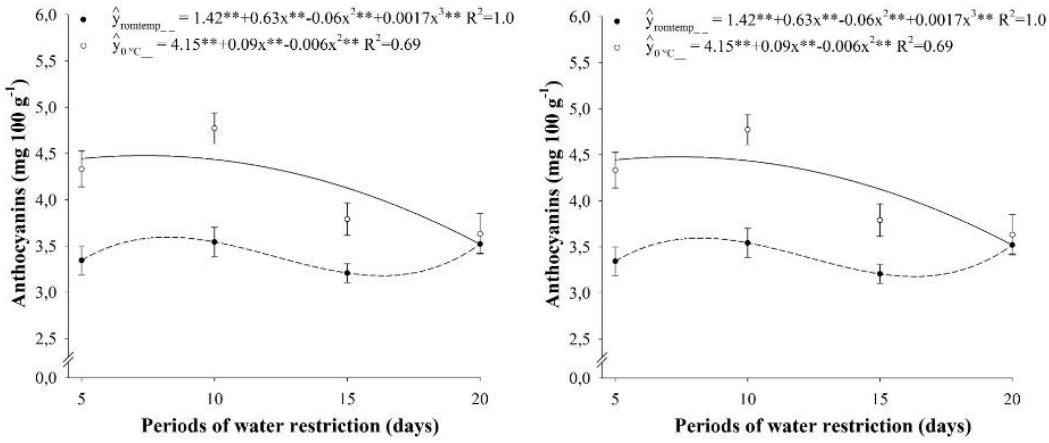
Regarding the submission of plants to water restriction supply, Leão et al. (2019), when evaluating the antioxidant activity by the citronella ORAC method, obtained 534.10 µmol Eq.Trolox g-1 of plants collected in a natural environment without the submission of any type of treatment or storage. In this work, similar values were found at five days of water stress; however, at 10 and 15 days of water stress, higher values were observed.

For the antioxidant activity by the DPPH method, results obtained were similar to those found by the ORAC method. The best scores were observed at 10 and 15 days of water restriction supply without refrigeration (691.54 and 662.76 µmol Eq.Trolox g-1, respectively). When the plants were subjected to water restriction supply and storage at 0 ºC, the best results were obtained at five and 10 days, with 686.32 and 782.10 µmol Eq.Trolox g-1, respectively (Figure 1C). Note that there was no drop in the averages of treatments that were subjected to water stress without refrigeration over the 20 days. However, for samples subjected to both water stress and storage at 0 ºC, there was a decrease in this activity after 10 days of water restriction supply, that is, at 15 and 20 days, which were not favorable to maintain the antioxidant activity of citronella. Thus, Leão et al. (2019), when evaluating the antioxidant activity by the DPPH method of citronella, obtained values of 879.28, 1,171.17, and 1,167.54 µmol Eq.Trolox g-1 when storing citronella leaves at 0 ºC at 30, 60, and 90 days, respectively, values higher than those here presented, confirming the importance of storage at 0 ºC to obtain high antioxidant potential; plants harvested in a natural environment showed a DPPH value of 528.41 µmol Eq.Trolox g-1.

Regarding carotenoids, averages of 4.14 µg 100 g-1 and 4.12 µg 100 g-1 were observed at 10 days of water restriction supply without and with storage at 0 ºC, respectively. When the plants were submitted to 15 days of water restriction supply with and without refrigeration at 0 ºC, averages of 4.00 and 3.91 µg 100 g-1 were observed (Figure 1D). The treatment that most stood out was when the plants were subjected to 10 days of water restriction supply without freezing. Thus, it may be seen that water restriction supply was essential to optimize these components of citronella leaves, both in isolation and associated with storage at 0 ºC. Leão et al. (2019), when evaluating the carotenoid content in citronella, obtained values similar to those found in this work (3.67, 4.88, and 4.87 µg 100 g-1) when the leaves were stored at 0 ºC at 30, 60, and 90 days, respectively. Therefore, for the carotenoid content, in this work, the water stress, together with storage at 0 ºC, was efficient.

In the analysis of phenolic compounds, there were different behaviors among water restriction treatments with and without cold storage at 0 ºC (Figure 1E). The water restriction supply was positive to obtain higher values of phenolic compounds, with the best results at 10 and 15 days of restriction, with an average of 450.00 mg of EAG 100 g-1. The water restriction supply associated with the storage at 0 ºC presented the best results when performed at 5 and 10 days with an average of 480 mg of EAG 100 g-1. After this period, this association negatively interfered with the content of phenolic compounds. Selmar and Kleinwächter (2013), when cultivating the species Hypericum brasiliense under drought conditions, observed that stress improved the concentration and the total amount of phenolic compounds, with an increase of up to 10% in the total quantity of these products.

(A) (B)



(C) (D)

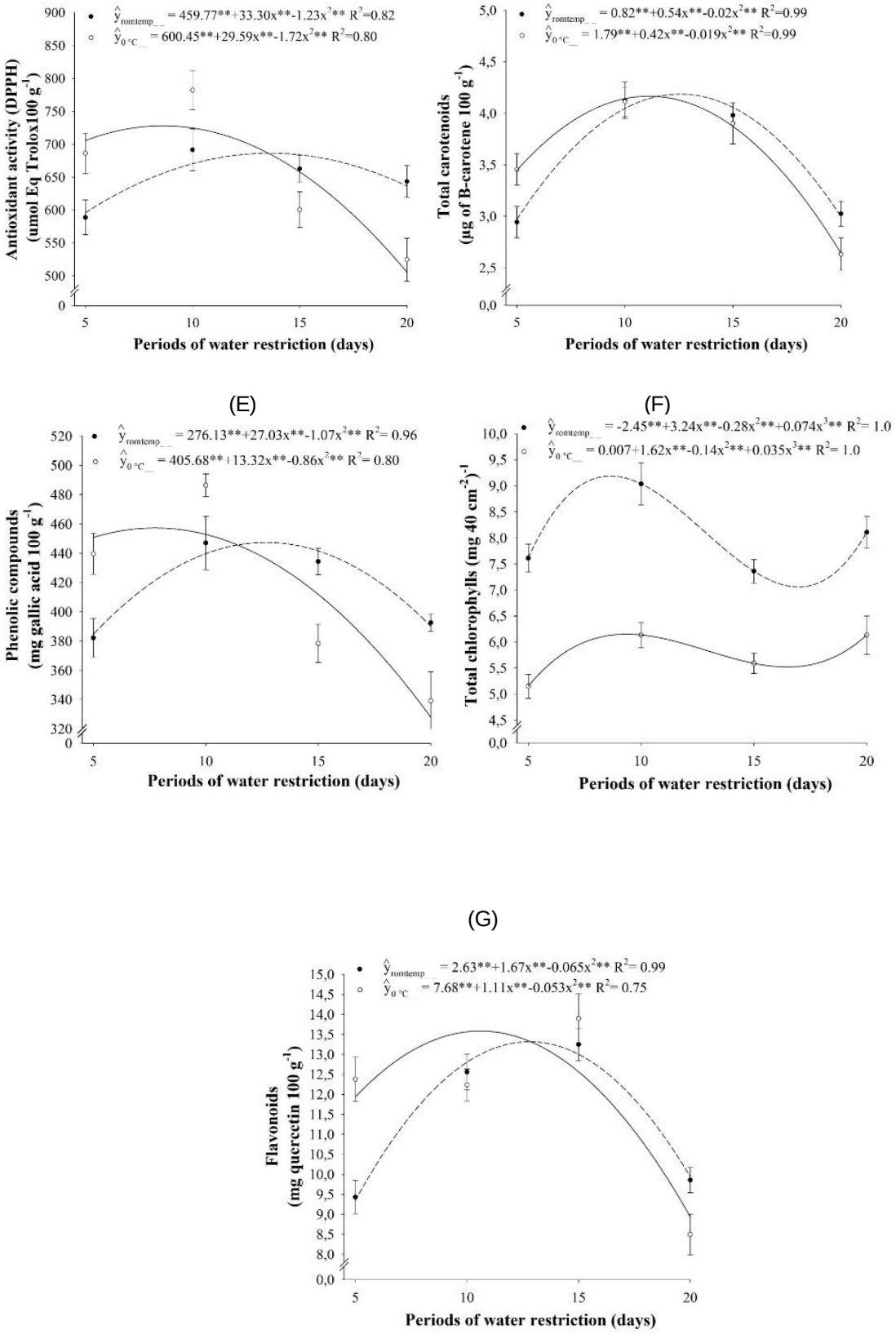


Figure 1. Total anthocyanins (A), Antioxidant activity (ORAC) (B), Antioxidant activity (DPPH) (C), Carotenoids (D), Phenolic compounds (E), Total chlorophylls (F), and Flavonoids (G) of *Cymbopogon nardus* plants subjected to water restriction supply and stored at room temperature (32 ± 2 ºC and 75 ± 3% of R.H.) or under cold (0 ± 0.5 ºC and 90 ± 2% of R.H.). Boa Vista county, Roraima State, Brazil (2019/2020).

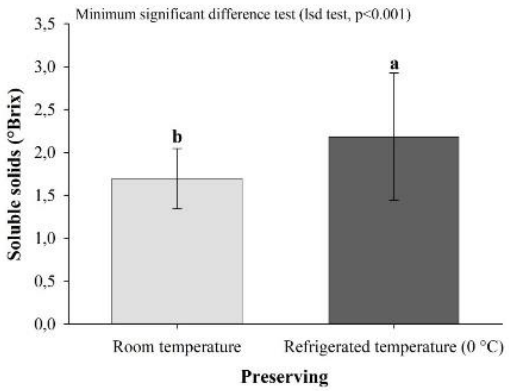
The highest levels of total chlorophylls for citronella were found when the plants were subjected to 10 and 20 days of water restriction supply at room temperature, with values of 9.02 mg (40 cm^2)$^{-1}$ and 8.13 mg (40 cm^2)$^{-1}$, respectively (Figure 1F). Therefore, water stress was a positive factor to optimize the synthesis of chlorophyll. However, associated with cold storage at 0 ºC, the chlorophyll content was negatively affected, thus obtaining much lower values for this compound. Therefore, when the chlorophyll contents were low, there may have been an interference of the pH of the plants, and in the treatments in which the leaves were stored at 0 ºC, the pH values found for citronella showed a different behavior from the values without refrigeration. This is a factor that directly affects the action of the enzyme, such as temperature. Lins et al. (2015), when quantifying the bioactive compounds in the species *C. citratus* sold in open markets, obtained total chlorophyll values of 4.52 mg 100 g^{-1}. In this work, lower values were obtained due to chlorophyll degradation because under conditions of great stress, the intensity of green leaf color tends to decrease. Silva et al. (2013), when submitting beets to different water depths, reported that there was a degradation of chlorophyll due to stress, resulting in a considerable reduction in the photosynthetic rate and, as a consequence, reduced productivity.

Regarding the flavonoid contents, the highest averages were found when the plants were subjected to 15-day water restriction supply, both for samples submitted to storage at 0 ºC and for those kept at room temperature, with values of 13.88 and 13.30 mg 100 g^{-1}, respectively (Figure 1G). In this sense, Lins et al. (2015), when quantifying the levels of flavonoids in the species *C. citratus* subjected to the stress caused by solar radiation, obtained values of 7.17 mg 100 g^{-1}. As this species is from the same citronella family, it is considered that their characteristics are similar, but in this work, higher values were obtained for citronella submitted to water restriction supply in the dry season. Therefore, it is possible to see that plants may respond in a similar way, both to the stress caused by exposure to excessive solar radiation and by drought. In this sense, Jaafar et al. (2012) reported that the concentration of total phenolics and flavonoids per plant is improved in plants that suffer water stress. In this work, it was also possible to notice the increase of these constituents until 15 days of water restriction supply.

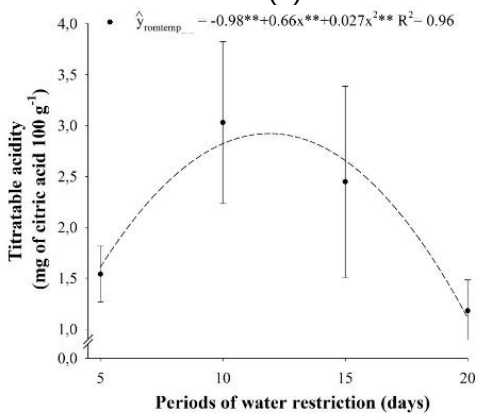
In the analysis of soluble solids contents (SS), it appears that there was no significant difference between the treatments regarding the days of water restriction supply, but there was a significant difference regarding the postharvest treatments. Thus, the analyzes of the leaves submitted to the cold storage showed better results in terms of SS contents compared to those storage at room temperature (Figure 2A).

According to Silva et al. (2013), plants subjected to water restriction supply have a reduced capacity to perform their metabolic and physiological processes, such as photosynthesis, thus decreasing the synthesis of sugars. Santos et al. (2014) observed an increase in the content of soluble solids when subjecting the leaves of the species *C. citratus* to the lyophilization process, resulting in initial values higher than those found in this work of 3.0 ºBrix. The water restriction treatments without cold storage did not result in an increase in the synthesis of soluble solids over the days of stress, with an average of 1.70 ºBrix, without significant differences. Menezes et al. (2005), when evaluating lettuce leaves stored at 5 ºC for 5 days, observed that there were no significant variations in the soluble solid contents. In this work, it may consider the fact that both cold storage at 0 ºC and water stress, in isolation, did not interfere with the soluble solids content, only when associated.

(A)



(B)



(C)

(D)

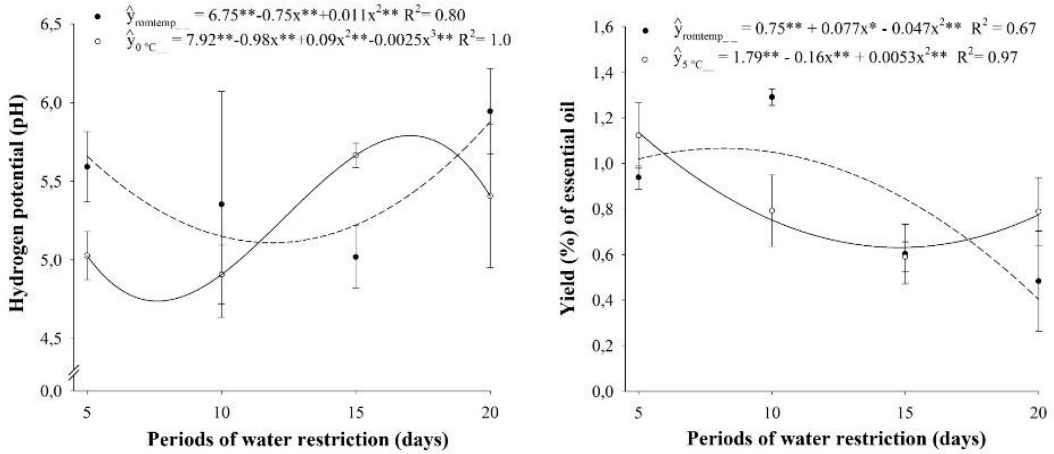


Figure 2. Soluble solids (A), titratable acidity (B), pH (C), and yield (D) of *Cymbopogon nardus* plants subjected to water restriction supply and stored at room temperature (32 ± 2 ºC and 75 ± 3% of R.H.) or under cold (0 ± 0.5 ºC and 90 ± 2% of R.H.). Boa Vista country, Roraima State, Brazil (2019/2020).

About the titratable acidity, there were significant differences regarding the periods of water restriction supply, with a significant increase observed after 10 days and a possible reduction in the following periods (Figure 2B). The water restriction supply, associated or not with the cold storage, presented similar behaviors to the logo of the period, being able to attribute this oscillation basically to the water stress. It is known that metabolic reactions may reduce the levels of titratable acidity, similar to the ripening processes in fruits, for example. Thus, the titratable acidity levels tend to decrease over time, even when the citronella leaves were stored under cold storage. The initial increase in titratable acidity may be associated with the increase in H^+ ions and organic acids due to the stress suffered by plants caused by water restriction supply. Then, with acclimatization, energy dissipation, and alternative energy routes, there is a considerable reduction in these ions and consequently a reduction in titratable acidity. The averages of titratable acidity changed from 1.5% at five days to 3.0% at 10 days of water restriction supply, 2.5% at 15 days and reduction to 1.0% at 20 days of restriction. The values for the titratable acidity of citronella leaves are scarce. However, Santos et al. (2014), when evaluating the aqueous and lyophilized extracts of *C. citratus*, obtained titratable acidity averages of 3.0% and 1.73% (g of citric acid 100 g^{-1}), respectively. These differences are due to the treatments to which these plant materials were submitted, with a significant decrease in the citric acid content due to the lyophilization process. Therefore, with the dehydration of the leaves, appreciable losses of citric acid are expected.

In concern to the pH of citronella leaves, both water restriction supply and cold storage did not cause major interference in the averages obtained, but at 10 days of restriction, there was a small reduction, possibly due to the increase in titratable acidity in that same period, with averages between 5.0 and 5.9 (Figure 2C). This little variation can be considered a positive factor since the increase or sudden drop in these values could compromise cellular metabolism. Santos et al. (2014), when evaluating the physicochemical characteristics of aqueous and lyophilized extracts of the species *C. citratus*, obtained mean pH values of 5.08 and 6.32, respectively. Lins et al. (2015) observed pH means of 5.81 in the process of physical-chemical quantification of the same species.

(A) (B)

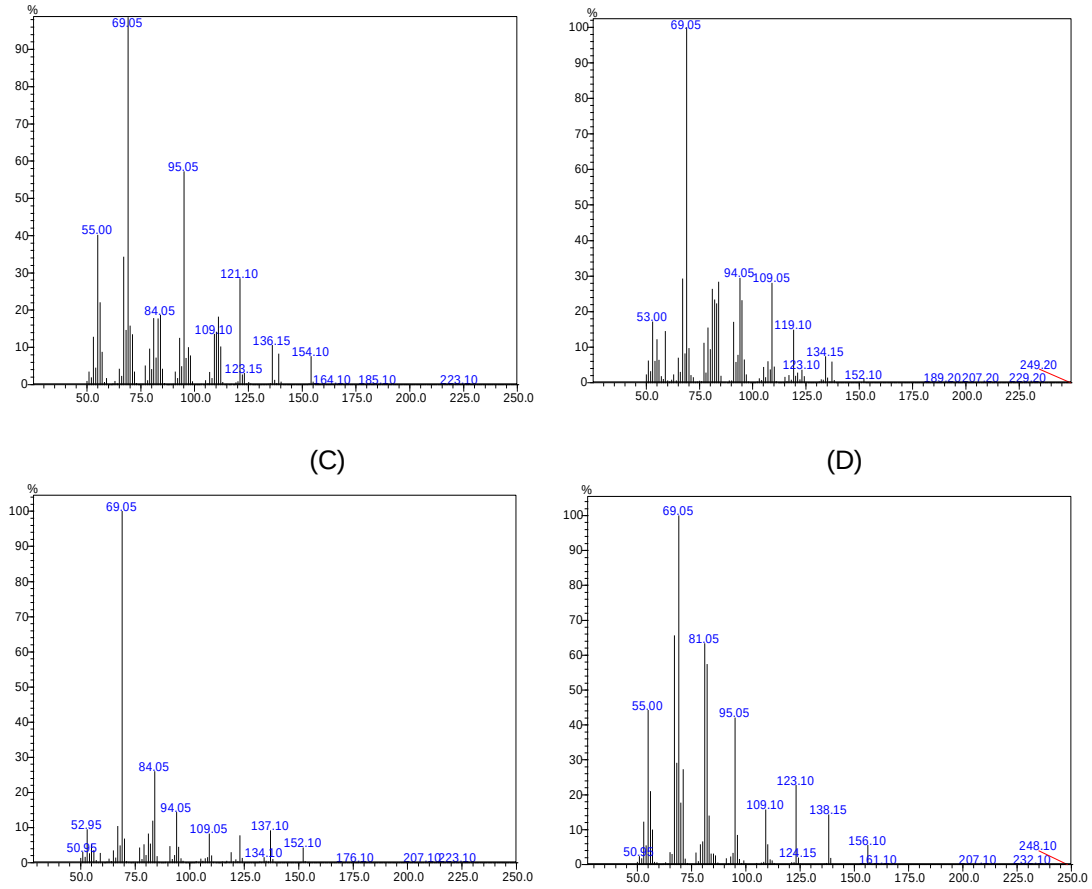


Figure 3. Chromatograms (CG-FID and CG-MS) mass spectra of the major peaks corresponding to Citronellal TR=17.99 min (A), Neral TR= 25.46 min (B), Geranial TR= 27.36 min (C), and Citronellol TR= 28.90 min (D) and other metabolites identified in *Cymbopogon nardus* plants subjected to water restriction supply and stored at room temperature (32 ± 2 ºC and 75 ± 3% of R.H.) or under cold (0 ± 0.5 ºC and 90 ± 2% of R.H.). For peak assignment, see Table 1. Boa Vista county, Roraima State, Brazil (2019/2020).

The lower pH values are ideal for the growth of fungi, approximately 2.0 to 5.0, and values above 6.0 favor the proliferation of bacteria. In the present experiment, the main objective of water restriction supply, as well as cold storage, was to maximize the synthesis of secondary citronella metabolites, and pH stability is an important factor. In addition, large changes in pH may negatively affect the quality of these secondary constituents. Plant tissues are considered acidic, so pH is a factor that may interfere in both chlorophylls and anthocyanins.

Yield, identification, and quantification of essential oils

The citronella in general showed an excellent yield of essential oil, and from most treatments, the amount was satisfactory.

Table 1. Chromatographic characteristics (CG-FID and CG-MS) of compounds identified in *Cymbopogon nardus* plants subjected to water restriction supply and stored at room temperature (32 ± 2 ºC and 75 ± 3% of R.H.) or under cold (0 ± 0.5 ºC and 90 ± 2% of R.H.). Relative abundance (%), retention time (min) and Kovats index (KI) were calculated. Boa Vista county, Roraima State, Brazil (2019/2020).

Peak #	RT min	ʛI calculated	Relative abundance (%) Water restriction supply (days) and storage at room temperature				Assignment
			5	10	15	20	
1	8.034	1193	1.0	1.0	1.4	0.8	limonene
2	18.718	1477	17.7	19.3	13.4	18.8	citronellal
3	19.403	1496	0.5	0.5	0.2	0.4	p-menth-8-en-3-ol
4	21.485	1551	0.7	0.8	0.8	0.6	linalool
5	21.962	1564	0.5	0.6	0.6	0.4	isopulegol
6	22.612	1581	1.7	1.0	1.7	1.9	β-karyophylene
7	25.569	1660	0.2	0.3	0.4	0.3	isoborneol
8	26.140	1675	7.1	7.0	0.6	6.0	neral
9	27.994	1724	10.1	9.8	1.0	9.2	geraniol
10	28.514	1738	1.6	0.8	1.4	1.8	γ-muuroleno
11	28.947	1749	0.5	0.5	0.7	0.5	geranyl acetate
12	29.413	1762	10.8	10.2	14.1	13.8	citronellol
13	30.490	1791	0.4	0.4	0.4	1.1	cis-geraniol
14	32.226	1837	39.3	41.4	55.8	36.4	trans-geraniol
15	35.802	1932	1.4	1.3	2.2	1.6	karyophylline oxide
16	43.684	2141	0.6	0.4	0.3	0.5	t-muurolol
17	44.740	2170	0.5	0.5	0.2	0.4	citronellic acid
18	47.213	2235	0.2	0.2	0.1	0.1	farnesol
other	-	-	5.2	4.1	4.6	5.3	-

Peak #	RT min	K calculated	Peaks (%) Water restriction supply (days) and storage at 0 ºC				Assignment
			5	10	15	20	
1	8.034	1193	0.7	0.8	0.9	0.7	limonene
2	18.718	1477	17.5	18.7	19.2	18.9	citronellal
3	19.403	1496	0.5	0.4	0.4	0.5	p-menth-8-en-3-ol
4	21.485	1551	0.7	0.6	0.6	0.6	linalool
5	21.962	1564	0.4	0.4	0.4	0.4	isopulegol
6	22.612	1581	1.2	1.2	1.5	1.6	β-karyophylene
7	25.569	1660	0.3	0.3	0.3	0.3	isoborneol
8	26.140	1675	6.5	3.9	3.7	6.6	neral
9	27.994	1724	9.8	6.3	5.9	10.4	geraniol
10	28.514	1738	1.1	0.9	1.3	1.6	γ-muuroleno
11	28.947	1749	0.5	0.5	0.6	0.6	geranyl acetate
12	29.413	1762	11.0	11.9	12.4	12.8	citronellol
13	30.490	1791	0.4	0.3	0.4	1.0	cis-geraniol
14	32.226	1837	42.0	46.4	45.4	35.5	trans-geraniol
15	35.802	1932	1.4	1.6	2.1	2.0	karyophylline oxide
16	43.684	2141	0.5	0.4	0.3	0.5	t-muurolol
17	44.740	2170	0.6	0.3	0.4	0.6	citronellic acid
18	47.213	2235	0.2	0.2	0.2	0.3	farnesol
other	-	-	4.8	5.1	3.7	5.2	-

However, from longer water restriction treatments, this yield fell significantly, with a higher value only after 10 days of restriction associated with storage at room temperature, with a yield of 1.29% (Figure 2D).

Storage at 0 ºC was not positive to the yield of essential oil, with this treatment not obtaining good results. In a study evaluating the effect of irrigation depths on citronella essential oil yield, Pinto et al. (2014) observed that higher values of irrigation depth resulted in lower essential oil yield, indicating that the water deficit may favor the production of this oil.

In this present work, prolonged stress had a negative effect on the essential oil yield, and water restriction supply for 15 and 20 days associated or not with cold storage had the worst oil yield. On the other hand, if the plant is in a rainy environment, there will be little essential oil synthesis, probably due to leaching losses of secondary constituents. In this sense, Marchese et al. (2010), when testing different intensities and durations of water stress on two species of the *Cymbopogon* genus, namely, *C. pendulus* and *C. nardus*, noted that this factor had a positive influence on the composition of monoterpenes. Another very relevant factor is that in both species, there was a reduction in growth, directly affecting the development of plants, but there was also a large accumulation of secondary metabolites in plant tissues, which may be a response to water scarcity.

Eighteen chemical components were identified in the citronella essential oil (Table 1 and Figure 3). The major components were citronellal (19.3%), with the highest percentage at 10 days of water restriction supply, stored at room temperature, geraniol (10.4%) at 20 days of water restriction supply and stored at 0 ºC, citronellol (14.1%), and trans-geraniol (55.8%) at 15 days of water restriction supply and stored in the postharvest at room temperature. However, trans-geraniol was the component found in greater quantity among all components and from all treatments.

In general, evaluating the influence of treatments on major components and of great importance, the highest concentrations of geraniol and citronellol were observed in the leaves of plants subjected to water restriction supply from days. But, for geraniol synthesis, the plants responded better to the water restriction treatment for 20 days and stored at 0 ºC. It is noted that plants responded positively to stress in the synthesis of major active principles.

When observing the constituents present in citronella essential oil, Andrade et al. (2012) identified 17 constituents, and the major components were the acyclic monoterpenes citronellal (47.12%), geraniol (18.56%), and citronellol (11.07%). Oliveira et al. (2011) identified 13 constituents in the essential oil citronella, citronellal (34.61%), followed by geraniol (23.18%), and citronellol (12.10%), while Oliveira et al. (2010), when studying the essential oil of citronella, found as major components, citronellal (34.60%), geraniol (23.17%), and citronellol (12.09%).

Thus, in comparative terms, it may be seen in this work that a greater number of constituents and values were also found for trans-geraniol, equal and even higher values for citronellol, and only for citronellal, lower values were observed. This difference in results, both for the majority and minority of components, is mainly due to edaphoclimatic factors, in addition to the treatments imposed on the plants in this study, since even these plants belong to the same species, the essential oil components are affected by harvest season, climatic conditions, age and growth, temperature, soil and fertilization, and storage conditions (Andrade, 2012).

It is possible to observe that the water stress results in a massive increase in the concentration of monoterpenes. When performing water restriction supply with *Salvia officinalis*, Nowak et al. (2010) observed a massive increase in the concentration of monoterpenes, compensating for the reduction in biomass. According to Kleinwachter and Selmar (2016), in this same species subjected to moderate drought, the synthesis of monoterpenes was significantly greater than that in plants without water restrictions supply.

Cold storage at 0 ºC was positive only when associated with water stress for 20 days. Thus, the stress caused by low temperatures, on its own, is already a factor that may optimize the secondary constituents of essential oils. However, in the other treatments, storage at 0 ºC did not have the expected effect when associated with water restriction supply.

Bioassay of inhibitory activity of the enzyme acetylcholinesterase

In the acetylcholinesterase enzyme inhibition bioassays (AChE), positive results were observed for citronella essential oil, but none of the treatments exceeded the standard control sample of eresin (Table 2). Among the samples that showed significant results, leafs in the treatments collected in the period with water restriction supply at 5 and 20 days and stored at room temperature, presented the highest means of enzymatic inhibition, 64.07 and 56.62%, respectively.

Vinutha (2007), when studying AChE inhibitors, proposed the following classification: potent inhibitors (>50%); moderate inhibitors (30-50%); and weak inhibitors (<30%). However, one may then classify them as potent inhibitors (>50%) and potential sources of compounds to be used in the health field due to their relevant actions against the enzyme AChE and inhibitors of free radicals.

Table 2. Acetylcholinesterase (AChE) enzyme inhibition (± standard deviations) and classification of *Cymbopogon nardus* plants subjected to water restriction supply and stored at room temperature (32 ± 2 ºC and 75 ± 3% of R.H.) or under cold (0 ± 0.5 ºC and 90 ± 2% of R.H.). Boa Vista county, Roraima State, Brazil (2019/2020).

Treatments (water restriction periods and storage conditions)	**AChE inhibition (%)**	**Classification [a]**
Eserine (stardard)	83.07 ± 02.49	-
5 days and stored at room temperature	64.07 ± 12.02	powerful
10 days and stored at room temperature	28.44 ± 09.17	weak
15 days and stored at room temperature	46.30 ± 05.94	moderate
20 days and stored at room temperature	56.62 ± 05.00	powerful
5 days and stored at 0 ºC	23.19 ± 09.46	weak
10 days and stored at 0 ºC	20.56 ± 09.16	weak
15 days and stored at 0 ºC	45.84 ± 07.05	moderate
20 days and stored at 0 ºC	46.97 ± 06.17	moderate

[a]Classification according to Vinutha (2007).

Table 3. Inhibition of microorganisms by extracts of *Cymbopogon nardus* plants subjected to water restriction supply and stored at room temperature (32 ± 2 ºC and 75 ± 3% of R.H.) or under cold (0 ± 0.5 ºC and 90 ± 2% of R.H.). Boa Vista county, Roraima State, Brazil (2019/2020).

	Microorganisms				
Treatments	*Staphylococus aureus*	*Escherichia coli*	*Salmonella typhimurium*	*Bacillus cereus*	*Candida albicans*
WR5dRT	0.00	0.00	0.00	0.00	72.00^{ns}e ±2.31
WR5d0ºC	4.76***f ±1.95	0.64***c ±0.51	0.00	0.00	82.37^{ns}c ±4.26
WR10dRT	8.33***e ±0.28	10.43***b ±2.96	4.30***d ±0.95	0.00	76.16^{ns}d ±0.39
WR10d0ºC	17.45***c ±0.28	24.34***a ±4.41	13.62***bc	0.00	98.36**a ±16.27
WR15dRT	18.53***c ±0.42	24.60***a ±1.30	11.06***c ±1.61	0.00	97.26**a ±8.52
WR15d0ºC	30.62***a ±0.60	23.21***a ±1.77	16.35***b ±1.62	5.86***b ±0.41	82.0^{ns}cd ±6.71
WR20dRT	11.34***d ±0.35	11.71***b ±0.51	5.96***d ±2.42	6.28***b ±5.14	86.66^{ns}b ±1.55
WR20d0ºC	24.89***b ±1.49	24.17***a ±1.95	20.80***a ±0.08	77.90***a ±0.73	83.5^{ns}bc ±2.80
Ampicilin	97.87 ±0.52	95.39 ± 0.21	95.86 ± 0.62	90.37 ± 1.26	nd
Miconazol	nd	nd	nd	nd	68.51 ± 3.12

Means followed by different letters differ from each other by Tukey's test ($p<0.05$).
***($p<0.001$); **($p<0.01$) differ by Dunnett's test. ns($p>0.05$) do not differ by Dunnett's test.
WR: water restriction; d: days; RT: stored at room temperature; 0°C: stored at 0°C.
nd = not determined.

Therefore, it is noteworthy that the treatments described above had good results for the sum of all constituents in general, and plants under water restriction supply for five days and leaves stored at room temperature showed even better results for the majority of constituents, neral (7.1%), and geraniol (10.1%) (Table 1). The treatment of water restriction supply for 20 days and storage at room temperature showed the highest content of β-karyophylene (1.9%) and γ-muurolene (1.8%). Although it is not a large difference in relation to the other treatments, this may have contributed to the good result of inhibiting the enzyme.

According to Souza et al. (2013), many of the constituents of essential oils are AChE inhibitors, such as neral, geraniol, and linalool, and with that it is possible that the best performances of the water stress treatments for five days and storage at room temperature (Table 2), either due to the high levels of neral and geraniol found (Table 1). According to Ferreira et al. (2016), several constituents of essential oils have different biological activities in the central nervous system of humans; however, in this work, it was difficult to find possible answers or to define which constituents contributed to greater or lesser inhibition of AChE.

Water stress was positive in the inhibitory activity of the AChE enzyme when the plants were subjected to water restriction supply for five and 20 days and stored at room temperature, with potent inhibition for both treatments. However, when associating these treatments with cold storage at 0

ºC, all values decreased; that is, the water restriction supply associated with cold storage of leaves is not indicated to have a good result in inhibiting AChE. According to Alcantara et al. (2010), the monoterpenes and sesquiterpenes elemol, linalool, and α-pinene have proven AChE inhibitory activity. However, the joint action of these substances may either reduce or intensify the inhibitory activity of the enzyme due to the synergistic effect and the competitive inhibition between them. Therefore, it cannot be inferred whether any substance alone or in conjunction with others is responsible for such activity.

Bioassay of antimicrobial activity

It appears that the essential oils studied showed an inhibitory effect for gram-positive, gram-negative, and an antifungal activity against yeasts (Table 3). All samples showed antimicrobial activity against *Candida albicans* strains. The water restriction supply for 10 and 15 days associated with cold storage at 0 ºC and room temperature, presented an inhibitory percentage statistically superior to the other treatments ($p<0.05$). The values observed for samples of treatments submitted to 10 days of water restriction supply and storage at 0 ºC, as well as the samples submitted to water restriction supply during 15 days and storage in natural conditions were higher than the standard control value for miconazole ($p<0.001$). The other treatments applied did not show significant differences in relation to the standard miconazole control ($p>0.05$).

Santos et al. (2009) proposed values of minimum inhibitory concentration (MIC), considered a reference for antimicrobial activity against essential oils: high inhibition - MIC up to 0.5 mg mL^{-1}; moderate inhibition - MIC between 0.6 and 1.55 mg mL^{-1}; low inhibition - MIC above 1.65 mg mL^{-1}. However, the inhibition results for *C. albicans* proved to be promising compared to the treatments, since the concentration used was 0.25 mg mL^{-1}. Therefore, citronella oil is considered to have high inhibition for that yeast. Duarte et al. (2014) state that the effect of a substance considered medicinal in inhibiting microorganisms in concentrations below 100 µg mL^{-1} presents antimicrobial action considered good; between 100 - 500 µg mL^{-1} is considered moderate; and between 500 and 1,000 µg mL^{-1} is considered to be of low activity. Considering these parameters, the concentration of 250 µg mL^{-1} used in this test classifies citronella oil as a moderate inhibitor. However, considering the good result of treatments for *C. albicans*, the use of lower concentrations of this oil for this microorganism may have positive feedback.

Citronella oil showed antibacterial inactivity in relation to some treatments, and for water restriction supply for five days and storage at room temperature, inactivity was observed for all bacteria tested. The water restriction supply for five days associated with cold storage showed inactivity for *Salmonella typhimurium* ATCC 14028 and *Bacillus cereus*, and as for the other bacteria, this treatment showed low inhibition compared to the other treatments and ampicillin.

For *B. cereus*, only the water restriction treatments for 15 and 20 days associated with cold storage and the water restriction supply associated with storage at room temperature demonstrated antibacterial activity. These treatments showed high levels of geraniol and citronellol, a fact that may have contributed to this result, since these alcohols act as dehydrating agents and solvents causing protein denaturation (Andrade et al., 2012).

Geraniol, citronellal, and citronellol found in essential oils, such as citronella oil, are compounds that have high antimicrobial activity. Therefore, products that have a high content of these constituents can be used efficiently in the control of microorganisms; thus, citronella oil may prove to be an alternative to synthetic antimicrobials (Scherer et al., 2009).

In general, among all the tested bacteria, *B. cereus* showed the greatest resistance to the inhibitory effect of citronella essential oil. In addition, it was also shown to be more resistant to the effect of the antibiotic ampicillin compared to other bacteria.

S. typhimurium has also been shown to be resistant to citronella essential oil, having no inhibitory effect on water restriction treatments for five days associated or not with cold storage. For the other treatments, there was inhibition of the bacteria in relation to the oil, but this effect was low, considering ampicillin and the other tested bacteria (*Staphylococcus aureus* and *Escherichia coli*).

For *S. aureus* and *E. coli*, the inhibition values were more significant. The water restriction supply of 15 days associated or not with cold storage and the water restriction supply of 20 days at room temperature were the treatments that showed the best results, but lower when compared to ampicillin.

In this work, the dosage used for citronella essential oil was 250 µg mL^{-1} or 0.25 mg mL^{-1}. Oussalah et al. (2006), when evaluating the antimicrobial effect of citronella essential oil, obtained minimum inhibition concentrations for *E. coli* of 8.0 µg mL^{-1}, for *S. typhimurium* of 4.0 µg mL^{-1}, and for *S. aureus* of 0.5 µg mL^{-1}. Scherer et al. (2009) presented an MIC of 0.8 µg mL^{-1} for *E. coli*, of 0.4 µg mL^{-1} for *S. typhimurium*, and 0.05 µg mL^{-1} for *S. aureus* when using different concentrations of citronella essential oil.

In most cases, Gram-negative bacteria are more sensitive to essential oils compared to Gram-positive bacteria because of the cell wall of these Gram-negative bacteria, which is rich in polysaccharides, and therefore makes it easy for products to penetrate, such as antimicrobials (Duarte et al., 2014). However, in this work, the Gram-negative (*E. coli* and *S. typhimurium*) and Gram-

positive (*S. aureus* and *B. cereus*) species evaluated showed different sensitivity patterns. In general, the microorganisms tested, with the exception of *C. albicans*, showed less resistance to ampicillin and more resistance to citronella essential oil, with *B. cereus* being the most resistant bacteria, both to antibiotics and to essential oil.

Conclusions

The water restriction supply of citronella plants for 10 and 15 days associated with cold storage of the leaves at 0 ºC (0 ± 0.5 ºC and 90 ± 2% of R.H.) induced the major production of secondary metabolites in *Cymbopogon nardus* essential oil. The concentration of carotenoids and chlorophyll was superior when plants were subjected to water restriction supply for 10 days and stored at room temperature (32 ± 2 ºC and 75 ± 3% of R.H.). For phenolic compounds and antioxidant activity, also water restriction supply for 10 days and storage at 0 ºC resulted in the highest averages, while for flavonoids, water restriction supply for 15 days and storage at 0 ºC promoted the best results.

The major components identified in *Cymbopogon nardus* essential oil were citronellal (19.3%) under water restriction supply for 10 days and stored at room temperature, and citronellol (14.1%) and trans-geraniol (55.8%) under water restriction supply for 15 days stored also at room temperature. In the essential oil, most of the treatments used confirmed to be effective in inhibiting the enzyme acetylcholinesterase and fungicidal and bactericidal effects were observed in the minimum inhibitory concentrations of CMIs. The treatment that most stood out was when plants were subjected to 10 days of water restriction supply without freezing at 0 ºC. Thus, it may be seen that water restriction supply was essential to optimize these components of citronella leaves, both in isolation and associated with storage at 0 ºC.

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