Water suppression indicates the prevalence of the secondary defense system in *Piper aduncum*

Fernanda Ventorim Pacheco[1], Ivan Caldeira Almeida Alvarenga[1*], Suzan Kelly Vilela Bertolucci[2], José Eduardo Brasil Pereira Pinto[1], Amauri Alves de Alvarenga[3]

[1]Laboratory of Plant Tissue Culture and Medicinal Plants, Department of Agriculture, Federal University of Lavras, 37200-000, Lavras, Brazil
[2]Laboratory of Phytochemistry and Medicinal Plants, Department of Agriculture, Federal University of Lavras, 37200-000, Lavras, Brazil
[3]Laboratory of Plant Growth and Development, Department of Biology, Federal University of Lavras, 37200-000, Lavras, Brazil.
Corresponding author: pytchouai@yahoo.com.br

ABSTRACT

The aim of this work was to evaluate the response of *Piper aduncum* to water suppression. The experiment was conducted in a greenhouse, in an entirely randomized blocks, with five treatments: 0, 2, 4, 6, and 8 days without irrigation. After this period, dry matter, photosynthetic pigments (chlorophyll *a*, *b*, and carotenoids), leaf temperature, activity of the enzymes ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) were evaluated. The essential oil content of leaves and roots was also quantified through hydrodistillation, as well as the identification of constituents by CG-MS. The period of water suppression influenced the content of chlorophyll *a*, carotenoids, and enzymatic activity of APX and CAT. The activities of APX and CAT were reduced under low water availability (CAT only increased after 4 days of suppression). Meanwhile, SOD had its activity increased under eight days of water suppression. In addition, there was an increase in essential oil content when subjected to stress. The predominant classes of constituents in the leaves were sesquiterpenes (32.56-36.54%) and phenylpropanoids (33.12- 44.97%) in the roots. *E*-nerolidol was the major constituent of leaves (23.56-26.75%) and apiol (17.57-32.78%) of the roots. Thus, water suppression favors the secondary metabolism of the species.

Keywords: Terpenes**,** phenylpropanoids, medicinal plants, water stress, essential oil.

INTRODUCTION

The monkey-pepper (*Piper aduncum* L.; Piperaceae) is a shrub native of Americas and has a high economic potential due to its bioactive properties (Rocha et al. 2005). Among these properties, the species shows antimicrobial, insecticidal and antioxidant activities (Fazolin et al. 2005; Sousa et al. 2008). These activities are mainly attributed to the compound dillapiole, which is a substance that has been reported by some authors, as the main component of the essential oil obtained from the leaves of this species (Rali et al. 2007; Araújo et al. 2020). In addition, according to Parmar et al. (1997), the biological activities attributed to essential oil can be associated with the presence of other components such as phenolic compounds, flavonoids and sesquiterpenes. However, the literature reports variations in the chemical composition of its essential oil, due to external factors (Potzernheim et al. 2006; Oliveira et al. 2013).

The agronomic cultivation of medicinal species allows obtaining vegetal raw material with higher quality and safety. Since there is a greater control of some factors that influence the production of active ingredients. In addition, the cultivation of medicinal plants helps in the preservation of native species that suffer extraction (Lee et al. 2020). The variation in production and composition of essential oil is linked to factors extrinsic to the plant, such as radiation, mineral nutrition, latitude, altitude, geographic orientation and water availability (Gobbo Netto and Lopes 2007).

Water limitation influences practically the entire physiology of the plant, mainly altering photosynthesis and activating antioxidant systems, due to the formation of reactive oxygen species (ROS) that can degrade cell membranes, causing cell death (Foyer, 2018). To mitigate the effect of ROS, plants have enzymes that act as reactive oxygen inactivators, especially Superoxide Dismutase (SOD), Catalase (CAT) and Ascorbate Peroxidase (APX). SOD is responsible for performing, through

Received: June 27, 2022
Accepted after revision: August 20, 2022
Published on line: September 14, 2022
ISSN 1983-084X

catalysis, the dismutation of ROS in H_2O_2 and O_2. Hydrogen peroxide (H_2O_2) that is formed by the action of SOD is considered a toxic compound for cells and that can be converted into even more reactive radicals such as hydroxyl (Perl-Treves and Perl 2002). Then H_2O_2 becomes a substrate for ascorbate peroxidase (APX) and catalase (CAT) enzymes that are responsible for converting H_2O_2 into H_2O and O_2. CAT has one of the highest turnover rates among all enzymes; a CAT molecule can convert 6 million molecules from H_2O_2 to H_2O and O_2 per minute (Gill and Tuteja 2010).

The water limitation in the environment can change quantitatively and qualitatively the production of secondary compounds and the growth of species. Studies related to water availability in the cultivation for medicinal species have shown specific responses in relation to the growth and production of metabolites of economic interest. Lopes et al. (2011) evaluating different irrigation depths in *Lippia sidoides* Cham. observed increasing responses both for growth and for the production of essential oil with increasing water availability. In *Melissa officinalis* L. Meira et al. (2013) observed a reduction in the essential oil content with the increase of the applied water depths. Alvarenga et al. (2018) observed that water limitation influences the profile and contents of the volatile constituents of *Achillea millefolium* L. Thus, studies on irrigated cultivation in medicinal species are incipient, lacking technical and physiological information that allows combining high production of dry matter, with adequate levels of active ingredients (Abdelmajeed et al. 2013).

In this context, the objective was to evaluate the effect of water suppression for different periods of time (0, 2, 4, 6, and 8 days) on dry matter production, production of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids), leaf temperature, activity of antioxidant enzymes (SOD, CAT, and APX), production and quality of essential oil from leaves and roots of *P. aduncum*.

MATERIALS AND METHODS

Plant material

The experiment was conducted at the Federal University of Lavras, Brazil. *P. aduncum* seedlings were cultivated from seeds in the Department of Biology. The seeds were pre-germinated in petri dishes on three sheets of filter paper and kept in a Mangelsdorf germination chamber at 25 °C and for a 12-h photoperiod for 30 days. After this period, the seedlings were transferred to polypropylene trays containing the commercial substrate Tropstrato HA® (Vida Verde©, Brazil) and kept in greenhouse with 50% shading until they reached 2.5 cm height. The seedlings were transplanted to 6 l plastic pots containing a substrate comprised of subsoil, sand, and bovine manure; in a 2:1:1 ratio (v/v). The physicochemical characteristics of the soil were analyzed in the Soil Testing Laboratory, being that: pH: 5.4; P: 4.13 mg/dm^3; K: 73.32 mg/dm^3, Ca: 2.30 cmolc/dm^3, Mg: 0.30 cmolc/dm^3, Al: 0.10 cmolc/dm^3, H + Al: 2.90 cmolc/dm^3, V: 49.00%; organic matter: 2.10 dag/kg, Clay: 70.00 dag/kg; Silt: 16.00 dag/kg, and Sand: 14.00 dag/kg.

The plants keeped in greenhouse of 50% of irradiance until start the experiment, with average measures: 32.3 cm heigh, 9.79 mm diameter of collar and 11 pairs of leaves. During the growing period, the irrigation was performed daily, and the soil was kept under field conditions. After 180 days, was apllied de treatments, which consisted of suppressing irrigation for periods of 2, 4, 6, 8, and one treatment that remained irrigated for all period. The leaf temperature was monitored using an Incotherm® infrared thermometer, being measured on three fully expanded leaves of all plants in each treatment. The average air temperature during the suppression days was 30.62 ºC, measured with a maximum and minimum thermometer.

After the period of water suppression, the dry matter of the aerial part, root and total, the photosynthetic pigments, the activity of antioxidant enzymes (SOD, CAT and APX), the content and quality of the essential oil of the leaves and roots of *P. aduncum* was measured. For the analysis of the activity of antioxidant enzymes, the plant material was collected, immediately frozen in liquid nitrogen and kept refrigerated at -86 °C (ultrafreezer) until the moment of the analysis.

Dry matter and photosynthetic pigments

The dry matter production in the different water suppression treatments was evaluated in 10 plants per treatment. Aerial dry matter (ADM), root dry matter (RDM) and total dry matter (TDM = ADM + RDM) were evaluated. The dry matter was obtained by drying the aerial part (leaves and stems) and roots, previously separated. For this, the material was dried in an oven with forced air circulation, at a temperature of 45 °C, until constant weight.

The photosynthetic pigments analyzed were chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids. The extraction was performed according to the methodology reported by Lichtenthaler and Buschmann (2001), being collected fully expanded leaves located at the third node in last day of experiment; after collection, leaves were placed in aluminum foil and transported in polystyrene boxes containing ice for immediate extraction and quantification of pigments. For extraction, weighed 200 mg of fresh leaves and homogenized with 10

ml of 80% acetone (v/v), filtered through glass wool, completing the volume for 30 ml 80% acetone. Immediately following this procedure was carried the reading of the absorbance at 663.2 nm, 646.8 nm and 470 nm in spectrophotometer, respectively. The entire procedure was performed in the dark, to prevent degradation of chlorophylls. The content of chlorophyll *a*, *b* and carotenoids were expressed in mg/g fresh weight. After determining the contents, were done the total chlorophylls, summing the contents of chlorophyll *a* and *b*.

Antioxidant enzymes activity

The antioxidant enzyme evaluated were superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). These were extracted according Biemelt et al. (1998): 0.2 g of fresh leaf were placed in liquid nitrogen and homogenized in 1.5 ml of extraction buffer containing 1.47 ml of potassium phosphate 0.1 M (pH 7.0), 15 µl of EDTA 0.1 M (pH 7.0), 6 µl of DTT 0.5 M, 12 µl of PMSF 0.1 M, ascorbic acid 0.001 M and 22 mg polyvinylpolypyrrolidone (PVPP). The extract was centrifuged at 12,000 *x* g for 30 min at 4 °C, and the supernatant was collected and stored at -20 °C during the analysis period. The SOD activity was measured by the ability of the enzyme to inhibit the photoreduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries 1977). Aliquots (10 µl) of enzymatic extract were added to the incubation medium, which contained 100 µl of potassium phosphate 100 mM (pH 7.8), 40 µl of methionine 70 mM, 3 µl EDTA 10 µM, 31 µl of water, 15 µl of NBT 1 mM, and 2 µl of riboflavin 0.2 mM.

Tubes containing the reaction medium and 10 µl of sample were illuminated for 7 min with a 20 W fluorescent lamp. The same reaction medium without a sample was illuminated as a control. Readings were taken at 560 nm, and the calculation of the enzyme was performed with the following equation: % inhibition = (A560 sample with enzyme extract – A560 control without enzyme extract)/(A560 control without enzyme). One unit of SOD can inhibit 50% of the photoreduction of NBT under the assay conditions.

CAT was evaluated according Havir and McHale (1987) as follows: aliquots (10 µl) of enzyme extract were added to 170 µl of incubation medium containing 90 µl of potassium phosphate 200 mM (pH 7.0), 71 µl of water and 9 µl of hydrogen peroxide 250 mM, incubated at 28 °C. Enzyme activity was determined by the decrease in absorbance at 240 nm every 15 s for 3 min, monitored by the consumption of hydrogen peroxide. The molar extinction coefficient used was 36 mM/cm. The APX activity was determined by monitoring the rate of oxidation of ascorbate at 290 nm every 15 s for 3 min. Aliquots (10 µl) of enzyme extract were added to 170 µl of incubation buffer, consisting of 90 µl of potassium phosphate 200 mM (pH 7.0), 9 µl ascorbic acid 10 mM, 62 µl of water and 9 µl of hydrogen peroxide 2 mM (Nakano and Asada 1981). The molar extinction coefficient was 2.8 mM/cm.

Essential oil content

To determine the essential oil content, plants were dried in a circulating forced oven, at temperature of 45 ºC, to constant weight. The hydrodistillation by Clevenger apparatus (Vidrolabor®, Poá, Brazil.) was used, with the time of 90 min. For each treatment, three samples of 30 g were used. The material before hydrodistillation it was cut into ~1 cm with a scissor and placed in volumetric balloons of 2 l of capacity. After the hydrodistillation time, essential oil and hydrolate were collected. The condenser and the apparatus collector were washed 3 times, with the aid of a pisete containing dichloromethane, to collect oil adhered in glassware. Essential oil, hydrolate and washes of dichloromethane were kept in separation balloon, allowed to stand for 15 min and then separated liquid–liquid partition. The solution was treated with anhydrous magnesium sulfate to remove possible moisture residues. After treatment, the solution was filtered, and the solvent evaporated under gas exhaust hood. The isolated oils were weighed in analytic balance and stored in tightly closed vials at 4 ºC until analysis. The content of essential oil was calculated in percent (w/w) in dry mass basis of sample.

Chromatographic analysis

The essential oils from leaves and roots were analyzed in the Phytochemical Laboratory of the Department of Agriculture at UFLA. The samples were prepared through the dilution of the essential oils in ethyl acetate (1%, v/v), prior to the analyses. A GC/MS Agilent 7890 A equipment, operated by the HP GC ChemStation Ver. A.01.14 software, equipped with an automatic injector/sampler CombiPAL Autosampler System (CTC Analytic AG, Switzerland) and with a flame ionization detector (FID) was used. A HP-5MS capillary column (30 m × 250 µm, 0.25 µm film thickness) and Helium as the carrier gas were used. The analyses were under the following conditions: 1.0 µl sample injection, in split mode, and at 1:20 injection flow. The selective mass detector operates through impact ionization at 70 eV, in scan mode, at 1.0 scan/s, with mass acquisition interval of 40-400 *m/z*. Regarding FID analyses, the initial oven temperature was at 60 ºC, which was maintained for 1 min, followed by a ramp of 3 ºC/min until it reached 240 ºC, then followed by a ramp of 10 ºC/min until it reached 250 ºC, maintaining isothermal conditions for 1 min. The

injector and transference line temperatures for the mass spectrum were maintained at 250 ºC. The flow of the gas carrier (Helium) was 1.0 ml/min.

The constituents were identified for comparison of relative retention index, relative a *n*-alkanes series (C_8-C_{20}) (Sigma-Aldrich®,St. Louis,USA) and mass spectrum of literature (NIST, 2008; Adams, 2007). The retention index was calculated using the Van den Dool and Krats (1963) equation. The results of the analyses are shown in Table 3 and 4 with the calculated retention index, and the compounds relative percentage and classes.

Statistical analyses

In this experiment, an entirely randomized blocks design was used, with five treatments (0, 2, 4, 6, and 8 days without irrigation) with 5 repetitions, where each repetition consisted of five plants. To determine the content of essential oil, pigments and enzymes, three triplicates for each treatment were used. The data were compared using the Tukey test ($p < 0.05$) by the SAEG program (SAEG, 2007).

RESULTS AND DISCUSSION

The periods of water suppression did not affect the dry matter production of *P. aduncum* (Table 1). The low water availability can lead to stomatal closure, thus reducing gas exchange and consequently decreasing CO_2 fixation. In addition, it is possible to mention an increase in respiration in relation to photosynthesis, which can contribute to the decrease in dry matter (Taiz el al. 2017; Yang et al. 2019). However, the non-alteration of the observed dry matter production may be related to the short period that the plants were exposed to water suppression. Additionally, the species *P. aduncum* has adaptations to conditions of low water availability, being considered a pioneer plant present in the clearings of the forests (Yunker 1975). However, Santos et al. (2006) analyzing water suppression for *Hyptis pectinata* (L.) Poit., observed a decrease in leaf dry matter under 6 days of water suppression.

The chlorophyll *a*, total and carotenoids pigments increased in plants (Table 1) under suppression (mainly, in treatments with 6 and 8 days) while chlorophyll *b* production was not affected. The increase in chlorophyll *a* levels in plants under longer water suppression period may be related to an increase in the *PSII* reaction center, maintaining the photosynthetic efficiency of the plant. Since chlorophyll *a* is the pigment used to perform the photochemical step (the first stage of the photosynthetic process). While the other pigments help to absorb light and transfer radiant energy to the reaction centers, they are called accessory pigments (Streit et al. 2005). Thus, even under water suppression, *P. aduncum* maintains its photosynthetic efficiency, as it increases the production of chlorophyll *a*. Contrary results were observed for *Cymbopogon flexuosus* (Nees ex Steud.) W.Watson and *Annona muricata* L., where the water deficit reduced the amount of chlorophyll (Oliveira et al. 2013; Singh et al. 2017).

An increase in the levels of pigments such as chlorophyll *a* under water deficit implies an increase in the ability to capture light (Mafakheri et al. 2010). When there is an excess of energy, absorption by the photosynthetic apparatus (photosystems and antenna complex), and this can cause photoxidation. In this study, an increase in carotenoid levels was also observed. These are considered accessory pigments in the photosynthetic process and protectors of chlorophyll (Lima et al. 2004). Thus, the observed increase may indicate a species defense mechanism to support water suppression and maintain CO_2 assimilation, avoiding oxidative damage in the photosynthetic apparatus (Souza et al. 2013). Similar results were observed for medicinal species like *Plantago* spp. and *Achillea millefolium* L., where there was a greater accumulation of pigments in plants grown under water deficit (Gonçalves et al. 2017; Alvarenga et al. 2018).

The higher leaf temperature (Table 1) observed at eight days of water suppression,

Table 1. Dry matter, photosynthetic pigments and leaf temperature of *Piper aduncum* under 0, 2, 4, 6, and 8 days of water supression.

Days	Dry matter (g/plant)			Pigments (mg/g/mf)				Leaf Temp ºC
	Aerial Part	Roots	Total	Cloro *a*	Cloro *b*	Cloro Tot	Carot	
0	10.9671^{ns}	4.3164^{ns}	15.28358^{ns}	1.36c	1.06 ^{ns}	2.42c	0.96 b	24.25b
2	10.4429	3.9765	14.41942	1.46c	0.85	2.31bc	0.88 b	24.74b
4	10.3527	4.1524	14.50506	1.71bc	0.82	2.53bc	0.95 b	24.96 b
6	11.1158	3.5434	14.65924	2.53ab	1.11	3.64 ab	0.99 b	25.62 ab
8	9.5337	3.5025	13.03622	2.90 a	1.20	4.11 a	1.60 a	26.46 a

*Means followed by the same letter in the column do not differ by the Tukey test at 5% probability.^{ns} Not significant.

indicates that the plant reduces transpiration and, consequently, reduces its heat exchange capacity with the environment, which mainly changes the enzyme complex of the plants (Yang et al. 2019). This result indicates that the species may have other defense mechanisms to maintain its photosynthetic rates in stress situations.

Water restriction caused a significant decrease ($p < 0.05\%$) in APX activity (Figure 1A). This reduction may be associated with a large production of ROS. The APX is an enzyme that has increased activity in low concentrations of ROS and attenuates oxidative stress generated under inadequate conditions (Shvaleva et al. 2006). An increase in APX activity was observed for *Gossypium herbaceum* L. and *Fraxinus ornus* L. under moderate water stress (Fini et al. 2012; Yi at al. 2016).

The CAT also had reduced activity under water deficit, except for treatment with four days of suppression (Figure 1B). The increase in the activity of this enzyme may have been induced by the accumulation of H_2O_2, and the reduction may be related to the participation, again, of other molecules of protection against oxidative stress. This enzyme is involved in antioxidant protection and in maintaining membrane integrity (Carvalho et al. 2011). On the other hand, SOD had its activity increased with water suppression, being greater in the treatment with two, four and six days of suppression (Figure 1C). Similar results were observed by Masoumi et al. (2010) while working with soy.

SOD is considered the most efficient antioxidant enzyme. It plays a key role in suppressing active oxygen by catalyzing the dismutation of O_2^- into H_2O_2, which is eliminated by CAT or other oxidizing enzymes or molecules (Fu and Huang, 2001). The positive regulation of CAT prevents the accumulation of H_2O_2 in the cytosol and protects plant cells against oxidative damage (Prochakova et al. 2001). Therefore, in conditions of water limitation, the greater SOD activity accompanied by increased CAT activity is highly desirable to satisfy the increase in H_2O_2, a result not observed in the present study. This fact, which can be attributed to the characteristic of the study species being a species that produces secondary molecules. The increase in H_2O_2 promoted by high SOD activity may be related to higher production of secondary metabolites. Furthermore, the H_2O_2 can act as a messenger or is required in the synthesis of several secondary compounds (Taiz et al. 2017). The low activity of CAT can indicate a decline in the enzymatic antioxidant system, being possibly triggering of other metabolic pathways (Foyer and Noctor 2003).

Thus, for *P. aduncum*, water suppression leads to a variation in the activation of antioxidant enzymes. However, the enzymatic antioxidant system is not its main route of oxidative protection, since it maintained the production of dry matter and showed increased activity only SOD. It is assumed that there is an overproduction of oxidizing compounds such as hydrogen peroxide, which has its effect blocked by molecules such as carotenoids and other secondary compounds. A study carried out on *F. ornus*, another medicinal species, the enzyme antioxidant system was also not the main antioxidant defense route when it was subjected to water stress (Fini et al. 2012).

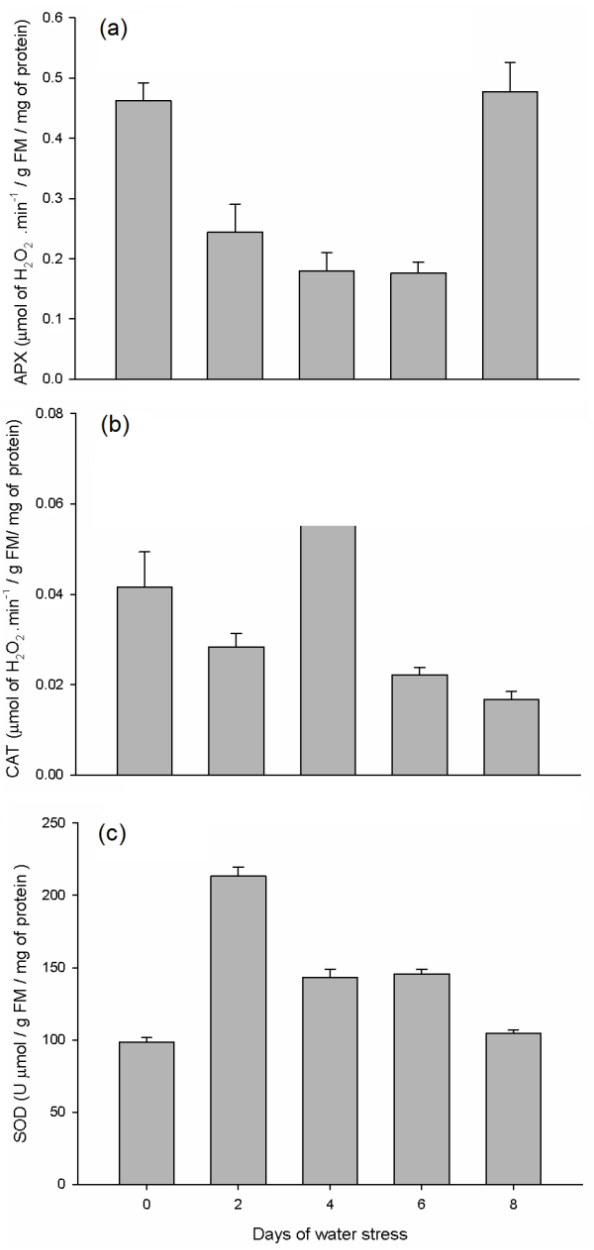

Figure 1. Activity of the enzymes ascorbate peroxidase-APX (a), catalase-CAT (b) and superoxide dismutase-SOD (c), in *Piper aduncum* grown under 0, 2, 4, 6, and 8 days of water supression.

Water suppression influenced the essential oil content of leaves and roots (Table 2). Both in the leaf and in the roots, the oil content increased in the plants subjected to water deficit (highest content observed at eight days of deficit). According to Selmar and Kleinwächter (2013), most medicinal species increase the productivity of secondary compounds in conditions of water deficit. The increases of essential oil production in water stress conditions can be attributed to greater production of secondary metabolites as a means of defense against oxidative stress exhibited in these conditions.

Other species also had their essential oil content favored by water stress, as observed for *H. pectinata*, when subjected to 4 days of water suppression (Santos et al. 2006) and for *L. sidoides* after 8 days of suppression before harvest (Alvarenga et al. 2011). For *P. aduncum*, Jacinto et al. (2018) observed decreases in essential oil production when the plant was submitted to water soil tensions more than 60 kPa.

The percentage of identification of the constituents of the essential oil achieved in both the leaf and the root was greater than 87.6% (Table 3 and 4). Regarding the number of oil constituents, it is noted that the oil composition of the leaves is more complex than that of the roots, with 38 compounds identified in the 34, respectively. Other studies have also found a greater number of constituents in the essential oil of *P. aduncum* leaves under different growing conditions (Ralli et al. 2007; Pacheco et al. 2016).

Table 2. Essential oil content of leaves and roots of *Piper aduncum* under 0, 2, 4, 6, and 8 days of water suppression.

Days	Essential oil content (%)		
	Leaves	Roots	Total
0	0.1576 c E	0.2580 b B	0.4156 a C
2	0.3120 b D	0.2614 b B	0.5735 a B
4	0.3309 b C	0.3066 b B	0.6375 a B
6	0.3553 b B	0.2622 b B	0.6175 a B
8	0.3659 c A	0.5273 b A	0.8932 a A

*Means followed by the same lowercase letter in the line, and caps letter in column do not differ by the Tukey test at 5% probability.*Means followed by the same lowercase letter in the line, and caps letter in column do not differ by the Tukey test at 5% probability.

Table 3. Chemical constituents (%) of essential oil from the leaves of *Piper aduncum* under 0, 2, 4, 6, and 8 days of water suppression

Compounds	**RI**	**Relative area (%)**				
		Days of stress				
		0	**2**	**4**	**6**	**8**
Elimicin	1559	0.49	0.41	0.48	0.46	0.53
Dillapiole	1630	0.63	0.53	0.64	0.58	0.49
Phenylpropanoids		***1.12***	***0.94***	***1.12***	***1.05***	***1.02***
1,8 Cineol	1032	0.31	0.13	0.22	0.11	0.44
cis-Furanolinalol oxide	1073	1.64	1.96	1.34	1.74	2.64
trans–Furanolinalol oxide	1090	0.18	0.21	0.18	0.15	0.31
Linalool	1101	14.52	16.96	13.15	16.34	16.77
Camphor	1145	0.15	0.14	-	0.14	0.15
Borneol	1166	0.12	-	-	-	0.11
α-Terpineol	1191	0.50	-	0.45	0.57	0.53
Geranial	1289	0.67	0.72	0.45	1.05	1.37
2-Undecanona	1294	-	-	-	0.14	0.14
Oxygenated Monoterpenes		***18.08***	***20.61***	***15.78***	***20.23***	***22.46***

Table 3. continued

Compounds	RI	Relative area (%)				
		Days of stress				
		0	2	4	6	8
α-Copaene	1377	1.26	1.36	1.15	1.32	1.19
Caryophyllene	1421	11.94	11.98	13.16	13.03	11.59
β-Copaene	1430	-	-	0.21	0.21	-
Aromadrendene	1440	0.32	0.23	0.23	0.21	0.18
α**-**Humulene	1455	14.39	14.36	14.86	14.95	13.60
Alloaromandrendene	1462	0.57	0.52	0.53	0.44	0.44
γ-Muuorolene	1478	0.70	0.63	0.58	0.65	0.51
α-Selinene	1496	2.57	2.50	2.56	2.57	2.41
α-Muurolene	1501	0.21	0.15	0.23	0.20	0.15
γ-Cadinene	1515	1.01	1.03	0.91	0.87	0.93
δ-Cadinene	1525	1.80	1.67	1.58	1.72	1.45
trans-Cadina-1,4-diene	1533	0.13	0.13	0.14	0.13	0.12
α-Calacorene	1544	0.14	0.10	0.12	0.24	-
Sesquiterpenes		***35.03***	***34.64***	***36.24***	***36.54***	***32.56***
eudesma-4(14),11-diene	1487	1.58	1.48	1.30	1.42	1.31
E-nerolidol	1533	25.46	25.46	26.75	24.76	23.56
Caryophyllene oxide	1567	1.66	1.25	1.64	1.32	1.24
Guaiol	1579	0.48	0.36	0.37	0.32	0.25
Ledol	1600	0.38	0.33	0.32	0.27	0.28
epi-Globulol	1604	1.88	1.64	1.90	1.62	1.45
1-*epi*-Cubenol	1610	0.24	0.19	0.30	0.12	0.13
γ-Eudesmol	1633	0.75	0.69	0.73	0.56	0.59
cis-Cadin-4-en-7-ol	1636	0.12	0.11	0.11	-	-
epi-α-Cadinol	1642	1.96	1.61	1.49	1.58	1.44
α-Muurolol	1647	0.43	0.35	0.45	0.33	0.32
α-Cadinol	1655	1.26	0.99	1.11	0.95	0.90
(3*Z*)-caryophylla-3,8(13)-diene-5α-ol	1659	0.24	0.21	0.23	0.19	0.17
n-hexadecanoic acid	1960	0.25	0.19	0.20	-	-
Oxigenated Sesquiterpenes		***36.67***	***34.87***	***36.92***	***32.01***	***31.65***
Not identified (*m/z* = 218)	1673	1.86	1.22	1.24	0.99	0.91
Number of compounds		38	35	36	36	34
Total Identification (%)		90.90	91.07	90.06	89.90	87.90

*RI: retention indexes in relation to C_8–C_{20}*n*-alkanes on the HP-5MS column, in order of elution

Table 4. Chemical constituents (%) of essential oil from the roots of *Piper aduncum* under 0, 2, 4, 6, and 8 days of water stress.

Compound	RI	Relative area (%)				
		Days of stress				
		0	2	4	6	8
Elemicin	1559	5.37	4.30	4.74	5.42	5.16
Dillapiole	1674	9.69	9.22	13.52	5.44	22.25
Apiole	1686	22.97	19.60	26.61	32.78	17.57
Phenylpropanoids		***38.02***	***33.12***	***44.87***	***43.64***	***44.97***
1,8 cineol	1032	0.41	0.55	0.32	0.28	0.35
Mircenol	1101	0.19	0.24	0.23	0.20	0.21
Camphor	1145	0.74	0.94	0.79	0.23	1.11
Isoborneol	1157	0.32	0.46	0.38	-	0.51
Borneol	1166	0.19	0.25	0.22	-	0.27
α-Terpineol	1192	-	0.20	0.16	-	0.21
Piperitone	1255	-	0.20	0.25	-	0.25
Oxygenated Monoterpenes		***1.85***	***2.84***	***2.34***	***0.72***	***2.90***
Caryophyllene	1421	0.89	0.90	0.88	0.46	1.12
α-Humuleno	1455	0.86	1.02	0.77	0.44	1.20
Alloaromadrendene	1461	0.24	0.35	0.26	0.20	0.26
γ-Amorfene	1497	2.24	3.06	2.15	2.83	2.76
α-Selinene	1524	10.17	12.13	10.20	12.88	11.31
α-Calacorene	1542	0.80	0.96	0.53	0.48	1.07
Sesquiterpenes		***15.19***	***18.43***	***14.78***	***17.30***	***17.73***
E-nerolidol	1565	0.75	1.17	0.97	0.78	0.83
Geranyl-2-metil- butyrate	1599	9.92	11.78	7.09	6.19	14.73
epi-Globulol	1613	-	0.20	-	5.56	4.65
1-*epi*-Cubenol	1626	3.51	3.02	3.84	6.08	1.74
γ-Eudesmol	1633	0.61	0.81	0.50	0.37	0.78
cis-Cadin-4-en-7-ol	1640	0.13	0.18	0.13	-	0.19
α-Muurolol	1643	0.45	0.53	0.41	0.34	0.49
τ-Muurolol	1648	0.38	0.38	0.56	0.38	1.01
α-Eudesmol	1651	1.85	2.10	1.28	0.98	2.37
β-Eudesmol	1654	2.60	2.90	1.88	1.44	0.36
α-Cadinol	1659	5.47	3.89	4.77	4.82	0.74
Bulnesol	1669	4.72	5.53	3.32	2.62	6.17
n-hexadecanoic acid	1962	2.12	3.74	2.01	2.20	1.32
Oxigenated Sesquiterpenes		***32.50***	***36.22***	***25.75***	***31.76***	***35.00***
Number of compounds		27	30	29	25	30
Total Identification (%)		87.57	90.60	88.74	93.43	100

RI: retention indexes in relation to C_8–C_{20} *n*-alkanes on the HP-5MS column, in order of elution.

The constituents identified in both the leaf and the roots are of a chemical nature belonging to the class of phenylpropanoids, monoterpenes and sesquiterpenes. On the leaf, most of the compounds identified were oxygenated sesquiterpenes and sesquiterpenes (Figure 2A). While at the root the identified compounds are from the classes of oxygenated phenylpropanoids and sesquiterpenes (Figure 2B). The predominance of sesquiterpene constituents in the essential oil extracted from the leaves and phenylpropanoids in the oil extracted from the roots has also been observed in other studies with the species (Vieira et al. 2011; Pacheco et al. 2016).

The water deficit caused an increase in sesquiterpenes in the leaves, mainly in treatments with 4 and 6 days of water suppression. In the roots there was also an increase in relation to the contents of the phenylpropanoid constituents, after 4 days of water suppression. The increase in terpenes on the essential oil of leaves and phenylpropanoids in the roots has already been reported for other medicinal species under water deficit (Nowak et al. 2010; Manukyan 2011; Falahi et al. 2017). This increase in phenylpropanoid and terpenic constituents in leaves and roots in response to water deficit corroborates with the results found for antioxidant enzyme activity. Since the increase in SOD was not accompanied by an increase in CAT activity, indicating that secondary pathways were activated to neutralize the increase in H_2O_2.

The major compound found in the leaves (Figure 2 C) regardless of treatments, was *E*-neurolidol (23.56-26.75%), with other secondary components linalool (13.15-16.77%); α-humulene (13.60-14.95%); and caryophylene (11.59-13.16%). In the root, (Figure 2 D) apiol was the major constituent (17.57-32.78%). In this organ, dillapiole (5.44-22.25%), α-selinene (10.17-12.88%) and butanoate-2-methyl-geranyl (6.19-14, 73%). Pacheco et al. (2016) observed these major constituents in the leaves and roots of *P. aduncum* grown under different light conditions. However, Oliveira et al. (2013) analyzing the chemical composition of the oil of the leaves of the same species in two Cerrado environments, observed 1,8-cineol as the major component. Thus, it can be inferred that the chemical composition of this species varies depending on environmental conditions or may have chemotypes that have not yet been

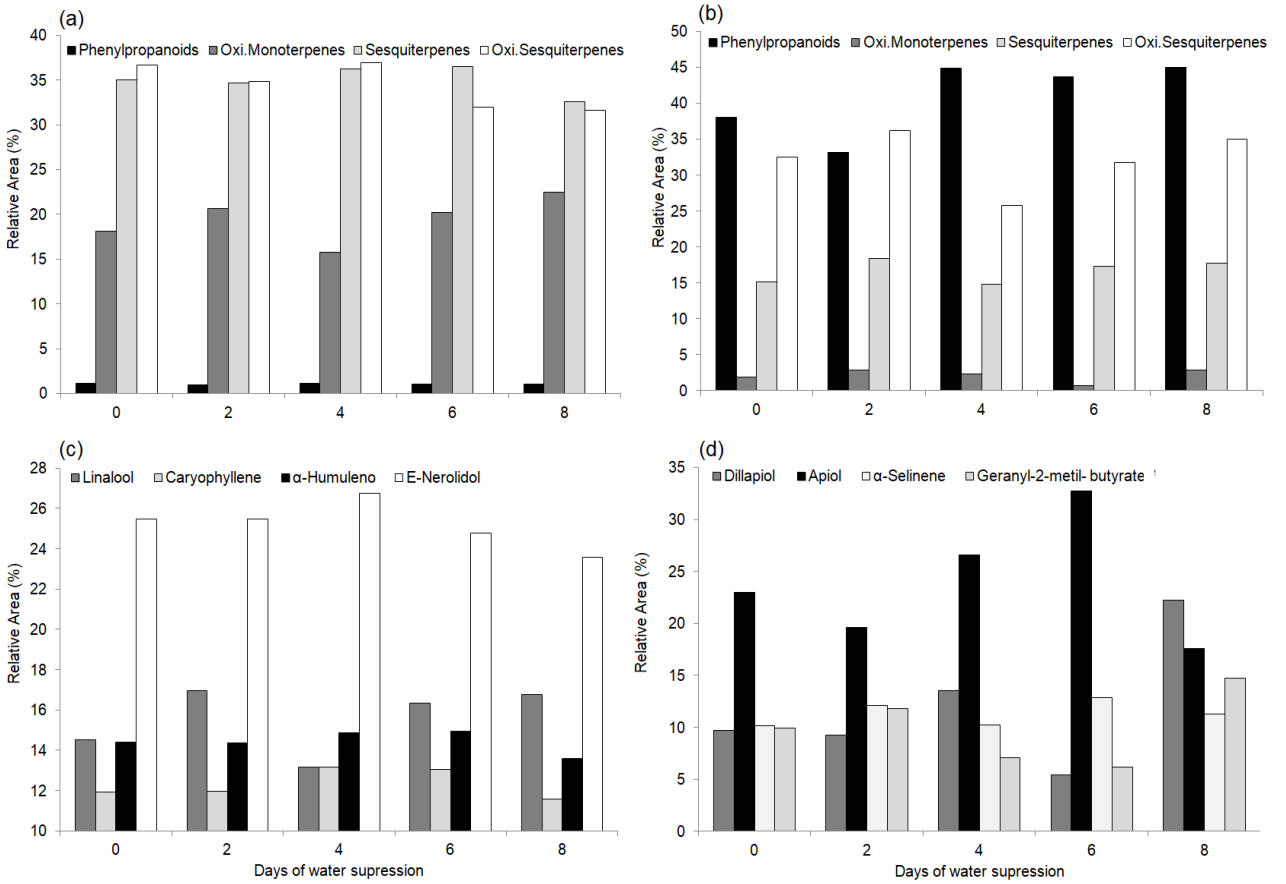

Figure 2. Classes of constituents of the essential oil of the leaves (a), roots (b) and major compounds of the leaves (c) and roots (d) of *Piper aduncum* grown under water suppression (0, 2, 4, 6, and 8 suppression days).

confirmed.

Most of the major compounds had their content increased under water suppression conditions. In leaves, *E*-neurolidol and linalool had their concentration increased, mainly in plants grown under four and eight days of suppression, respectively. While in the roots, apiol and dilapiol also increased in these treatments. In addition, the substances 2-undecanone identified in the leaves and epiglobulol in the roots were only observed at six and eight days of water suppression, indicating a possible need for stress to trigger its synthesis.

Thus, for *P. aduncum* the main form of defense against water deficit is through the induction of secondary defense pathways. Additionally, the results observed in the present work indicate a possible signaling provided by H_2O_2 in some secondary pathways of the species. Similarly, this result has also been reported for other species subjected to water deficit (Fini et al. 2012; Falahi et al. 2017). A moderate water suppression (from six to eight days) before harvesting in the of *P. aduncum* makes it possible to increase the production of essential oil and major constituents, without altering the production of plant material.

CONCLUSION

Water suppression in the time tested did not affect the dry matter production of *P. aduncum*. In plants subjected to eight days of suppression, there was an increase in the amount of chlorophyll *a*, carotenoids and leaf temperature. Regarding antioxidant enzymes, there were significant changes in their activities, with APX and CAT having their activities reduced under low water availability (CAT only increased after 4 days of suppression). While SOD had its activity increased in plants submitted to water stress. This increase in SOD activity indicates hydrogen peroxide as a possible messenger of secondary antioxidant defense pathways. Additionally, the decrease in APX and CAT activities under water limitation associated with the increase in secondary metabolites indicates a possible prevalence of secondary defense pathways against oxidative stress.

The production of essential oil in leaves and roots was observed with increased water suppression. There were also changes in the profile of constituents in plants subjected to treatments. The constituents found in the leaves and roots were predominantly sesquiterpenic and phenylpropanoic, respectively. E-nerolidol was the major constituent observed in the leaves and apiol in the roots. The low water availability promoted an increase in the production of these major constituents. Thus, for *P. aduncum*, water suppression up to eight days before harvest can be recommended to increase the production of essential oil and its bioactive molecules without altering plant production.

ACKNOWLEDGMENTS

The authors thanks to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Pesquisa do Estado de Minas Gerais (FAPEMIG) for the financial support; and to the Laboratory of Phytochemistry of the Department of Agriculture at UFLA, for the GC–MS analysis.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of the manuscript, and also drafting the work and revising it critically for important intellectual content.

DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

REFERENCES

Abdelmajeed NA, Danial EN, Ayad HS (2013) The effect of environmental stress on qualitative and quantitative essential oil of aromatic and medicinal plants. Arch Sci 66:100-120.

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry. 4.ed. Illinois: Allured Publish Corporation.804p.

Alvarenga ICA, Valadares RV, Martins ER, Oliveira FG, De Figueiredo LS, Kobayashi MK. (2011) Water stress before harvest of pepper-rosmarin. Pesqui Agropec Bras 46:706-711. http://dx.doi.org/10.1590/S0100-204X2011000700005

Alvarenga ICA, Pacheco FV, Alvarenga AA, Bertolucci SKV, Pinto JEB (2018) Growth and production of volatile compounds of yarrow (*Achillea millefolium* L.) under different irrigation depths. An Acad Bras Cienc 90:3901-3910. http://dx.doi.org/10.1590/0001-3765201820180092

Apostol I, Heinstein PF, Low OS (1989) Rapid stimulation of an oxidative burst during elicitation of cultured plant cells: role in defense and signal transduction. Plant Physiol 90:109-116. https://doi.org/10.1104/pp.90.1.109

Araújo MJD, Camara CAD, Moraes MM, Born FS, (2020) Insecticidal properties and chemical composition of *Piper aduncum* L., *Lippia sidoides* Cham. and *Schinus terebinthifolius* Raddi essential oils against *Plutella xylostella* L. An Acad Bras

Cienc 92:supl. 1:e20180895,1-14. http://dx.doi.org/10.1590/0001-3765202020180895

Baghalian K, Haghiry A, Naghavi MR, Mohammadi A (2008) Effect of saline irrigation water on agronomical and phytochemical characters of chamomile (*Matricaria recutita* L.). Sci Hortic116:437-441. https://doi.org/10.1016/j.scienta.2008.02.014

Carvalho FEL, Lobo AKM, Bonifacio A, Martins MO, Lima Neto, MC, Silveira, JAG (2011) Aclimatação ao estresse salino em plantas de arroz induzida pelo pré-tratamento com H_2O_2. Rev Bras Eng Agr Amb 15:416–423. http://dx.doi.org/10.1590/S1415-43662011000400014

De Oliveira LM, Da Silva JN, Coelho CCR, Neves MG, Da Silva RTL, De Oliveira Neto CF (2013) Pigmentos fotossintetizantes, aminoácidos e proteínas em plantas jovens de graviola submetida ao déficit hídrico. Rev Agroecoss 5:39-44. http://dx.doi.org/10.18542/ragros.v5i1.1409

Falahi H, Sharifi M, Maivan HZ, Chashmi NA (2018) Phenylethanoid glycosides accumulation in roots of *Scrophularia striata* as a response to water stress. Environ Exp Bot 147:13-21. https://doi.org/10.1016/j.envexpbot.2017.11.003

Fazolin M, Estrela JL, Catani V, Lima MSD, Alécio MR. 2005. Toxicity of *Piper aduncum* oil to adults of *Cerotoma tingomarianus* Bechyné (Coleoptera: Chrysomelidae). Neotrop Entomol 34:485-489. http://dx.doi.org/10.1590/S1519-566X2005000300018

Fini A, Guidi L, Ferrini F, Brunetti C, Di Ferdinando M, Biricolti S, Pollastri S, Calamai L, Tattini, M (2012) Drought stress has contrasting effects on antioxidant enzymes activity and phenylpropanoid biosynthesis in *Fraxinus ornus* leaves: an excess light stress affair. J Plant Physiol 169:929-939. https://doi.org/10.1016/j.jplph.2012.02.014

Foyer CH (2018) Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. Environ Exp Bot 154:134-142. https://doi.org/10.1016/j.envexpbot.2018.05.003

Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17:1866-1875. https://doi.org/10.1105/tpc.105.033589

Fu J, Huang B (2001) Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. Environ Exp Bot 45:105-114. https://doi.org/10.1016/S0098-8472(00)00084-8

Giannopolitis CN, Ries SK (1977) Superoxide dismutases: I. occurrence in higher plants. Plant Physiol 59:309-314. https://doi.org/10.1104/pp.59.2.309

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909-930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gobbo-Neto L, Lopes NP (2007) Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quim Nova 30:374-381. http://dx.doi.org/10.1590/S0100-40422007000200026

Gonçalves S, Martins N, Romano A (2017) Physiological traits and oxidative stress markers during acclimatization of micropropagated plants from two endangered *Plantago* species: *P. algarbiensis* Samp. and P. *almogravensis* Franco. In Vitro Cell Dev Pl 53:249-255. https://doi.org/10.1007/s11627-017-9812-y

Havir EA, McHale NA (1987) Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol 84:450-455. https://doi.org/10.1104/pp.84.2.450

Jacinto ACP, Souza LPD, Nakamura AT, Carvalho FJ, Simão E, Zocoler JL, Bergo CL (2018) Idioblasts formation and essential oil production in irrigated *Piper aduncum*. Pesqui Agropec Trop 48:447-452. http://dx.doi.org/10.1590/1983-40632018v4853165

Lee H, Jeon J, Yoon J, Kim SH, Choi HS, Kang JS, Lee YS, Lee M, Kim YH, Chang HB. 2020. Comparative metabolite profiling of wild and cultivated *Justicia procumbens* L. based on 1H-NMR Spectroscopy and HPLC-DAD Analysis Plants 9:1-12. https://doi.org/10.3390/plants9070860

Lichtenthaler HK, Buschmann C (2001) Chlorophylls and carotenoids: measurement and characterization by UV–VIS spectroscopy. Curr Protoc Food Anal Chem Sup. F4.3.1–F4.3.8. https://doi.org/10.1002/0471142913.faf0403s01

Lima MDGDS, Lopes NF, Bacarin MA, Mendes CR (2004) Efeito do estresse salino sobre a concentração de pigmentos e prolina em folhas de arroz. Bragantia 63:335-340. http://dx.doi.org/10.1590/S0006-87052004000300003

Lopes OD, Kobayashi MK, Oliveira FG, Alvarenga ICA, Martins E R, Corsato CE (2011) Determinação do coeficiente de cultura (Kc) e eficiência do uso de água do alecrim-pimenta irrigado. Rev Bras Eng Agr Amb 15:548-554. http://dx.doi.org/10.1590/S1415-43662011000600002

Mafakheri A, Siosemardeh AF, Bahramnejad B, Struik, PC, Sohrabl Y (2010) Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust J Crop Sci 4:580-585.

Masoumi A, Kafi M, Khazaei H, Davari K (2010) Effect of drought stress on water status, elecrolyte leakage and enzymatic antioxidants of kochia (*Kochia scoparia*) under saline condition. Pakistan J Bot 42:3517-3524.

Meira MR, Melo MTPD, Martins ER, Pinto MJDS,

Santana CS (2013) Crescimento vegetativo, produção de fitomassa e de óleo essencial de *Melissa officinalis* L. sob diferentes lâminas de irrigação. Cienc Rural 43:779-785. http://dx.doi.org/10.1590/S0103-84782013005000040.

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbatospecific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232.

Nist Speech Group Website. Topic detection and tracking evaluation (2008) Online. Available in:< http://webbook.nist.gov/chemistry> Acess in 15 fev. 2013.

Oliveira GL, Cardoso SK, Lara Junior CR, Vieira TM, Guimarães EF, Figueiredo LS, Martins ER, Moreira DL, Kaplan MAC (2013) Chemical study and larvicidal activity against *Aedes aegypti* of essential oil of *Piper aduncum* L. (Piperaceae). An Acad Bras Cienc 85:1227-1234. http://dx.doi.org/10.1590/0001-3765201391011

Pacheco F V, Avelar RP, Alvarenga ICA, Bertolucci SKV, De Alvarenga AA, Pinto, JEBP (2016) Essential oil of monkey-pepper (*Piper aduncum* L.) cultivated under different light environments. Ind Crop Prod 85:251-257. https://doi.org/10.1016/j.indcrop.2016.03.016

Parmar VS, Jain SC, Gupta S, Talwar S, Rajwanshi VK, Kumar R, Azim A, Malhotra S, Kumar N, Jain R, Sharma NK, Tyagi OD, Lawrie SJ, Errington W, Howarth OW, Olsen CE, Singh SK, Wengel J (1997) Phytochemistry of the genus *Piper*. Phytochem 46, 597-673. https://doi.org/10.1016/S0031-9422(97)00328-2

Perl-Treves R, Perl A (2002) Oxidative stress: an introduction. Oxidative stress in plants, p. 1-32, 2002.

Potzernheim MCL, Bizzo HR, Vieira RF (2006) Análise dos óleos essenciais de três espécies de Piper coletadas na região do Distrito Federal (Cerrado) e comparação com óleos de plantas procedentes da região de Paraty, RJ (Mata Atlântica). Rev Bras Farmacogn 16:246-251. https://doi.org/10.1590/S0102-695X2006000200019

Prochazkova D, Sairam RK, Srivastava GC, Singh DV (2001) Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Sci 161:765-771. https://doi.org/10.1016/S0168-9452(01)00462-9

Rali T, Wossa SW, Leach DN, Waterman PG (2007) Volatile chemical constituents of *Piper aduncum* L. and *Piper gibbilimbum* C. DC (Piperaceae) from Papua New Guinea. Molecules 12:389-394. https://doi.org/10.3390/12030389.

Rocha SF, Ming LC, Chaves FC, Scarda, FM (2005) Role of light and phytochrome on *Piper aduncum* L. germination: an adaptive and environmental approach. J Herbs Spices Med Plants 11:85-96. https://doi.org/10.1300/J044v11n03_08

SAEG. Sistema para análise estatística, Versão 9.1.Viçosa: Fundação Arthur Bernardes da Universidade Federal de Viçosa (2007). Available in <http://arquivo.ufv.br/saeg>. Acess in 30 Ago. 2020.

Santos TT, Santos MF, Mendonça MC, Silva Júnior CD, Silva-Mann R, Arrigoni-Blank MDF, Blank AF (2004) Efeito do estresse hídrico na produção de massa foliar e teor de óleo essencial em sambacaitá (*Hyptis pectinata* L.). In: Congresso Brasileiro De Olericultura. Campo Grande: Congresso de Olericultura, 1-4.

Selmar D, Kleinwächter M (2013) Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. Ind Crop Prod 42:558-566. https://doi.org/10.1016/j.indcrop.2012.06.020

Shvaleva AL, Silva FCE, Breia E, Jouve J, Hausman JF, Almeida M H, Maroco JP, Rodrigues ML, Pereira JS, Chaves MM (2006) Metabolic responses to water deficit in two *Eucalyptus globulus* clones with contrasting drought sensitivity. Tree Physiol 26:239-248. https://doi.org/10.1093/treephys/26.2.239

Singh M, Khan MMA, Uddin M, Naeem M, Qureshi MI (2017) Proliferating effect of radiolytically depolymerized carrageenan on physiological attributes, plant water relation parameters, essential oil production and active constituents of *Cymbopogon flexuosus* Steud. under drought stress. Plos One 12:e0180129. https://doi.org/10.1371/journal.pone.0180129

Sousa PJ, Barros CA, Rocha JCS, Lira DS, Monteiro GM, Maia JGS (2008) Avaliação toxicológica do óleo essencial de *Piper aduncum* L. Rev Bras Farmacogn 18:217-221. https://doi.org/10.1590/S0102-695X2008000200013

Souza GS, Oliveira UC, Silva JS, Lima JC (2013) Crescimento, produção de biomassa e aspectos fisiológicos de plantas de *Mentha piperita* L. cultivadas sob diferentes doses de fósforo e malhas coloridas. Global Sci Tech 6. http://dx.doi.org/10.14688/1984-3801.v06n03a04

Streit NM, Canterle LP, Canto MWD, Hecktheuer LHH (2005) As clorofilas. Cienc Rural 35:748-755. http://dx.doi.org/10.1590/S0103-84782005000300043

Taiz L, Zeiger E, Moller IM, Murphy A (2017) Fisiologia e desenvolvimento vegetal. 6.ed. Porto Alegre: Artmed.

Van Den Dool H, Kratz PDJA (1963) Generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr A 11:463-471. https://doi.org/10.1016/S0021-9673(01)80947-X

Vieira SCH, Paulo LFD, Svidzinski TIE, Dias Filho BP,

Nakamura CV, Souza AD, Mark MC, Cortez DAG (2011) Antifungal activity of *Piper diospyrifolium* Kunth (Piperaceae) essential oil. Braz J Microbiol 42:1001-1006. http://dx.doi.org/10.1590/S1517-83822011000300020

Yang Z, Liu J, Poree F, Schaeufele R, Helmke H, FrackenpohL J, Lehr S, Koskull-Doring P, Christmann A, Schnyder H, Schmidhalter U, Grill E (2019) Abscisic acid receptors and coreceptors modulate plant water use efficiency and water productivity. Plant Physiol 180:1066-1080. https://doi.org/10.1104/pp.18.01238

Yi XP, Zhang YL, Yao HS, Luo HH, Gou L, Chow WS, Zhang WF (2016) Rapid recovery of photosynthetic rate following soil water deficit and re-watering in cotton plants (*Gossypium herbaceum* L.) is related to the stability of the photosystems. J Plant Physiol 194:23-34. https://doi.org/10.1016/j.jplph.2016.01.016

Yuncker TG (1975) The Piperaceae of Brazil. Hoehnea 2:99-105.