Water stress before harvest of pepper-rosmarin

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Abstract – The objective of this work was to assess the effect of different periods of water stress before harvest of pepper-rosmarin (*Lippia sidoides*) on the contents of essential oil and flavonoids. The experiment was carried out during 270 days of cultivation, with drainage lysimeters, in a completely randomized block design with five treatments: 0, 2, 4, 6, and 8 days of water suppression before harvest, with four replicates. Fresh and dry matter yield, essential oil content, total flavonoids content, and water potential and temperature of leaves were determined. There was a decrease of approximately 50% in oil content and of 60% in total flavonoid content with the reduction of leaf water potential in 0.3 MPa. Essential oil is more sensitive to water stress than total flavonoids.

Index terms: *Lippia sidoides*, essential oil, flavonoids, leaf water potential, medicinal plant, water stress.

Estresse hídrico antes da colheita de alecrim-pimenta

Resumo – O objetivo deste trabalho foi avaliar o efeito de diferentes períodos de estresse hídrico antes da colheita de alecrim-pimenta (*Lippia sidoides*) sobre o conteúdo de óleo essencial e de flavonoides. O experimento foi realizado durante 270 dias de cultivo, com lisímetros de drenagem, em delineamento inteiramente casualizado, com cinco tratamentos: 0, 2, 4, 6 e 8 dias de supressão de irrigação antes da colheita, com quatro repetições. Foram determinados produção de matéria fresca e seca, teor de óleo essencial, teor de flavonoides totais, tensão e temperatura de folhas. Observou-se decréscimo de aproximadamente 50% no teor de óleo e de 60% no teor de flavonoides totais, com a diminuição do potencial hídrico foliar em 0,3 MPa. O óleo essencial é mais sensível ao estresse hídrico do que os flavonoides totais.

Termos para indexação: *Lippia sidoides*, óleo essencial, flavonoides, potencial hídrico foliar, planta medicinal, estresse hídrico.

Introduction

Pepper-rosmarin (*Lippia sidoides* Cham.) is an aromatic and medicinal species originated from the Brazilian Northeast and the semiarid regions of northern Minas Gerais state, Brazil. It is used to combat fungal infections, mouth inflammation, scabies, and bad odors of feet and armpits. Its essential oil, composed of a mixture of thymol and carvacrol, has antimicrobial properties against fungi and bacteria, and is effective in controlling *Aedes aegypti* larvae (Carvalho et al., 2003).

The behavior of medicinal plants is greatly influenced by water deficits, in terms of the production and the formation of compounds, such as alkaloids, flavonoids, parthenolides, and essential oils (Penka, 1978; Carvalho et al., 2003b; Castro et al., 2004; Bortolo et al., 2009). The level and duration of stress are fundamental in the response of aromatic and medicinal plants in terms of plant quality (Charles et al., 1990; Morvant et al., 1998). However, studies on the irrigated cultivation of these species are incipient, and plant behavior under stress conditions still needs further investigation (Carvalho et al., 2003a; Alvarenga et al., 2009).

Abiotic factors, such as temperature, cultivation season, time of harvest, and water availability, strongly influence medicinal plants. Therefore, studies related to this interaction are important to obtain information on the most appropriate irrigation management for the production of compounds of interest (Barros et al., 2009; Morais, 2009).

The objective of this work was to evaluate the effect of different periods of water stress before harvest of pepper-rosmarin on the contents of essential oil and flavonoids.

Materials and Methods

The experiment was carried out at the experimental site of Instituto de Ciências Agrárias of Universidade Federal de Minas Gerais, located at Montes Claros, MG, Brazil (16°44'02"S, 43°51'23"W, at 646 m of altitude). The climate is classified as Aw, semiarid tropical savanna with dry winter and rainy summer, according to Köppen (Leite et al., 2004).

The experiment was carried out with drainage lysimeters in a Red Oxisol (Santos et al., 2006), with the following characteristics: pH (H_2O), 5.9; 2.4 $cmol_c\ dm^{-3}$ of Ca^{2+}; 1.0 $cmol_c\ dm^{-3}$ of Mg; 6.72 $mg\ kg^{-1}$ of P Mehlich; 18.78 $mg\ L^{-1}$ of Prem; 90 $mg\ kg^{-1}$ of K; 1.4 $cmol_c\ dm^{-3}$ of Al; 4.04 $cmol_c\ dm^{-3}$ of H + Al; sum of bases of 3.63 $cmol_c\ dm^{-3}$; base saturation of 47%; 11.7 $g\ kg^{-1}$ of organic matter; 45 $g\ kg^{-1}$ of coarse sand; 355 $g\ kg^{-1}$ of fine sand; 300 $g\ kg^{-1}$ of silt; 300 $g\ kg^{-1}$ of clay.

The growing period occurred from 9/15/2009 to 6/11/2010, totaling 270 days of cultivation. Plants were derived from regrowth of plants pruned at 120 days after transplanting, and the seedlings were produced with cuttings. Treatments consisted of different periods of water suppression before harvest: T1, 0; T2, 2; T3, 4; T4, 6; and T5, 8 days without irrigation. A completely randomized block design, with four replicates, was used. At 95 days of cultivation, a supplemental fertilization of 12 $kg\ m^{-2}$ of cattle manure was applied (Assis et al., 2009). Before the beginning of the treatments, plants were characterized as to height and crown and stem diameter, measured at 5 cm above soil, using graduated tape and digital caliper, respectively. Plants were grown in row spacing of 0.4x0.4 m, with nine plants per lysimeter.

At the beginning of the experiment, the soil at the bottom of the lysimeters was saturated and kept at field capacity until irrigation suppression. However, the total water volume consumed by the culture could not be determined, since there was no coverage in the area and, when the rainy season started, it was not possible to determine the percolated blade. Irrigation was carried out based on the reference evapotranspiration (ETo) calculated according to the Hargreaves-Samani equation:

$$Et_o = [(Tmed + 17.8) \times 0.0023 \times Q_o (Tmax - Tmin)^{0.5}],$$

in which Eto is the potential evapotranspiration in millimiters per day; Tmed is the average daily temperature in °C; Tmax is the daily maximum temperature in °C; Tmin is the daily minimum temperature in °C; Q_o is the incident radiation in mm per day (Pereira et al., 1997). Meteorological data used to determine the ETo were collected with the automatic weather station Davis Vantage Pro 2 (Davis Instruments Corp., Hayward, CA, USA). The soil was maintained at field capacity with irrigation frequency of three days until the onset of treatments.

An infrared thermometer Infraterm, (Incoterm, Porto Alegre, RS, Brazil) was used to monitor leaf temperature, which was measured at 6, 9, 12, 15, and 18 h in fully developed leaves. A Scholander pressure chamber (Scholander et al., 1964) equipped with a digital manometer was used to evaluate leaf water potential, and nitrogen gas was used as a pressurizer. Top branches with about 15 cm were collected at 3:00 am on alternate days, and leaf tension was measured in all treatments. The water stress day index (WSDI) and the relation between the relative yield and water stress in the culture were determined by the method described by Katerji et al. (2000), using the equation $Y = a - (b \times WSDI)$, in which: Y is the relative productivity in %; a is the value of the ordinate (relative yield), which should be approximately equal to 100; b is the percent loss in productivity per unit of increase in the WSDI, which is dimensionless. The WSDI was determined by the equation $WSDI = \sum_{i=1}^{n} (\psi_{ci} - \psi_{si})/n$, in which: ψ_{ci} is the daily leaf water potential before dawn in the control treatment over the stress period; ψ_{si} is the daily leaf water potential before dawn for each treatment during the stress period; n is the number of days with measurements. In the present study, the variable relative yield referred to essential oil content and total flavonoid content, i.e., only the levels of fresh matter (FM), dry matter (DM), and oil production were considered, since these factors may be more important in short periods of stress applied close to harvest.

After water suppression, plants from each plot were collected at 10 cm above ground and then sampled for determination of FM, weighed, and taken to an air forced circulation oven for determination of dry material. Moisture content of each treatment was based on DM. Essential oil was determined from fresh samples of about 100 g, which were taken to a Clevenger machine and hydrodistilled during 4 hours to determine the mass of essential oil on an analytical scale. Plant material from the extraction was subjected to drying process in a forced air oven at 65°C until constant weight to determine DM. The oil percentage was expressed as w w^{-1} regarding DM.

After grinding the dried material, total flavonoids were extracted from leaves according to a method adapted from Santos & Blatt (1998). The standard curve was prepared with increasing concentrations of rutin, and expressed in mg g^{-1}. Data were subjected to mean test for comparison of the average yield of DM, FM, oil, and leaf temperature and tension among treatments, using the software SAEG (SAEG, 2007). Means were compared by Tukey's test, at 5% probability.

Results and Discussion

The highest temperatures, close to 35°C, were observed at the beginning of the experiment (Figure 1). There was a significant decrease in temperature in the last 60 days of cultivation, in which the minimum temperatures were close to 17°C, coinciding with the period of data collection on leaf water potential and temperature. The minimum and maximum reference evapotranspiration values were close to 2 and 7 mm per day, respectively, which can be attributed to the wider temperature range. There was a decrease in the evapotranspiration rate at 100 days before harvest, due to the extended rainy season of the year. The air relative humidity was relatively low in the first 40 days of cultivation, with values below 50%, but was close to 70% with the start of the dry season, around April for the region.

After cultivation, the plants had the following average values: height of 1.67±0.26 m (mean ± standard deviation), crown diameter of 1.07±0.29 m, and stem diameter of 14.00±3.14 mm. Figueiredo et al. (2009) observed heights exceeding 1.80 m, in the same conditions, at 300 days of cultivation. Pepper-rosmarin yield, considering FM and DM, showed no significant differences between treatments, with average productivity of 8.38±0.98 Mg ha^{-1} for FM and 4.26±0.48 Mg ha^{-1} for DM. No differences were expected between these variables due to the short period of treatment.

Pepper-rosmarin FM yield can reach 15.96 Mg ha^{-1} under the same edaphoclimatic conditions (Figueiredo et al., 2009). According to Lopes (2010), the best irrigation depth is 1.9 ETo, with productivity of 9.59 Mg ha^{-1} for FM, with a growing period of 120 days. In the present study, the average productivity was 8.38 Mg ha^{-1} after 270 days, keeping soil at field capacity. There

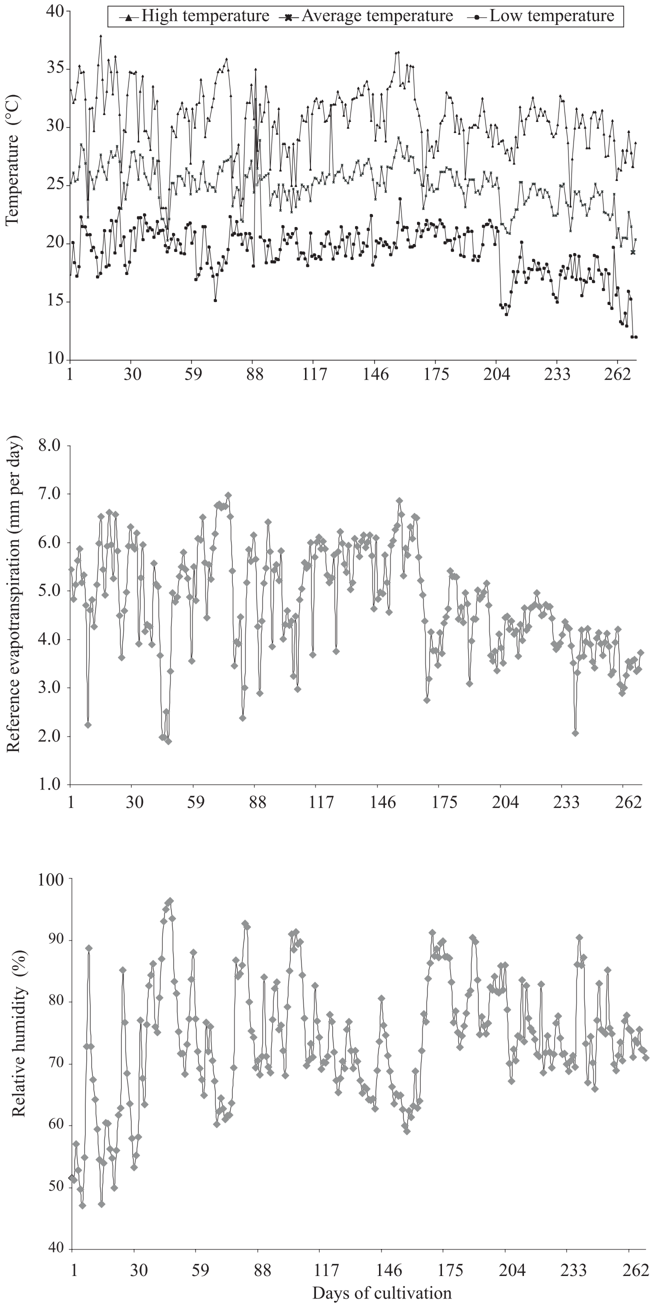

Figure 1. Oscillation temperature, evapotranspiration, and relative humidity during the crop cycle of pepper-rosmarin (*Lippia sidoides*).

were no significant differences regarding essential oil production, which had an average production of 42.47±13.47 kg ha^{-1}, and moisture content, which ranged from 47.17 to 51.84%.

Leaf temperature varied significantly after different periods of stress (Table 1). The highest average temperature was observed after eight days of irrigation supression, possibly due to less water availability for transpiration and, consequently, less capacity for heat exchange with the environment. In coffee plants (*Coffea arabica* L.), proper leaf temperatures enhance productivity, since high temperatures limit photosynthesis, causing injuries, such as leaf scald and flower abortion (Damatta, 2004). Leal et al. (2006) evaluated coffee in intercropping systems as an alternative to decrease leaf temperature, and found differences of up to 5ºC in intercropped plants when compared to plants under sun, during the hottest hours of the day. Therefore, leaf temperature behavior regarding ambient temperature can be used as an indicator of water status in plants, which is a possible indicator for irrigation (Testi et al., 2008).

There were no significant differences among treatments for essential oil content (Table 1), which ranged from 1.27 to 0.56%. Lopes (2010) observed lower values, of 0.40 and 0.60%, depending on the irrigation regime. These results indicate that water suppression can be managed in order to save energy and water in the production process. Dunford & Vasquez (2005) evaluated the influence of water stress in oregano mexicano (*L. berlandieri* Schauer) at different stages of plant development and found levels of essential oil ranging from 0.7 to 2.5%. However, there were no significant differences among treatments, as observed in the present study. Oil composition was also monitored, and no significant differences were observed for levels of thymol and carvacrol.

Table 1. Average values of leaf water potential, temperature, and essential oil content in pepper-rosmarin (*Lippia sidoides* Cham.) under water stress before harvest[(1)].

Days of water stress	Water leaf potential (MPa)	Leaf temperature (ºC)	Oil content (%)
0	-0.44b	19.79b	1.17ab
2	-0.41b	19.53b	0.95ab
4	-0.47b	19.87b	0.56b
6	-0.54ab	19.94b	0.58b
8	-0.66a	21.06a	1.27a
CV (%)	12.27	26.27	31.10

[(1)]Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability.

Total flavonoid content ranged from 0.008 to 0.0139 g g^{-1} regarding DM, and linearly decreased with the prolongation of water stress (Figure 2). Bortolo et al. (2009), while assessing the concentration of flavonoids in flowers and plants of marigold (*Calendula officinalis* L.) as affected by different irrigation depths, found no significant differences in the metabolite of flowers, although flavonoid content decreased with the increase of irrigation depth. Flavonoids are attractive to pollinators, and, as water stress accelerates the cycle of annual plants, the concentrations of the active principle increases. However, this was not observed for pepper-rosmarin.

Values for leaf water potential were relatively low, ranging from -0.41 to -0.66 MPa (Table 1). Nogueira et al. (2001) found values between -1 and -5.5 MPa after 20 days of water stress, on acerola (*Malpighia emarginata* D.C.) plants. Plants without water restriction or subjected to water stress may have the same leaf water

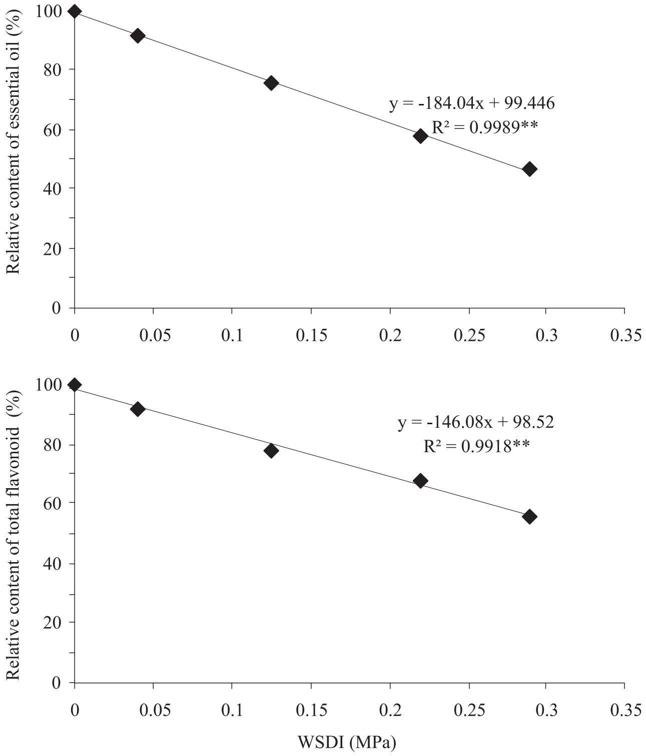

Figure 2. Essential oil and total flavonoid contents in pepper-rosmarin (*Lippia sidoides* Cham.) according to the water stress daily index (WSDI). **Significant by the F test, at 1% probability.

potential, according to the levels of water vapor loss through respiration (Schulze, 1986). In coffee, water potential ranged from -0.3 to -0.8 MPa for irrigated plants and from -0.6 to -1.5 MPa for plants under water stress (Nascimento, 2006). Pepper-rosmarin did not show great tensions in comparison to these other species, possibly due to the short period of stress.

The values found for the WSDI were also relatively low, ranging from 0.05 to 0.3 MPa, which could be related to the lower tensions observed during the experiment. Another possible explanation is that the method used to obtain the WSDI was adapted for essential oil and total flavonoid content, which have lower values in comparison to the production of FM, for example. Essential oil and total flavonoid contents decreased linearly, following the water stress magnitude, with daily decreases of approximately 50% for oil content and 60% for total flavonoids due to the reduction of 0.3 MPa in leaf water potential (Figure 2). Oliveira et al. (2005) observed production losses of about 10% for beans, for this same range of water stress, while Garcia et al. (2009) reported values of daily water stress with production losses of nearly 25%, at about 0.8 MPa. These results indicate that pepper-rosmarin plants are sensitive to water stress.

Conclusions

1. Pepper-rosmarin plants are very sensitive to water stress.
2. Water stress in pepper-rosmarin decreases essential oil content and total flavonoids up to 50 and 60%, respectively, with the reduction of 0.3 MPa in leaf water potential.
3. Essential oil from pepper-rosmarin is more sensitive to water stress than flavonoids.

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