Growth and essential oil production by Martianthus leucocephalus grown under the edaphoclimatic conditions of Feira de Santana, Bahia, Brazil

Crescimento e produção de óleo essencial de Martianthus leucocephalus cultivada nas condições edafoclimáticas de Feira de Santana, Bahia, Brasil

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ABSTRACT

The semiarid region of Brazil holds a great richness of medicinal and aromatic plants with considerable potential for pharmaceutical, food, cosmetic and biopesticide industries. Martianthus leucocephalus (Mart. ex Benth.) J. F. B. Pastore is endemic to this region, and its essential oils contain a principle compound, isobornyl formate, which demonstrates antimicrobial activity against Bacillus cereus, Staphylococcus aureus and Candida albicans. In spite of its significant pharmacological potential, little is known about its growth. In light of the influence of seasonality on plant growth, development, and secondary metabolism, the present study evaluated the growth and essential oil content of M. leucocephalus grown and harvested during different months of the year in the edaphoclimatic conditions of Feira de Santana, Bahia State, Brazil. The experimental design was entirely randomized, with twelve harvesting periods and five replicates. The study acquired monthly data of mean temperatures, relative humidity, rainfall, irradiance, and photoperiod from the National Institute of Meteorology (INMET) and quantified the fresh and dry weights of leaves, flowers and branches, as well as leaf area, and essential oil content. The data were submitted to Spearman correlation analysis and the means were compared using the Scott-Knott test. Total leaf masses and oil contents were higher as well as leaf area, and essential oil content. Higher total mean dry masses were recorded from September to January (except October), while oil content was higher in March.

INTRODUCTION

The Brazilian semiarid region, dominated by “Caatinga” vegetation, has many medicinal and aromatic plants, with considerable potential for pharmaceutical, food, cosmetic and biopesticide industries. The semiarid region of Brazil holds a great richness of medicinal and aromatic plants with considerable potential for pharmaceutical, food, cosmetic and biopesticide industries. Martianthus leucocephalus (Mart. ex Benth.) J. F. B. Pastore is endemic of this region, and its essential oils contain a principle compound, isobornyl formate, which demonstrates antimicrobial activity against Bacillus cereus, Staphylococcus aureus and Candida albicans. In spite of its significant pharmacological potential, little is known about its growth. In light of the influence of seasonality on plant growth, development, and secondary metabolism, the present study evaluated the growth and essential oil content of M. leucocephalus grown and harvested during different months of the year in the edaphoclimatic conditions of Feira de Santana, Bahia State, Brazil. The experimental design was entirely randomized, with twelve harvesting periods and five replicates. The study acquired monthly data of mean temperatures, relative humidity, rainfall, irradiance, and photoperiod from the National Institute of Meteorology (INMET) and quantified the fresh and dry weights of leaves, flowers and branches, as well as leaf area, and essential oil content. The data were submitted to Spearman correlation analysis and the means were compared using the Scott-Knott test. Total leaf masses and oil contents were higher during periods with longer photoperiods and higher solar irradiance. Rainfall and relative humidity reduced plant growth and essential oil content. Higher total mean dry masses were recorded from September to January (except October), while oil content was higher in March.

Key words: semiarid region, terpenes, biomass, endemic medicinal plant, Hyptis leucocephala.
The present study sought to evaluate the growth and production of essential oils by *M. leucocephalus* during a twelve month period and correlate these parameters with climatic factors to determine the most favorable season for cultivation and for obtaining essential oils under the edaphoclimatic conditions of Feira de Santana, Bahia State, Brazil.

**MATERIALS AND METHODS**

The experiments were conducted in the experimental unit (Horto Florestal) of the State Universidade Estadual de Feira de Santana (UEFS). This region is under the influence of a semiarid climate (KÖPPEN, 1931; THORNTHWAITE, 1948) in Bahia State, Brazil, (12°16’ S x 38°58’ W) at 234 meters above sea level, with a mean annual temperature of 24°C and a mean precipitation rate of 848mm per year. The test plants were obtained through the propagation of stocks kept in the Medicinal and Aromatic Plant Collection of the University; they were identified at the UEFS Herbarium (HUEFS, Voucher 25 322).

Apical cuttings were grown in polypropylene cups filled with Biomix® commercial substrate, with added organic material (1:1) following the methodology described by OLIVEIRA et al. (2011). Soil was chemically analyzed and fertilized before planting, using 6.160g of limestone powder, 732.6g of phosphorus (simple-superphosphate), and 10 liters of organic material per plot. After 60 days, the plants were replanted in eight rows per plot (1.2 x 10.0m), in full sunlight. The plots were weeded monthly and irrigated using a micro-sprinkler system. After 120 days of further cultivation, it was initiated random harvesting each month in 1m² subplots (five repetitions).

It was evaluated the following growth parameters on a monthly basis for one year: leaf area, fresh and dry masses of stems, leaves, and flowers, as well as the essential oil content. After weighing, the fresh material was placed in paper bags and dried by forced air circulation at 60°C to a constant weight. Leaf area was estimated from sub-samples, composed of three repetitions of 20 leaves randomly collected from each plot. Leaves were scanned in a HP4C deskjet scanner and their areas were quantified using QUANT software (VALE et al., 2003). Their corresponding dry weights of each sub-sample were obtained and the mean values were used to estimate the total leaf area of the samples.

Essential oil extractions from the dried leaves and flowers from each subplot were performed by hydrodistillation in a Clevenger apparatus, with 3 hours of distillation. After distillation, the oil was collected and anhydrous sodium sulfate was added to remove excess water. The essential oil content was
quantified based on its mass, as determined using an analytical balance, being expressed as a percentage (grams of oil per 100g of dry matter).

Monthly temperature, relative humidity, precipitation, photoperiod, and irradiance data were obtained from the Instituto Nacional de Meteorologia (INMET) in the years 2012 and 2013, and their means were used to correlate growth parameters with essential oil production.

The variables obtained were calculated using descriptive statistics: minimum and maximum values, means and standard deviations, coefficients of variation (%), and the Shapiro-Wilk normality test. It was also calculated the Spearman correlation coefficients using the CORR procedure of SAS. The correlation coefficients were tested by the Student t test, at a 5% level of significance. The analyses were performed using Statistical Analysis System – SAS software (SAS Institute Inc, 2004). Means were compared using the Scott-Knott test (P≤0.05) using the SISVAR statistical program (FERREIRA, 2010).

RESULTS AND DISCUSSION

Descriptive statistics showed high variability in the growth rate of *Martianthus leucocephalus*, especially in terms of the dry masses of the flowers (Table 1), with relatively high coefficients of variation (CV), indicating that this species was strongly affected by climatic factors during the different months of the year, and even within each harvest period. The oil content varied greatly (from 0.10% to 0.36%), with a yearly mean of 0.20%, similar to the results reported by LUCCHESI et al. (2005). Among the climatic variables, descriptive statistics demonstrated higher CV for precipitation and irradiance (Table 1), reflecting their irregularity during the year. The data demonstrated normality at a 5% level of significance by the Shapiro-Wilk test.

Growth parameters varied significantly during the twelve months of observation (Table 2), even under irrigated growing conditions. It is noteworthy that *M. leucocephalus* is a wild species, still in the process of domestication. According to TAIZ & ZEIGER (2013), plants at this stage adopt survival strategies of flexible growth patterns that allow their adaptation to locations that may differ from ideal, particularly with respect to solar radiation.

The total dry matter (TDM) per area was higher during the months of September, November, December, and January (Table 2) when day length and irradiance levels were higher (Figure 1). Correlation analysis demonstrated that these climatic variables tend to positively influence TDM growth, while high humidity tends to decrease this metric (Table 3); the same correlations were observed with stem dry mass (SDM) and flower dry mass (FDM). Higher temperatures increased flower

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**Table 1 -** Minimum, maximum, mean, standard deviation, coefficient of variation (CV) and normality tests for the variables TEMP (°C), HUM (%), RAINF (mm), RAD (kJ m⁻²), PHOTO (h/d), of the edaphoclimatic conditions, and the TDM (g), LDM (g), FDM (g), SDM (g), TLA (cm²) and OC (%) of *Martianthus leucocephalus* (Mart. ex Benth.) J.F.B. Pastore grown in Feira de Santana, Bahia State, Brazil, from November/2012 to October/2013.

| Variable | Minimum | Maximum | Mean | Standard deviation | CV (%) | Normality test |
|----------|---------|---------|------|--------------------|--------|---------------|
| TEMP     | 22.00   | 27.54   | 24.90| 1.91               | 7.68   | 0.8855ns      |
| HUM      | 64.51   | 84.52   | 74.30| 6.74               | 9.07   | 0.9150ns      |
| RAINF    | 2.00    | 122.00  | 60.53| 40.73              | 67.29  | 0.9015ns      |
| RAD      | 1361.97 | 2609.09 | 1996.07| 443.59             | 22.22  | 0.8822ns      |
| PHOTO    | 11.25   | 12.51   | 11.90| 0.48               | 4.08   | 0.8326ns      |
| TDM      | 97.65   | 423.27  | 238.14| 62.66              | 26.31  | 0.9857ns      |
| LDM      | 39.65   | 115.19  | 78.07| 19.47              | 24.95  | 0.9804ns      |
| FDM      | 1.77    | 80.24   | 22.45| 15.69              | 69.87  | 0.9147ns      |
| SDM      | 44.83   | 227.84  | 137.61| 39.59              | 28.77  | 0.9862ns      |
| TLA      | 2685.76 | 12670.90| 5894.34| 2029.96            | 34.44  | 0.9181ns      |
| OC       | 0.10    | 0.36    | 0.20 | 0.07               | 32.73  | 0.9647ns      |

*not significant by the Shapiro-Wilk test at 5% significance level.

TEMP (average temperature), HUM (relative humidity), RAINF (rainfall), RAD (irradiance) PHOTO (photoperiod), TDM (g) (total dry mass), LDM (g) (leaf dry mass), FDM (g) (flower dry mass), SDM (g) (stem dry mass), TLA (cm²) (total leaf area), and OC (%) (essential oil content).
production (Table 3). Total leaf area (TLA) also showed a positive correlation with temperature, irradiance, and photoperiod, while negatively correlated with relative humidity, following the same general pattern as TDM. The leaf dry mass (LDM) was not correlated with these same climatic variables, showing high production during most of the study period indicating that this species can grow well throughout the year if regularly watered.

Essential oil content (OC) showed a positive correlation with irradiance and a negative correlation with precipitation and relative humidity, with its production being highest during months of low rainfall and high levels of solar radiation. The highest essential oil content of *Otacanthus azureus* (Linden) Ronse was observed in treatments with 100% irradiance, with decreasing production as light levels decreased (SERUDO et al., 2013). Similarly, higher levels of oil production were observed in *Lippia citriodora* Lam. (GOMES et al., 2009) and *Hyptis marrubioides* Epl. (SALES et al., 2009) when grown in full sunlight. The negative correlation of essential oil production with rainfall and humidity has been observed in other studies, such as that undertaken by PRAVUSCHI et al. (2010) with basil (*Ocimum basilicum* L.). These authors established that excessive irrigation can negatively affect development, resulting in low oil productivity. Studies with *Plectranthus amboinicus* (Lourr.) Spreng. likewise showed increased essential oil contents in months with low rainfall, indicating that excess water can reduce oil production (CARNEIRO et al., 2010).

The results presented here established that higher essential oil content can be obtained from *Martianthus leucocephalus* under the edaphic and climatic conditions of Feira de Santana, Bahia State, Brazil by planting in May and June (the period in which essential oil production is low, but vegetative growth is higher), with harvesting between September and March when essential oil production is high.

### Table 2 - Monthly variations of TDM (g), LDM (g), FDM (g), SDM (g), TLA (cm²) and OC (%) of *Martianthus leucocephalus* (Mart. Ex Benth.) J.F.B. Pastore grown under the edaphoclimatic conditions of Feira de Santana, Bahia State, Brazil.

| Month/year | TDM (g) | LDM (g) | FDM (g) | SDM (g) | TLA (g) | OC (%) |
|------------|---------|---------|---------|---------|---------|--------|
| Nov/12     | 291.95a | 97.66a  | 32.57b  | 161.73a | 6713.85b| 0.21c  |
| Dec/12     | 293.95a | 85.51a  | 38.27a  | 170.18a | 7618.96b| 0.16d  |
| Jan/13     | 307.55a | 86.01a  | 47.10a  | 174.44a | 9461.10a| 0.18d  |
| Feb/13     | 212.68b | 63.40b  | 22.95c  | 126.33b | 7749.38b| 0.25b  |
| Mar/13     | 229.69b | 58.13b  | 28.07b  | 143.50b | 7104.53b| 0.31a  |
| Apr/13     | 193.11b | 64.04b  | 19.67c  | 109.40b | 5031.71c| 0.17d  |
| May/13     | 168.18b | 68.80b  | 12.64d  | 86.74b  | 3983.39c| 0.10e  |
| Jun/13     | 203.13b | 81.18a  | 4.18d   | 117.76b | 4059.00c| 0.12c  |
| Jul/13     | 222.58b | 84.48a  | 10.59d  | 125.51b | 5285.01c| 0.23c  |
| Aug/13     | 222.45b | 82.58a  | 6.52d   | 133.35b | 5046.43c| 0.21c  |
| Sep/13     | 300.24a | 100.37a | 17.43c  | 182.44a | 5520.35c| 0.25b  |
| Oct/13     | 212.66b | 62.72b  | 29.46b  | 119.98b | 4058.35c| 0.26b  |
| Mean       | 238.14  | 78.07   | 22.45   | 137.61  | 5894.34 | 0.20   |

*Means followed by same letter in the column do not differ by the Scott-Knott test at a 5% level of probability.

TDM (g) (total dry mass), LDM (g) (leaf dry mass), FDM (g) (flower dry mass), SDM (g) (stem dry mass), TLA (cm²) (total leaf area), and OC (%) (essential oil content).
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|          | TDM   | LDM   | FDM   | SDM   | OC    | TEMP  | HUM   | RAINF | RAD   | PHOTO |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| TLA      | 0.7024 | 0.5104 | 0.4730 | 0.6631 | 0.3373 | 0.5490 | -0.5390 | -0.2120 | 0.5760 | 0.5910 |
| TDM      | 0.7319 | 0.5140 | 0.9581 | 0.1945 | 0.1714 | 0.2796 | 0.2197 | 0.0824 | -0.0747 | 0.0493 |
| LDM      | 0.0041 | 0.6142 | -0.0160 | -0.0741 | 0.6514 | 0.1957 | 0.1681 | -0.9161 | 0.4869 | 0.2406 |
| FDM      | 0.4469 | 0.1777 | 0.2392 | 0.3361 | 0.3581 | -0.3361 | 0.1681 | -0.9161 | 0.4869 | 0.2406 |
| SDM      | 0.2392 | 0.1681 | 0.2557 | 0.1957 | 0.1681 | -0.9161 | 0.4869 | -0.9161 | 0.4869 | 0.2406 |
| OC       | 0.2392 | 0.1681 | 0.2557 | 0.1957 | 0.1681 | -0.9161 | 0.4869 | -0.9161 | 0.4869 | 0.2406 |
| TEMP     |       |       |       | 0.8112 |       |       |       |       | 0.7273 |       |
| HUM      |       |       |       |       |       |       |       |       |       |       |
| RAINF    |       |       |       |       |       |       |       |       |       |       |
| RAD      |       |       |       |       |       |       |       |       |       |       |
| PHOTO    |       |       |       |       |       |       |       |       |       |       |

Table 3 - Spearman correlation coefficients between climate variables and increased dry matter of Martianthus leucocephalus (Mart. Ex Benth.) J.F.B. Pastore in one year of exposure to the edaphoclimatic conditions of Feira de Santana, Bahia State, Brazil.

### CONCLUSION

Essential oil content and dry mass accumulation in Martianthus Leucocephalus is influenced by the edaphoclimatic condition of Feira de Santana, and is especially favored by the high irradiance levels during the Austral summer months.

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