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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

Bianca Oliveira de Azevedo[I*] Lenaldo Muniz de Oliveira[II] Angélica Maria Lucchese[III] Daniel de Jesus da Silva[I] Carlos Alberto da Silva Ledo[IV] Marilza Neves do Nascimento[II]

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* ***Bacilus 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***.

RESUMO

O semiárido brasileiro apresenta grande riqueza de espécies medicinais e aromáticas, com intenso uso na indústria farmacêutica, alimentícia, de cosméticos e de biopesticidas. A espécie ***Martianthus leucocephalus*** *(Mart. ex Benth.) J. F. B. Pastore é endêmica dessa região. Seu óleo essencial apresenta como composto majoritário o formiato de isobornila, com atividade antimicrobiana frente à* ***Bacilus cereus***, ***Staphylococcus aureus*** *e* ***Candida albicans***. *Devido à grande influência da sazonalidade sobre o crescimento e produção vegetal, sobretudo quando envolve o metabolismo secundário de plantas, o objetivo deste trabalho foi avaliar o crescimento e a produção de óleo essencial de* ***M. leucocephalus*** *cultivada e colhida em diferentes meses do ano, nas condições edafoclimáticas de Feira de Santana, BA. O experimento foi conduzido em delineamento inteiramente casualizado (DIC), com doze tratamentos, constituídos dos diferentes meses de colheita, com cinco repetições. Mensalmente, foram obtidos os valores médios de temperatura, umidade relativa do ar, precipitação pluviométrica, irradiância e fotoperíodo, através do Instituto Nacional de Meteorologia (INMET), quantificando-se também a massa fresca e seca das folhas, flores e caule, a área foliar e o teor de óleo essencial das plantas. Os dados foram submetidos à análise de correlação de Spearman e as médias foram comparadas pelo teste de Scott-Knott. A massa seca total e o teor de óleo aumentaram em função da irradiância e reduziram em função da precipitação pluviométrica e umidade relativa do ar. As maiores médias de massa seca total foram encontradas durante os meses de setembro a janeiro, exceto outubro, e de teor de óleo no mês de março.*

Palavras-chave: *região semiárida, terpenos, biomassa, planta medicinal endêmica,* ***Hyptis leucocephala***.

INTRODUCTION

The Brazilian semiarid region, dominated by "Caatinga" vegetation, has many medicinal and

[I]Programa de Pós-graduação em Recursos Genéticos Vegetais (PPGRGV), Universidade Estadual de Feira de Santana (UEFS), 44036-900, Feira de Santana, BA, Brasil. E-mail: biancaoz@yahoo.com.br. *Corresponding author.
[II]Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana (UEFS), Feira de Santana, BA, Brasil.
[III]Departamento de Ciências Exatas, Universidade Estadual de Feira de Santana (UEFS), Feira de Santana, BA, Brasil.
[IV]Centro Nacional de Pesquisas em Mandioca e Fruticultura (CNPMF/EMBRAPA), Cruz das Almas, BA, Brasil.
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aromatic plants, including the ***Lamiaceae*** family with many endemic taxa (GIULIETTI et al., 2004). Several species of this family have proven pharmacological capabilities, including antimicrobial, cytotoxic, anti-inflammatory, anti-HIV, and insecticide activities (FALCÃO & MENEZES, 2003).

Martianthus leucocephalus (Mart. ex Benth.) J. F. B. Pastore (***Lamiaceae***), formerly recognized as ***Hyptis leucocephala*** Mart. ex Benth., is an endemic medicinal plant of the Brazilian semiarid region (HARLEY & PASTORE, 2012). It is a decumbent aromatic herb, up 20cm tall, with chartaceous leaves, flowers with a green calyx and purple corolla (OLIVEIRA et al., 2011). Leaves, flowers and branches produce essential oils (0.2% average yield, with isobornyl formate being the principal compound) with proven antimicrobial activity against ***Bacillus cereus***, ***Staphylococcus aureus,*** and ***Candida albicans*** (LUCCHESE et al., 2005).

Essential oils are volatile compounds, consisting mainly of mono- and sesquiterpenes, with wide applications in the pharmaceutical, food, cosmetic, and bio-pesticide industries. These oils derive from the secondary metabolism of plants, and accumulate in trichomes or secretory cavities (TAIZ & ZEIGER, 2013). Their production depends mainly on genetic and environmental factors, although they can be influenced by cultivation techniques (CASTRO et al., 2002), with the harvest season appearing to have a marked effect, as oil volumes and active constituents are not constant throughout the year (GOBBO-NETO & LOPES, 2007).

Environmental factors such as temperature, rainfall, relative humidity, irradiance, and photoperiod can affect the production of these secondary compounds. In ***Lippia alba*** (Mill), for example, the yields of essential oil were higher in the summer when the temperature was elevated (BARROS et al., 2009). BOTREL et al. (2010) came to similar conclusions in their research with ***Hyptis marrubioides*** Epl., observing that metabolite production was greater during the summer (when day length was longer). Although these results demonstrate the influence of the growth environment, each species tends to respond in a unique fashion, with different levels of essential oil production throughout the year.

Plant growth analysis has been widely used to quantify the rate of assimilation and utilization of organic compounds, and can be very useful in understanding how environmental factors influence the growth and production of essential oils in aromatic species.

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 ($P \leq 0.05$) using the SISVAR statistical program (FERREIRA, 2010).

RESULTS AND DISCUSSION

Descriptive statistics showed high variability in the growth rate of ***M. 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 LUCCHESE 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

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	0.8855^{ns}
HUM	64.51	84.52	74.30	6.74	9.07	0.9150^{ns}
RAINF	2.00	122.00	60.53	40.73	67.29	0.9015^{ns}
RAD	1361.97	2609.09	1996.07	443.59	22.22	0.8822^{ns}
PHOTO	11.25	12.51	11.90	0.48	4.08	0.8328^{ns}
TDM	97.65	423.27	238.14	62.66	26.31	0.9857^{ns}
LDM	39.65	115.19	78.07	19.47	24.95	0.9804^{ns}
FDM	1.77	80.24	22.45	15.69	69.87	0.9147^{ns}
SDM	44.83	227.84	137.61	39.59	28.77	0.9862^{ns}
TLA	2658.76	12670.90	5894.34	2029.96	34.44	0.9181^{ns}
OC	0.10	0.36	0.20	0.07	32.73	0.9647^{ns}

^{ns}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).

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	6718.96b	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	0.12e
Jul/13	222.58b	86.48a	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	212.16b	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).

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).

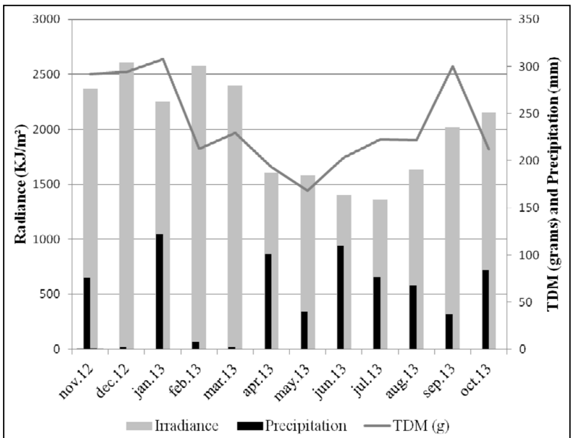

Figure 1 - Irradiance levels and precipitation in relation to increases in total dry matter (TDM) of ***Martianthus leucocephalus*** (Mart. Ex Benth.) JFB Pastore, exposed to the edaphoclimatic conditions of Feira de Santana, Bahia State, Brazil.

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 (with irrigation) 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 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.

	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^{ns}	0.5760^{*}	0.5910^{*}
TDM		0.7319^{*}	0.5140^{*}	0.9581^{*}	0.1945^{ns}	0.1714^{ns}	-0.2844^{*}	-0.1026^{ns}	0.4084^{*}	0.5241^{*}
LDM			0.0041^{ns}	0.6142^{*}	-0.0160^{ns}	0.2796^{*}	0.2197^{ns}	0.0824^{ns}	-0.0747^{ns}	0.0493^{ns}
FDM				0.4469^{*}	0.1777^{ns}	0.6514^{*}	-0.7491^{*}	-0.1373^{ns}	0.6777^{*}	0.8044^{*}
SDM					0.2392^{ns}	0.1957^{ns}	-0.2938^{*}	-0.1514^{ns}	0.4240^{*}	0.4895^{*}
OC						0.1681^{ns}	-0.2557^{*}	-0.3361^{*}	0.3581^{*}	0.2406^{ns}
TEMP							-0.9161^{*}	-0.3678^{*}	0.8112^{*}	0.7273^{*}
HUM								0.4869^{*}	-0.9231^{*}	-0.8741^{*}
RAINF									-0.5604^{*}	-0.2627^{*}
RAD										0.9021^{*}

^{ns}not significant by t test at 5% significance.

*Significant.

TDM (total dry mass), LDM (leaf dry mass), FDM (flower dry mass), SDM (stem dry mass), TLA (total leaf area), OC (essential oil content), TEMP (average temperature), HUM (relative humidity), RAINF (rainfall), RAD (irradiance) and PHOTO (photoperiod).

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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