Original Article

SEASONAL VARIATION OF VEGETATIVE GROWTH, ESSENTIAL OIL YIELD AND COMPOSITION OF MENTHOL MINT GENOTYPES AT SOUTHERN BRAZIL

VARIAÇÃO SAZONAL DO CRESCIMENTO VEGETATIVO, PRODUTIVIDADE E COMPOSIÇÃO DE ÓLEO ESSENCIAL EM GENÓTIPOS DE MENTA NO SUDESTE BRASILEIRO

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ABSTRACT: Menthol has economic importance to the flavor, food and pharmaceutical industries. Ten menthol mint (Mentha spp) genotypes were assessed for essential oil content and composition at Southern Brazil environmental conditions at two harvest times (February and May). The experimental design was in completely randomized blocks with a 10 x 2 factorial for genotypes and harvest time. The essential oil was obtained by hydrodistillation in a Clevenger apparatus. The essential oil content varied from 0.8 to 5.3% and was greater in February for all the investigated genotypes. The main constituents identified in the essential oil samples were menthol (12 - 92.7%), mentone (2.2 - 56.9%), and neomenthol (2.9 - 12.1%). Menthol levels were superior in May and showed a negative correlation with mentone and neomenthol, which in turn were higher in February. Menthol levels were positively correlated with menthyl acetate. Pulegone, 1.8 cineol, and limonene were also detected in lower concentrations in some genotypes. Thirteen other essential oil constituents were identified as trace elements in essential oil. *Mentha canadensis* L. showed the highest essential oil content (5.3 % - February and 3.5% - May) as well as the highest menthol content (89.6% - February, 92.7% - May) in both harvests. From the analyzed results, Southern Brazil local environmental conditions are appropriated for menthol production, with two harvests and *M. canadensis* L. can be recommended as a promising genetic source. The summer harvest (February) favored oil yield, although with a slight decrease in menthol content. The challenge of achieving higher essential oil and menthol yields depends on strategies to increase herb yield by developing innovative agronomic practices.

KEYWORD: Lamiaceae. Mentha. Genetic resources. Biomass. Menthol. Menthone.

INTRODUCTION

The mint family is composed by different species that are grown worldwide to explore their fresh or dried leaves, as well as their essential oils, as flavoring or spices in a wide variety of food (MORRIS, 2007). India, China and United States are the main mint producer countries. Although Brazil has been the world's leading menthol producer in the early 1970's, it now imports annually approximately US$ 10 million of mint essential oil (BIZZO et al., 2009). The major p-menthane monoterpene compound is (-)-menthol, which is important to the pharmaceutical, food and confectionery industries. Peltate glandular trichomes have been identified as the sites of essential oil production and storage in mint (DESCHAMPS et al., 2006; CROTEAU et al., 2005). The eight steps of the menthol biosynthesis pathway and the specific enzymes and gene properties have been described (CHANG et al., 2010; GERSHERZON et al., 2000). The understanding of the regulation of gene expression distributed in four subcellular compartments and the influence of plant ontogeny on oil production have begun to be explained (CHANG et al., 2010; RIOS – ESTEPA et al., 2008; CROTEAU et al., 2005; GERSHERZON et al., 2000). However, the correlation between genotypic acquirements with ecological interactions in physiological plant regulation plays a decisive role in essential oil modulation. (GOBBO-NETO; LOPES, 2007; SANGWAN et al., 2001).

The genotypic variability of the genus *Mentha* is favored by cytomixis and consequent polyploidy, transgressive segregation and the ease of hybridization, resulting in significant chemical polymorphism (TUCKER; NACZI, 2007). In addition to genotypic traits, it has been demonstrated that genetic expression for oil production is also affected by plant ontogeny and environmental regulation, such as soil and seasonal variations (RIOS-ESTEPA et al., 2008; SANGWAN et al., 2001). Due to the economic importance of menthol, great emphasis has been

Received: 12/11/11
Accepted: 03/05/12

given to the conservation of mint genetic materials as a valuable bioresource to increase essential oil yield and quality, allowing economic production of mint-related commodities (KHANUJA et al., 2000; FRANZ, 2010).

This work is part of a research program directed to mint production in Brazil which includes germplasm evaluation in different edaphoclimatic conditions throughout the country. The main objective of this work was to evaluate the vegetative growth, essential oil yield and composition of different mint genotypes at the environmental conditions of Southern Brazil.

MATERIAL AND METHODS

Plant material and experimental conditions

Ten mint genotypes were obtained from the germplasm bank of Embrapa Genetic Resources and Biotechnology, Brazil (Table 1), by vegetative propagation using stem cuttings with 5 to 7 cm in length, Plantmax HT® as substrate, and grown under greenhouse conditions at Federal University of Parana (UFPR), on styrofoam trays with three intermittent mist irrigations during the day. Plants were transferred to field in November 2009, at the Catarinense Federal Institute, Campus Araquari (S 26º23'56'', W 48º44'30'', 4m), on sandy Espodossolo soil (EMBRAPA, 1999). They were harvested twice, in February (90 days after planting) and May 2010 (180 days after planting). The experimental design was in completely randomized blocks with a 10 x 2 factorial, four replications and sixteen plants each.

Before transferring the plants to the field, the experimental area was fertilized with 40 kg/ha of nitrogen, 30 kg/ha of phosphorus and 30 kg/ha of potassium, with two additional nitrogen fertilization (20 kg/ha) 30 days after transplanting and after the first harvest. Plants were spaced by 60 cm between rows and 30 cm between each other. The total area of the experimental unit was 4.2 m^2 and the samples were collected from 0.72 m^2 of this area. Plant height, fresh and dry weights were determined. The samples were dried at 65ºC in a FANEN (São Paulo, Brazil) model 320SE circulation oven until constant weight. The essential oil was obtained by hydrodistillation of 100 g of fresh leaves in a Clevenger apparatus for 2 hours.

Table 1. Identification of genotype from mint collection (MC) evaluated in this study

GENOTYPE	DENOMINATION	CIENTIFIC NAME	ORIGIN
MC* 3	Chocolate mint	*Mentha* x *piperita* L.	Purdue University - USA
MC 23	Pepermint	*Mentha* x *piperita* L.	Purdue University - USA
MC 34	EMATER 2	*Mentha sylvestris* L.	UnB - Brazil
MC 37	EMATER 3	*Mentha canadensis* L.	UnB - Brazil
MC 43	Hortelã 560	*Mentha piperita* L.	CPQBA - Brazil
MC 57	IAC 9	*Mentha* sp.	IAC - Brazil
MC 69	UFC 5	*Mentha* x *piperita* L.	UFC - Brazil
MC 75	Hortelã – PR1	*Mentha arvensis* L.	PR1 Brazil
MC 76	Hortelã – PR2	*Mentha arvensis* L.	PR2 Brazil
MC 78	Hortelã – PR3	*Mentha arvensis L.*	PR3- Brazil

EMATER – Empresa de Assistência técnica e Extensão Rural,CPQBA - Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas - CPQBA , Instituto Agronômico de Campinas - IAC - , Universidade Federal do Ceará – UFC, Universidade de Brasília - UnB, Genótipos de produtores do Paraná (PR1, PR2 e PR3).MC* = Mint collection from Embrapa Genetic Resources and Biotechnology

Chemicals

Dichloromethane spectroscopic grade (Tedia, Fairfield, USA) was used as solvent. The standards were purchased from Aldrich (Milwaukee, USA).

Analysis of the essential oils

The oils were analyzed in an Agilent (Palo Alto, USA) 6890N gas chromatograph fitted with a 5% phenyl - 95% methylsilicone (HP5, 25m X 0.32mm X 0.25µm) fused silica capillary column. The oven temperature was programmed from 60ºC to 240ºC at 3ºC/min, and hydrogen was used as carrier gas (1.5 mL/min). The oils were diluted to 1% in dichloromethane and 1.0µL of this solution was injected in split mode (1:100). The injector was kept at 250ºC and the detector (FID) at 280ºC.

Mass spectra were obtained in an Agilent 5973N system operating in electronic ionization mode (EI) at 70 eV, with scan mass range of 40-500 *m/z*. The sampling rate was 3.15 scans/s. The ion source was kept at 230ºC, mass analyzer at 150ºC and transfer line at 260ºC. The mass detector was coupled to an Agilent 6890 gas chromatograph fitted with a low bleeding 5% phenyl - 95%

methylsilicone (HP-5MS, 30m X 0.25mm X 0.25µm) fused silica capillary column. The injection procedure and oven temperature program were the same as above. Helium was the carrier gas, at 1.0 mL/min.

Linear retention indices (LRI) were measured (DOOL; KRATZ, 1963) by injecting a series of n-alkanes (C_7-C_{26}) in the same column and conditions indicated above for GC analyses.

Identification of the oil components was based on a computer search using the Wiley 6th ed. library of mass spectral data and by comparison of their calculated linear retention indices with data from the literature (ADAMS, 2007). Standard solutions of menthol and menthone were injected for confirmation.

Statistical Analysis

For each genotype and harvest time the average oil content and the percentage of the identified compounds were calculated using the average mean of the four blocks of replications. MSTAT-C (NISSEN, 1993) was used to verify mean homogeneity with a Barlett Test and data was analyzed using the analysis of variance (ANOVA) of the factorial design by the randomization method. The main plot treatments were the ten genotypes and the subplots consisted of the two harvest times. A Tukey honestly significance difference test at $p<0.05$ compared means of biomass yield, oil content and yield as well as the percentage of the identified constituents.

RESULTS AND DISCUSSION

The *M. arvensis* genotypes exhibited the highest plant height (64.17 to 64.83 cm - Table 2). *M. x piperita* (MC 3 and MC 23), cultivated in Santa Catarina lead to higher plant heights (28.25 and 21.92cm), when compared to those from Brasilia (18.3 and 15.6 cm), respectively (GRIZI et al., 2006). *M. canadensis* (MC 37) in this study presented lower plant height than the cultivars Cornmint and Sakhalin mint studied in Finland (AFLATUNI, 2005).

The leaf and herb dry yield were affected by both genotypes and harvest time (Table 2). Plants harvested in February showed higher vegetative growth than those harvested in May despite the fact that the vegetation period was very similar (95 and 90 days). The environmental conditions of temperature and precipitation, probably affected the vegetative growth. In February, the average temperature was 6.9ºC higher with a long-day photoperiod of 14 hours, increasing carbon assimilation and biomass production. In addition, precipitation rates lower than 60 mm in April and May could also have limited plant growth in the second harvest. Similar results have been reported for mint species in Curitiba, Brazil, with superior biomass production in summer than autumn (MONTEIRO, 2009; DESCHAMPS et al., 2008).

Table 2. Plant height, dry leaf yield and total dry yield (herb yield) of mint species at two harvests

Mint genotypes	Height (cm)	Leaf dry yield kg/ha			Total dry yield (herb yield) kg/ha		
		Feb	May	Total	Feb	May	Total
MC 3	28.25	2163 ^{Aab}	585 ^{Bab}	2748 ^{a}	2905 ^{Aab}	1119 ^{Bab}	4024 ^{b}
MC 23	21.92	1177 ^{Ac}	257 ^{Bb}	1434 ^{c}	1566 ^{Ac}	475 ^{Bb}	2041 ^{d}
MC 34	28.83	1532 ^{Aabc}	548 ^{Bab}	2080 ^{b}	2128 ^{Abc}	967 ^{Bab}	3095 ^{c}
MC 37	38.50	1829 ^{Aabc}	1219 ^{Ba}	3048 ^{a}	2395 ^{Abc}	1867 ^{Ba}	4262 ^{b}
MC 43	28.08	1749 ^{Aabc}	286 ^{Bb}	2035 ^{b}	2374 ^{Abc}	549 ^{Bb}	2923 ^{c}
MC 57	27.92	1632 ^{Aabc}	692 ^{Bab}	2324 ^{b}	2198 ^{Abc}	1673 ^{Bab}	3871 ^{bc}
MC 69	19.25	1463 ^{Aabc}	512 ^{Bab}	1975 ^{b}	1929 ^{Abc}	984 ^{Bab}	2913 ^{c}
MC 75	64.25	2261 ^{Aa}	972 ^{Bab}	3233 ^{a}	3735 ^{Aa}	2161 ^{Ba}	5896 ^{a}
MC 76	64.83	1656 ^{Aabc}	1306 ^{Ba}	2962 ^{a}	2657 ^{Aabc}	1810 ^{Ba}	4467 ^{b}
MC 78	64.17	1736 ^{Aabc}	1243 ^{Ba}	2979 ^{a}	2690 ^{Aabc}	2053 ^{Ba}	4743 ^{b}
Average		1720	762		2458	1366	

Coefficient of Variation –CV Leaf dry yield =29.82%. Total dry yield CV= 26.33%; Means followed by the same capital letters on lines and the same lowercase letters on columns are not significantly different for Tukey test at the 0.05 level.

Highest herb and leaf yield were observed in *M. arvensis* (MC 75, MC 76, MC 78), *M. canadensis* and *Mentha* x *piperita* L. (MC 3) genotypes. The *M. arvensis* genotypes and *M. canadensis* (MC 37) were harvested at full bloom, when plant structure is commonly changed due to leaf senescence. This did not occur with *M. canadensis*, as demonstrated by the leaf-herb ratios

(Table 3). In spite of the leaf senescence, the correlation with essential oil production was positive due to the total herb yield, justifying harvesting at full bloom (ROHLOFF et al., 2005). *M. piperita* L. (MC 69) in turn exhibited the lowest herb and leaf yield due to its dwarf characteristics and prostrate growth, evidenced by the plant height and the leaf-herb ratio. Although *M. arvensis* cultivars achieved the highest values of dry herb yield in this study, they are significantly lower than those obtained commercially in India, which ranged from 5.14 to 8.25 t/ha (SRIVASTAVA, et al., 2002).

Table 3. Means of leaf/herb ratio of mint species at two harvests

Mint genotypes	Dry leaf / dry herb		
	Feb	May	Average
MC 3	0,73 ^{Aa}	0,50 ^{Bab}	0,62 ^{ab}
MC 23	0,74 ^{Aa}	0,56 ^{Bab}	0,65 ^{ab}
MC 34	0,72 ^{Aa}	0,59 ^{Bab}	0,65 ^{ab}
MC 37	0,76 ^{Aa}	0,66 ^{Ba}	0,71 ^{a}
MC 43	0,73 ^{Aa}	0,49 ^{Bab}	0,61 ^{ab}
MC 57	0,75 ^{Aa}	0,42 ^{Bb}	0,58 ^{b}
MC 69	0,74 ^{Aa}	0,51 ^{Bab}	0,63 ^{ab}
MC 75	0,60 ^{Aa}	0,54 ^{Bab}	0,57 ^{b}
MC 76	0,63 ^{Aa}	0,59 ^{Bab}	0,61 ^{ab}
MC 78	0,63 ^{Aa}	0,60 ^{Bab}	0,61 ^{ab}
Average	0,70	0,55	

Dry leaf / dry herb CV=13.89%. Means followed by the same capital letters on lines and the same lowercase letters on columns are not significantly different for Tukey test at the 0.05 level.

Significant differences were observed in essential oil content among harvest times and genotypes (Table 4). All of the genotypes had relatively high essential oil contents (0.8 to 5.3%). The essential oil content was higher in February (2.7%) than in May (1.5%). Summer environmental conditions (February), such as high temperature and long days favored essential oil biosynthesis. This is explained by the increase in biomass associated to plant modulation of photosynthetic carbon production into the metabolic machinery of monoterpene biosynthesis (KHANUJA et al., 2000). Similar results have been reported at high altitude conditions in southern Brazil, where a 50% decrease in essential oil content among seven mint genotypes was observed when plants were harvest in winter compared to summer (DESCHAMPS et al., 2008).

Table 4. Essential oil content (%) and essential oil yield (L/ha) of mint genotypes at two harvests time

Mint genotypes	Essential oil content (%)		Essential oil yield (L/ha)			
	Feb	May	Feb	May	Average	Total
MC 3	2.8 ^{Abc}	1.2 ^{Bbc}	81.9 ^{Ab}	13.9 ^{Bbc}	47.6 ^{b}	95.1
MC 23	2.1 ^{Abcd}	1.3 ^{Bbc}	31.8 ^{Ade}	5.8 ^{Bc}	18.8 ^{c}	37.6
MC 34	2.5 ^{Abc}	1.4 ^{Bc}	51.5 ^{Acd}	12.0 ^{Bbc}	31.8 ^{bc}	63.5
MC 37	5.3 ^{Aa}	3.5 ^{Ba}	125.8 ^{Aa}	61.1 ^{Ba}	93.45 ^{a}	186.9
MC 43	2.9 ^{Abc}	2.2 ^{Bab}	67.4 ^{Abc}	11.4 ^{Bbc}	39.4 ^{bc}	78.8
MC 57	3.4 ^{Ab}	0.8 ^{Bc}	72.9 ^{Abc}	14.8 ^{Bbc}	43.8 ^{bc}	87.7
MC 69	1.6 ^{Acd}	0.9 ^{Bc}	27.6 ^{Ae}	8.1 ^{Bc}	17.8 ^{c}	35.7
MC 75	2.1 ^{Abc}	1.2 ^{Bc}	81.9 ^{Ab}	23.3 ^{Bbc}	52.6 ^{bc}	105.2
MC 76	2.2 ^{Abc}	1.5 ^{Bbc}	56.8 ^{Ac}	33.6 ^{Bb}	45.6 ^{b}	90.4
MC 78	2.2 ^{Abc}	1.4 ^{Bbc}	57.4 ^{Ac}	28.1 ^{Bbc}	42.8 ^{bc}	85.4
Average	2.7	1.5	65.4	21.2		

Essential oil content CV= 25.33%. Essential oil yield CV=23.13%. Means followed by the same capital letters on lines and the same lowercase letters on columns are not significantly different for Tukey test at the 0.05 level.

In the northern hemisphere, *M. spicata* also presented higher essential oil during the summer months from July to September (13). Study on photoperiodic influence in mint have correlated long days to flowering induction (SANGWAN et al., 2001). In fact, in February, all of the mint genotypes

were at bloom stage, while in May only the *M. arvensis* and *M. canadensis* had attained the stage of bud formation. Besides environmental conditions, senescence of older leaves in February due to flowering induced a reduction of leaf area, with greater oil gland density resulting in higher oil accumulation.

Mentha canadensis L. (MC 37), presented the highest essential oil content in both harvests (5.3 % in February and 3.5% in May). When studied in Curitiba, (940 m) Brazil, this genotype had lower oil content (2.8%) (MONTEIRO, 2009). The effect of the altitude correlated to higher UV radiation in secondary metabolites is described for *M. piperita*, increasing oil content from UV-A radiation during the day, while at night a decrease was observed, (MAFFEI et al., 2000) which may indicate that essential oil biosynthesis can be negatively affected by the altitude and consequent higher UV exposure. Two *M. canadensis* evaluated in Finland (AFLATUNI, 2005) achieved essential oil content ranging from 1.7 to 2.8% and two other *M. canadensis* studied in Brasilia, Brazil, presented 2.03 and 4.17% of oil content during the dry season (GRACINDO et al., 2006).

The Indian reports on oil and menthol yield for *M. arvensis* are greatly superior to the values obtained here. Srivastava et al. (2002) describe oil yields of 99.5 to 165 kg/ha and menthol yields of 72.5 to 101 kg/ha. However, their oil content levels ranged from 0.7 to 0.9 and menthol content from 66.7 to 76% which are lower than those found in this study. Therefore, the component responsible for the limitation of oil and menthol yield here was the herb yield, which was two to three times lower than in India.

Essential oil (Table 4) and menthol yield (Table 5) data were also influenced by genotype and harvest time. For the material harvested in February, both yields are increased. *M. canadensis* L. (MC 37) presented the highest oil and menthol yield values. The reports of Monteiro (2009) with the same cultivar were higher for oil (149.4 L/ha) and menthol (126.7 L/ha) yield, although oil content (2,7%) and menthol content (85.6%) were lower. This means that the difference was a consequence of a higher herb yield. In Finland, Cornmint and Sakhalin mint, both *M. canadensis* L, achieved oil yields ranging from 10 to 51 Kg/ha at three different harvest times. In this case, lower oil content and herb yield limited the values of oil yield (AFLATUNI, 2005). *Mentha* x *piperita* L. (MC 3) and *Mentha* sp. (MC 57) showed potential for essential oil production, however, their menthol yield was low.

Table 5. Menthol yield (L/ha) means of mint genotypes at two harvests time

Genotype	Feb	May	Average	Total
MC 3	32.8 ^{Acde}	9.3 ^{Bcd}	21.1 ^{cd}	42.1
MC 23	12.7 ^{Ag}	3.3 ^{Bd}	7.9 ^{e}	16.0
MC 34	24.5 ^{Aef}	5.6 ^{Bd}	15.1 ^{de}	30.1
MC 37	110.2 ^{Aa}	56.6 ^{Ba}	83.4 ^{a}	166.8
MC 43	28.7 ^{Ade}	5.5 ^{Bd}	17.1 ^{de}	34.2
MC 57	8.7 ^{Ag}	5.5 ^{Bd}	7.6 ^{e}	14.2
MC 69	13.9 ^{Afg}	4.2 ^{Bd}	9.1 ^{e}	18.1
MC 75	62.2 ^{Ab}	17.9 ^{Bbc}	46.1 ^{b}	80.1
MC 76	40.5 ^{Ac}	25.1 ^{Bb}	32.8 ^{b}	65.6
MC 78	38.8 ^{Acd}	20.1 ^{Bbc}	29.5 ^{bc}	58.9
Average	37.3 A	15.3 B		

Menthol yield CV= 19.89%. Means followed by the same capital letters on lines and the same lowercase letters on columns are not significantly different for Tukey test at the 0.05 level.

The main components identified in the oil are presented in Table 6. The highest menthol contents were obtained at the second harvest (May) for all genotypes, except *M. sylvestris* L., while its direct precursor, menthone and the isomer neomenthol were lower. In contrast, characteristics of essential oil in February can be described as "immature" with lower menthol and higher menthone levels (WILDUNG; CROTEAU, 2005)

The enzyme menthone-reductase is responsible for converting menthone into menthol (CROTEAU et al. 2005). This enzymatic reaction occurs during the late leaf development (15-55 days) while high menthone content is typical at the early stages of leaf development (RIOS – ESTEPA et al., 2008; WILDUNG; CROTEAU, 2005). This might explain the inverse relationship between menthol/menthone results since at the February harvest a significant senescence of the older leaves

occurred with concomitant flowering, reducing the ratio of old (mature) to young (immature) leaves (data not presented). The negative correlation of menthol content with menthone and neomenthol observed in February, has been reported as a result of increased photoperiodic influence of long day in *M. arvensis* (SANGWAN et al., 2001). Menthyl acetate is described as an undesirable compound because it promotes changes in organoleptic properties and oil oxidation (PAULUS et al., 2007). This compound is also used as a commercial indicator of an "over mature" oil (WILDUNG; CROTEAU, 2005). This second stage of oil maturation occurs at late bloom increasing the menthol and menthyl acetate levels as occurred during the the harvest of May (RIOS – ESTEPA et al., 2008; ROHLOFF et al., 2005). The higher menthyl acetate contents in May might suggest that an earlier harvest can decrease the level of mentyl acetate and improve oil quality. In the case of *Mentha* x *piperita* L. (MC 69) the higher levels of menthyl acetate seems due to a specific genotypic characteristic.

Table 6. Qualitative and quantitative composition of the essential oil from ten mint genotypes at two harvests

	February						
Genotype	Menthol	Mentone	Neomenthol	Menthyl Acetate	Pulegone	1.8 cineol	Limonene
MC* 3	40.4 d	35.9 b	9.0 ab	1.44 b	2.80 c	4.20 a	0.46 bcd
MC 23	39.7 d	32.9 bc	12.0 a	1.82 b	-	4.22 a	-
MC 34	47.6 d	26.5 c	8.9 ab	1.57 b	4.97 b	3.58 a	-
MC 37	89.6 a	2.6 e	3.0 c	-	-	-	0.51 abcd
MC 43	42.6 d	29.9 bc	10.9 ab	1.52 b	3.39 bc	3.80 a	0.50 abcd
MC 57	12.0 e	56.8 a	8.6 ab	1.75 b	14.01 a	3.64 a	0.11 d
MC 69	50.5 cd	13.0 d	8.4 bc	9.92 a	2.51 c	1.95 a	0.85 abc
MC 75	75.9 ab	10.4 de	6.4 bc	3.47 b	-	-	0.24 cd
MC 76	71.2 b	16.2 d	7.2 abc	2.35 b	-	-	0.63 abc
MC 78	67.6 bc	17.1 d	8.9 ab	3.07 b	-	-	0.94 ab
	May						
Genotype	Menthol	Mentone	Neomenthol	Menthyl Acetate	Pulegone	1.8 cineol	Limonene
MC* 3	66.93 bcd	15.36 b	5.57 b	1.95 e	0.30 c	3.28 a	1.15 a
MC 23	57.53 cde	14.80 bc	3.30 b	10.35 bc	-	1.20 a	-
MC 34	46.70 ef	10.73 bcd	6.03 ab	10.93 b	4.04 a	2.40 a	-
MC 37	92.69 a	2.20 e	2.95 b	-	-	-	0.31 bc
MC 43	48.44 ef	11.56 bcd	10.41 a	9.80 bc	3.59 a	2.69 a	0.92 ab
MC 57	37.33 f	26.00 a	4.57 b	4.70 de	5.84 a	2.34 a	0.09 c
MC 69	51.78 ef	3.63 de	5.89 ab	25.92 a	1.28 b	1.90 a	1.13 ab
MC 75	76.76 ab	4.98 de	6.86 ab	9.07 bcd	-	-	0.60 ab
MC 76	74.59 bc	6.69 cd	7.23 ab	5.76 cd	-	-	0.36 bc
MC 78	71.93 bc	6.30 cde	7.09 ab	9.50 bc	-	-	0.62 ab
CV%	13.88	20.95	25.81	15.43	13.60	18.39	24.75

Means followed by the letters are not significantly different for Tukey test at the 0.05 level.

Low contents of pulegone and 1,8-cineol were observed in the essential oil of *M. piperita* genotypes and these compounds were absent in *M. canadensis* and *M. arvensis* genotypes. Pulegone along with menthofuran (not present) are produced and accumulated according to the level of environmental stress (WILDUNG; CROTEAU, 2005). Therefore it is reasonable to presume that the *M. canadensis* and *M. arvensis* genotypes exhibited good adaptation to local environmental conditions.

Limonene, an earlier precursor of the menthol biosynthesis pathway appears in low levels (approximately 1%) probably due to the plants ontogenic stage at harvest time (RIOS – ESTEPA et al., 2008). Other constituents identified at amounts lower than 0.3% in oil analysis were: isomenthol, isopulegone, isomenthone, linalool, α-pinene, β-pinene, sabinene, mircene, piperitone, 3-octanol, (*E*)-caryophyllene, β-germacrene and bicyclogermacrene.

The genotype *M. canadensis* L. (MC 37) showed the highest menthol content at both harvests (89.6 and 92.7). The results were higher than those reported by Monteiro, (2009) with the same genotype (85.6%) in Curitiba, Brazil and also higher than the 18 genotypes of the mint collection studied in India by Shasany et al. (2010) when the highest menthol content was 83.8%. At Brasilia, Brazil

another *M. canadensis* L. (MC 20) was characterized with 65% of menthol (GRACINDO et al., 2006). These data express the potential of this *M. canadensis* L. genotype for menthol production under the local conditions.

The result of menthol content of the *M. arvensis* (MC 75, 76 and 78) genotypes was also high. These values are consistent with that reported by Srivastava et al. (2002) for three cultivars at ten locations, varying from 66.7% to 76%, of menthol content but were lower than that described by Anwar et al.(2010) ranging from 77.6. to 82.4 on six cultivars, in India.

Gracindo et al. (2006) also studied the oil composition of *Mentha* x *piperita* L. (MC 3 and MC 23) in Brasilia, and found that the levels of menthol varied from 38.0 to 43.0%, menthone from 11.4 to 20.6% and neomenthol from 1.9 to 4.7%, respectively. In contrast, they also detected 16.2% of carvone in MC 3 and 1.0% of limonene in MC 23, which were not detected in this work.

CONCLUSIONS

From the results it can be stated that the environmental conditions in Southern Brazil are viable for menthol production, with two harvests. The summer harvest (February) favored oil yield, although with a slight decrease in menthol content. *M. canadensis* L. (MC 37) can be recommended for local menthol production. The challenge of achieving higher essential oil and menthol yields depends on strategies to increase herb yield by developing innovative agronomic practices.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. Roberto F. Vieira, from Embrapa Genetic Resources and Biotechnology, for providing the plant material for the experiment.

RESUMO: O mentol, constituinte majoritário do óleo essencial de menta é usado nas indústrias farmacêutica, alimentícia e de aromas. Onze genótipos de *Mentha s*p. foram estudados em relação ao desenvolvimento vegetativo, rendimento, produtividade e composição de óleo essencial nas condições edafoclimáticas do litoral Norte Catarinense, em duas épocas (fevereiro e maio). O trabalho foi conduzido em delineamento experimental de blocos ao acaso, em esquema fatorial 10 x 2 para genótipos e épocas de colheita. A extração do óleo essencial foi realizada por hidrodestilação em aparelho graduado Clevenger. O teor de óleo essencial variou entre 0,8 e 5,3%, sendo maior em fevereiro para todos os genótipos. Os constituintes majoritários identificados foram mentol (12 – 92,7%), mentona (2,2 – 56,9%), e neomentol (2,9 – 12,1). Os maiores teores de mentol foram observados em maio, apresentando correlação negativa com mentona e neomentol, os quais foram superiores em fevereiro. Os teores de acetato de metila apresentaram correlação positiva com os de mentol. Pulegona, 1,8 cineol, e limoneno foram identificados em menores concentrações e outros treze constituintes foram detectados como elementos traço em alguns genótipos. *Mentha canadensis* L. apresentou os maiores teores de óleo essencial, (5,3 % - Fevereiro e 3,5% - Maio) e mentol (89,6% - Fevereiro, 92,7% - Maio) em ambas as colheitas. Os resultados obtidos permitem concluir que as condições edafoclimáticas do litoral Norte Catarinense são adequadas para a produção de mentol, com duas colheitas, recomendando-se o genótipo *M. canadensis* L. A colheita de verão (fevereiro) favorece a produtividade de óleo essencial reduzindo o teor de mentol. O desafio para aumentar a produtividade de óleo essencial e de mentol depende de estratégias que aumentem a produção de biomassa através do desenvolvimento de práticas agronômicas inovativas.

PALAVRAS - CHAVE: Lamiaceae. Hortelã. Recursos genéticos. Biomassa. Mentol. Mentona.

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