Original Article

DEVELOPMENT, ESSENTIAL OIL YIELD AND COMPOSITION OF MINT SPECIES AND CHEMOTYPES UNDER DIFFERENT RADIATION AND NITROGEN LEVELS

DESENVOLVIMENTO, PRODUÇÃO E COMPOSIÇÃO DO ÓLEO ESSENCIAL DE ESPÉCIES E QUIMIOTIPOS DE MENTA SOB DIFERENTES NÍVEIS DE RADIAÇÃO E NITROGÊNIO

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ABSTRACT: Radiation and soil nutrient levels affect essential oil production in aromatic plants. The objective of this work was to evaluate the vegetative development, essential oil yield and composition of *Mentha aquatica* L. (linalool chemotype) and *Mentha* x *piperita* L. (linalool and menthol chemotypes) cultivated under different radiation levels (100%, 46% and 23%), and with or without nitrogen fertilization. The essential oil samples were obtained from leaves by 3 hours hydro-distillation and analyzed by GC/MS. Reduced leaf area, stem number and total dry mass accumulation was found in all genetic materials submitted to the lowest level of radiation. Nitrogen requirements were different in mint chemotypes, being *M.* x *piperita*, linalool chemotype, the only genetic material where nitrogen fertilization resulted in higher stem number and total dry mass under full radiation. Although reduction in radiation levels decreased essential oil yield and the percentage of its major constituents menthol, menthone, linalool and linalil acetate, no correlation between essential oil production and plant development was observed.

KEYWORDS: *Mentha aquatica*. *Mentha* x *piperita*. Menthol. Linalool

INTRODUCTION

Mint essential oils are of economic importance around the world especially because of the extensive use of terpene menthol in the food, pharmaceutical and perfumery industries (FAROOQI; SANGWAN; SANGWAN, 1999). Another terpene of economic interest is linalool, which is also produced by the *Mentha* species. Such monoterpenes (C_{10}), and many other in the Lamiaceae family, are synthesized by the mevalonic acid pathway (TURNER; CROTEAU, 2004) and constitute the major components of plant derived essential oils (TURNER; CROTEAU, 2004; TAIZ; ZEIGER, 1998).

Essential oil synthesis and storage in mint species occur in glandular peltate trichomes which are predominantly distributed on the leaf abaxial surface, and in lower density on the adaxial surface (TURNER; GERSHENZON; CROTEAU, 2000a,b; DESCHAMPS et al., 2006b). It has also been demonstrated that essential oil production in glandular trichomes of mint (MCCONKEY; GERSHENZON; CROTEAU, 2000) and basil (DESCHAMPS et al., 2006a) is regulated by plant vegetative growth stage.

Environmental conditions such as nutrition and radiation result in differentiated essential oil yield and composition in aromatic plants (SANGWAN et al., 2001).

Nitrogen is extremely important for plant metabolism, principally for protein and nucleic acid biosynthesis. Nitrogen fertilization has been shown not only to improve vegetative growth and leaf development, but also to alter both yield and composition of mint (SAXENA; SINGH, 1998) and basil (SIFOLA; BARBIERI, 2006) essential oils.

The influence of different radiation conditions on essential oil composition of *Mentha* species has also been demonstrated, where a significant increase in menthol yield was observed at high photoperiodic exposure (FAHLEN; WELANDER; WENNERSTEN, 1997) and no UV-B radiation (MAFFEI; SCANNERINI, 2000).

In this work, we investigated the influence of radiation and nitrogen levels on plant development and on essential oil yield and composition of *Mentha aquatica*, linalool

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chemotype, and *Mentha* x *piperita*, menthol and linalool chemotypes.

MATERIAL AND METHODS

Plant material

Mentha aquatica, whose major constituent is linalool, and *Mentha* x *piperita*, whose major constituents are linalool and menthol, were obtained from the "Genetics Resources and Biotechnology National Center" (CENARGEN), EMBRAPA, Brazil, where the plant vouchers are deposited.

Experimental design and growth conditions

Greenhouse cultivated 5-7 cm cuttings were selected and transplanted to vases containing soil chemically analyzed by the Soil Fertility Laboratory – Federal University of Parana (Table 1). As recommended by Raij et al. (1996), the soil pH was corrected by incorporating 6.2 kg ha^{-1} of limestone (100% PRNT) to achieve 70% of base saturation, and 40 kg ha^{-1} of P_2O_5 and 23 kg ha^{-1} of K_2O. A completely randomized design with six treatments and three replications (3 pots with 2 plants each) was used. Plants developed under different radiation levels (100%, 46% and 23%) and with the presence or absence of nitrogen fertilization. Treatments with nitrogen fertilization received two applications of 20 kg ha^{-1} of the element at planting and 23 days after. For the radiation treatments, screens which allowed 46% and 23% luminosity to pass through were used. A LI-COR 1600 porometer (LICOR Incorp., USA) was used in both sunny and cloudy days to monitor the radiation levels (Table 2). Plant development was evaluated 60 days after planting, leaf area being determined by the leaf discs method (RADLEY, 1963) and total dry mass after drying stems and leaves in an oven at 65°C until constant weight.

Table1. Chemical characteristics of the soil used in the greenhouse experiment.

pH	Al^{3+}	H + Al	Ca^{2+}	Mg^{2+}	K^+	SB	T	P	C	V	m	Ca/Mg
$CaCl_2$	$Cmol_c\ dm^{-3}$							$mg\ dm^{-3}$		%	%	
4,20	1,90	10,50	2,10	1,40	0,34	3,84	14,34	19,60	31,7	27	33	1,5

Table 2. Radiation levels in greenhouse conditions on sunny and cloudy days.

	Sunny days	Cloudy days
Growth conditions	Radiation level (quantum µmol s^{-1} m^{-2})	Radiation level (quantum µmol s^{-1} m^{-2})
Greenhouse	100% (869)	100% (144)
Greenhouse+Screens 46%	46,29% (400)	47,91% (69)
Greenhouse+Screens 23%	23,01% (202)	26,38% (38)

Essential oil isolation and volatile oil analysis

A total of 100 g of fresh leaves were used for hydrodistillation with a Clevenger type apparatus for three hours to determine the essential oil yield. The volatile oils were analyzed by gas chromatography coupled with mass spectrometry (Varian Inc., CP-3800 series, mass selective detector Saturn 2000 MS/MS). The inlet temperature was 200°C, while the column programmed temperature was 120°C min^{-1}, 10°C min^{-1}, and 223°C/20 min. The helium flow rate was at 49.5 psi. Individual compound identifications were made by matching spectra with those from a mass spectral library (Nist 98, Varian Inc.).

Statistical analysis

Analysis of variance and the Tukey's test ($P<0.05$) of mean comparison procedures were performed using MSTAT-C program (NISSEN, 1993).

RESULTS AND DISCUSSION

Vegetative development *of M. aquatica* and *M.* x *piperita*

The *Mentha* chemotypes responded differently to the radiation and nitrogen treatments. Even though a reduction of plant development was observed in all genetic materials exposed to the lowest radiation level, it was more intense in *M* x *piperita*, linalool chemotype, where plants exposed to 46% of radiation presented a decrease of aproximately 85% of total dry mass and 75% of leaf area (Tables 3 and 4). Total dry mass and stem number in this genetic material increased up to 60% with full radiation and nitrogen fertilization. A higher stem and leaves number and glandular trichomes density was also observed in *Salvia officinalis* and *Thymus vulgaris* plants developed under full radiation (LI; CRAKER; POTTER, 1996). The other two chemotypes, however, required no nitrogen fertilization once full radiation was provided. The nitrogen fertilization recommendation in which this experiment was based considered higher exigency levels among mint genetic material. The lack of significant results in terms of plant development in the nitrogen applied treatments may be due to the level of organic matter in the soil which supplied the plant exigences (Table 1). Ram et al. (2006) found increase in growth and essential oil yield of *M. arvensis*, menthol chemotype, after nitrogen fertilization. Leaf area of all evaluated genetic materials of this experiment was affected only by radiation levels and not by nitrogen fertilization.

Table 3. Leaf area (cm^2 $plant^{-1}$) of *Mentha* species under different radiation levels.

Species/chemotype	Radiation level (%)		
	100	46	23
M. aquatica / Linalool	796,33 Aa	681,35 Aa	225,74 Ba
M. x *piperita* / Linalool	711,74 Aa	180,78 Bb	208,81 Ba
M. x *piperita* / Menthol	803,42 Aa	803,98 Aa	389,45 Ba

* Means followed by the same capital letter in the rows and small letter within the columns are not significantly different by Tukey's test at P<0.05.

Table 4. Stem number, total dry mass (stems and leaves) of *Mentha* species with and without nitrogen fertilization under different radiation levels.

Species / chemotype	Radiation level (%)		
	100	46	23
M. aquatica / Linalool			
Stem number			
With nitrogen fertilization	13,83 Aa	15,22 Aa	13,78 Aa
Without nitrogen fertilization	15,11 Aa	9,88 Bb	12,11 Aa
Total dry mass			
With nitrogen fertilization	14,09 Aa	10,36 Aa	4,35 Ba
Without nitrogen fertilization	12,76 Aa	10,39 Aa	5,13 Ba
M. x *piperita* / Linalool			
Stem number			
With nitrogen fertilization	7,44 Aa	2,83 Ba	1,05 Ba
Without nitrogen fertilization	2,88 Ab	1,77 Aa	0,50 Aa
Total dry mass			
With nitrogen fertilization	20,57 Aa	3,00 Ba	1,46 Ba
Without nitrogen fertilization	8,53 Ab	4,83 ABa	1,82 Ba
M. x *piperita* / Menthol			
Stem number			
With nitrogen fertilization	15,11 Aa	18,22 Aa	15,44 Aa
Without nitrogen fertilization	16,66 Aa	15,27 ABa	11,66 Bb
Total dry mass			
With nitrogen fertilization	14,81 Aa	10,04 Ba	4,43 Ca
Without nitrogen fertilization	13,56 Aa	11,86 Aa	5,69 Ba

* Means followed by the same capital letter in the rows and small letter within the columns are not significantly different by Tukey's test at P<0.05.

Essential oil yield and composition of *Mentha aquatica* and *Mentha* x *piperita*

Similarly to obtained by Ram et al. (2006), when working with crescent nitrogen doses in *Mentha arvensis*, and Kiran and Patra (2003a,b) in *Mentha spicata*, the results obtained herein showed an increase in essential oil yield (63%) on *M. aquatica* leaves, chemotype linalool, developed with nitrogen supply under full radiation (Table 5). The lack of correlation between essential oil yield and vegetative development of this chemotype, which was not affected by nitrogen fertilization under full radiation, may be related to the glandular trichome density and/or rate of biosynthesis of specific terpene enzymes that were higher in presence of the nutrient. Sifola and Barbieri (2006) also obtained significant increase in essential oil yield when applying 100 and 230 kg ha^{-1} of nitrogen in basil plants. When radiation was limited to 46%, even with nitrogen, essential oil yield was reduced by 32%. The essential oil yield of *M.* x *piperita*, chemotype linalool, was higher on plants developed at full radiation but without nitrogen. In this case, a negative correlation was observed with plant development that was significatily higher in the presence of the nutrinet. Essential oils originate from different primary metabolic precursors (SANGWAN; FAROOQI; SHABIH, 2001), so the response observed in this chemotype is probably a result of increase in carbon flux to primary metabolism for plant growth to the detriment to secondary metabolite production. Nitrogen fertilization seems to compensate for the lower radiation level on this chemotype, as higher essential oil yield was obtained from nitrogen fertilized plants under 46% of radiation. Comparing the total dry mass accumulation and the essential oil yield of both linalool chemotypes, nitrogen fertilization did not interfere in *M. aquatica* development, only in essential oil yield, differently from *M.* x *piperita* where the higher essential oil yield was found on plants with lower total dry mass accumulation.

Table 5. Essential oil yield (µl.gr^{-1} of dry mass) of *Mentha* species under different radiation and nitrogen levels.

Species / chemotype	Radiation level (%)		
	100	46	23
M. aquatica / Linalool			
With nitrogen fertilization	3,99 Aa	1,27Ba	0,56Ca
Without nitrogen fertilization	1,46 Ab	0,93Ba	0,57Ca
M. x *piperita* / Linalool			
With nitrogen fertilization	2,76 Bb	3,79 Aa	-
Without nitrogen fertilization	3,64 Aa	2,09 Bb	-
M. x *piperita* / Menthol			
With nitrogen fertilization	2,63 Aa	2,21 Ba	1,42 Ca
Without nitrogen fertilization	2,50 Aa	1,93 Ba	1,56 Ca

* Means followed by the same capital letter in the rows and small letter within the columns are not significantly different by Tukey's test at P<0.05.

The results under radiation treatments herein were similar to those from Li; Craker and Potter (1996) comparing radiation levels (45, 27 e 15%) with full radiation on the development and essential oil yield of *Salvia officinalis* and *Thymus vulgaris.* The authors observed reduction of glandular trichomes density and essential oil production under limited radiation, but it was not related to the plants growth since plants of same size presented differences in composition and essential oil yield. Total dry mass accumulation of *M.* x *piperita*, menthol chemotype, did not differ in nitrogen treatment at full radiation, and only when plants were developed under limited radiation was nitrogen required. However, the essential oil yield was affected by a decrease in radiation levels and not by nitrogen. Furthrmore, nitrogen application did not affect the essential oil yield in *M. citrata* (MAY et al., 2010). On the other hand, in *M.* x *piperita* var. *piperita* the light intensity and the addition of fertilizer influenced the oil quality, showing the plants under full sunlight and fertilization with higher menthol content (PEGORARO et al., 2010).

M. aquatica, linalool chemotype, under 100% radiation, presented higher concentrations of the major components of the essential oil independently of nitrogen fertilization (Table 6). Even though *M* x *piperita*, linalool chemotype, was more affected by limited radiation and no nitrogen fertilization during vegetative growth, it did not present significant differences in essential oil composition under different environmental conditions. The *M* x *piperita*, menthol chemotype, presented reduction in menthol and menthone when radiation was reduced to 46%, independently of nitrogen. According to Sangwan et al. (2001), even though essential oil composition is genetically determined, environmental conditions may induce significant variations in essential oil yield and composition.

The obtained results show that development of mint chemotypes is reduced when plants are exposed to limited radiation levels. The reduction was higher in *M.* x *piperita*, linalool chemotype, indicating that in future studies on plant spacing allowing less radiation, competition would increase the production of this genetic material under field conditions. However, the observed lack of correlation between total dry mass accumulation and essential oil yield indicates that plant development characteristics alone do not determine the potential of mint genotypes and that other characteristics, such as leaf age and/or trichome density, may be more important.

Table 6. Essential oil constituents (%) of *Mentha* species under different radiation and nitrogen levels.

Species	Essential oil constituent (%)	Radiation level (%)					
		100	46	23	100	46	23
		With nitrogen			Without nitrogen		
M. aquatica	Linalool	54,84	54,76	19,60	56,23	29,82	23,51
	Linalil acetate	16,75	13,36	0,00	14,69	8,18	5,78
	Total	71,59	68,12	19,60	46,99	38,00	36,29
M. x *piperita*	Linalool	59,06	61,64	-	56,23	63,48	-
	Linalil acetate	18,84	21,23	-	15,84	16,50	-
	Total	77,90	82,94	-	72,07	79,98	-
M. x *piperita*	Menthol	35,18	25,77	40,61	33,86	22,76	29,63
	Menthone	23,36	19,65	1,43	38,39	31,15	28,04
	Total	58,54	45,42	42,04	72,25	53,91	57,67

- Not enough plant material for essential oil extraction.

Nitrogen fertilization under experimental conditions directly interfered in essential oil yield when full radiation was provided. Thus, nitrogen fertilized plants, although not showing any difference in plant development, presented higher essential oil production. Exception to *M.* x *piperita*, linalool chemotype, where the increase in plant development after nitrogen fertilization may have imposed a limited amount of primary precursors for essential oil production. Deschamps et al. (2012), found that nitrogen reduced the concentration of essential oil of *M.* x *piperita* when used at high doses, and concluded that nitrogen sources and doses affect essential oil yield and composition of *M.* x *piperita*.

The percentage of menthol and linalool in the essential oil samples were affected by radiation levels and not by nitrogen fertilization. Although many previous studies showed the effect of nitrogen on essential oil composition of mint species, including changes in menthol levels, the results presented showed that nitrogen exigences differ according to the mint chemotypes. High radiation levels under field conditions should be ensured to increase essential oil production and maintain menthol, menthone, linalool and linalil acetate levels.

CONCLUSIONS

The radiation level interferes in development, composition and essential oil yield of mint chemotypes.

Nitrogen exigences differ among the evaluated genetic materials, indicating that once full radiation is provided, optimal plant growth can be achieved at lower nitogen levels in the soil.

Specific plant spacing evaluation at field conditions of mint chemotypes can ensure high levels of essential oil production.

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RESUMO: Radiação e os níveis de nutrientes no solo afetam a produção de óleo essencial em plantas aromáticas. O objetivo deste trabalho foi avaliar o desenvolvimento vegetativo, rendimento e composição de óleo essencial de *Mentha aquatica* L. (quimiotipo linalol) e *Mentha* x *piperita* L. (quimiotipos linalol e mentol), cultivado sob diferentes níveis de radiação (100%, 46% e 23%), e com ou sem adubação nitrogenada. O óleo essencial foi obtido a partir de folhas por hidrodestilação durante três horas, e analisado por GC/MS. Observou-se redução da área foliar, número de hastes e massa seca total em todos os materiais genéticos submetidos ao menor nível de radiação. Os quimiotipos de menta apresentaram diferentes necessidades de nitrogênio, sendo M. x piperita, quimiotipo linalol, o único material genético onde a adubação nitrogenada resultou em maior número de haste e massa seca total, em plena radiação. Embora a redução nos

níveis de radiação tenha reduzido o rendimento de óleo essencial e o percentual de seus principais constituintes, mentol, mentona, linalol e acetato de linalila, nenhuma correlação entre a produção de óleo essencial e desenvolvimento da planta foi observada.

PALAVRAS-CHAVE: *Mentha aquatica*. *Mentha* x *piperita*. Mentol. Linalol

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