Development and essential oil yield and composition of mint chemotypes under nitrogen fertilization and radiation levels

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Abstract
Nitrogen fertilization and radiation levels affect essential oil production in aromatic plants. The objective of this study was to evaluate the vegetative development and the 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 of hydro-distillation and analyzed by GC/MS. Reduced leaf area, stem number, and total dry mass accumulation were found in all genetic materials subjected to the lowest level of radiation. Although reduction in radiation levels decreased essential oil yield and the percentage of its major constituents, namely, menthol, menthone, linalool, and linalil acetate, no correlation between essential oil production and plant development was observed. Nitrogen fertilization had different effects on mint chemotypes, with *M.* x *piperita*, linalool chemotype, being the only genetic material where nitrogen fertilization resulted in higher total dry mass under full radiation.

Key Words
Mentha aquatica, *Mentha* x *piperita*, menthol, linalool.

Introduction

The essential oils derived from mint are economically important, in particular due to the extensive use of the terpene menthol in the food, pharmaceutical, and perfumery industries (Farooqi *et al.* 1999). Another terpene of economic interest is linalool, which is also produced by *Mentha* species. Environmental conditions such as nutrition and radiation result in differentiated essential oil yield and composition in aromatic plants (Sangwan *et al.* 2001). Nitrogen fertilization has been shown not only to improve vegetative growth, but also to alter the essential oil yield and composition of mint (Saxena and Singh 1998). The radiation effect on essential oil composition of *Mentha* species has also been demonstrated (Fahlen *et al.* 1997). In this study, we investigated the influence of radiation and nitrogen levels on plant development and essential oil yield and composition of *Mentha aquatica*, linalool chemotype, and *Mentha* x *piperita*, menthol and linalool chemotypes.

Methods

Plant material

Mentha aquatica, linalool chemotype, and *Mentha* x *piperita*, linalool and menthol chemotypes 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, samples of which were chemically analyzed at the Soil Fertility Laboratory of UFPR (Table 1).
As recommended by Raij *et al.* (1996), the soil pH was corrected by incorporating 6.2 Mg/ha of limestone (100% PRNT), to achieve 70% of base saturation, and 40 kg/ha of P_2O_5 and 23 kg/ha of K_2O. A completely randomized design with six treatments and three replications (3 pots with 2 plants each) was used. Plants were developed under different radiation levels (100%, 46%, and 23%) and in the presence or absence of nitrogen fertilization. Treatments with nitrogen fertilization received two applications of 20 kg/ha of the element during planting and 23 days after planting. Plant development was evaluated 60 days after planting. Total dry mass was determined after stems and leaves were dried in an oven at 65^0C until constant weight.

Isolation of essential oils and analysis of volatile constituents
Samples with 100 g of fresh leaves were hydrodistilled for three hours with a Clevenger-type apparatus to determine the essential oil yield. The volatile oils were analyzed using gas chromatography coupled to mass spectrometry (Varian Inc., CP-3800 series, mass selective detector Saturn 2000 MS/MS). 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

Vegetative development
The mint chemotypes showed varying responses to the radiation and nitrogen treatments (Table 1). 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, wherein plants exposed to 46% of radiation presented a decrease of aproximately 85% of total dry mass. A higher development was also observed in *Salvia officinalis* and *Thymus vulgaris* developed under full radiation (Li *et al.* 1996). The other two chemotypes required no nitrogen fertilization once full radiation was provided. The lack of significant results in terms of plant development in the nitrogen application treatments may be due to the level of organic matter in the soil which supplied the plant's exigencies.

Table 1. 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			
With nitrogen fertilization	14,09 Aa	10,36 Aa	4,35 Ba
Without tnitrogen fertilization	12,76 Aa	10,39 Aa	5,13 Ba
M. x *piperita* / Linalool			
With nitrogen fertilization			
Without nitrogen fertilization	20,57 Aa	3,00 Ba	1,46 Ba
M. x *piperita* / Menthol	8,53 Ab	4,83 ABa	1,82 Ba
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.
An increase in essential oil yield in *M. aquatica* leaves, chemotype linalool, developed with nitrogen supply under full radiation was observed (Table 2). When radiation was limited to 46%, even with nitrogen, essential oil yield was reduced. The essential oil yield of *M.* x *piperita*, chemotype linalool, was higher in plants developed at full radiation but without nitrogen.

Table 2. Essential oil yield ($\mu l.gr^{-1}$ 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 essential oil composition of *M. aquatica* was affected by decrease of radiation levels and not by decrease of nitrogen. *M. aquatica*, linalool chemotype, under 100% radiation, presented higher concentrations of the major components of the essential oil, independently of nitrogen fertilization (Table 3). *M* x *piperita*, linalool chemotype, 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.

Table 3. Essential oil constituents (%) of *Mentha* species under different radiation and nitrogen levels.

Species	Essential oil constituent*	Radiation level (%)					
		With nitrogen			Without nitrogen		
		100	46	23	100	46	23
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 isolation.
* percentage.

Conclusions

The radiation levels interfere with development, essential oil yield, and composition of mint chemotypes. Nitrogen exigencies differ among the evaluated genetic materials, indicating that once full radiation is provided, optimal plant growth can be achieved with lower nitrogen levels in the soil. Specific plant-spacing evaluation under field conditions of mint chemotypes can ensure high levels of essential oil production and quality.

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References

Fahlen A, Welander M, Wennersten R. (1997) Effects of light-temperature regimes on plant growth and essential oil yield of selected aromatic plants. *Journal of Science of Food and Agriculture* **73**, 111-119.

Farooqi AHA, Sangwan NS, Sangwan RS (1999) Effect of different photoperiodic regimes on growth, flowering and essential oil in *Mentha* species. *Plant Growth Regulation* **29**, 181-187.

Li Y, Craker LE, Potter T (1996) Effect of light level on essential oil production of sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*). *Acta Horticulture* **426**, 419-426.

Nissen O (1993) 'MSTAT-C A microcomputer for design, management, and analysis of agronomic research experiments'. Version 2.11 (East Lansing: Michigan State University).

Raij B, Cantarella H, Quaggio JA, Furlani AMC (1996) Fertilization Guideline for São Paulo State. (Campinas: Instituto Agronômico e Fundação:Brazil) (in Brazil)

Saxena A, Singh JN (1998) Effect of irrigation, mulch and nitrogen on yield and composition of Japanese mint (*Mentha arvensis*) oil. *Indian Journal of Agronom* **43**, 179–182.

Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS (2001) Regulation of essential oil production in plants. *Plant Growth Regulation* **34**, 3-21.