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# Production and chemical composition of *Mentha* x *piperita* var. *citrata* (Ehrh.) Briq. essential oil regarding to different potassium concentrations in the hydroponic solution

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## ABSTRACT

*This work aimed to evaluate the production of fresh and dry mass of leaves, stems and aerial parts, and the content and quality of lemon mint (Mentha x piperita var. citrata) essential oil as a result of four potassium (K) concentrations (276, 414, 552 and 690 mg.L$^{-1}$) under hydroponic solutions. The experiment was carried out in the hydroponic NFT (Nutrient Film Technique) system. Leaves were separated and weighted to determine the fresh mass and part of them was used to extract oil in a Clevenger apparatus. The analysis of the oil chemical composition was performed in a gas chromatograph fitted with a mass spectrometer. The estimated concentration for the maximum fresh mass production of the leaves corresponded to 384 mg.L$^{-1}$ K. The greatest K concentration proportionated an increase in essential oil content and yield per plant, but decreased linalool and linalyl acetate in the oil. Under the conditions the experiment was carried out, in order to obtain an adequate quantity of leaves for a higher essential oil yield per plant and linalool and linalyl acetate accumulus, the K concentration of 414 mg.L$^{-1}$ is recommended in the hydroponic solution for the cultivation of lemon mint.*

**Key-words:** *Mint, Lamiaceae, hydroponic cultivation, linalool, linalyl acetate, medicinal plant.*

# Produção e composição química do óleo essencial de *Mentha* x *piperita* var. *citrata* (Ehrh.) Briq. em relação as concentrações de potássio na solução hidropônica

## RESUMO

Este trabalho objetivou avaliar a produção de massa fresca e seca de folhas, hastes e parte aérea, o teor e a qualidade do óleo essencial de hortelã-limão (*Mentha* x *piperita* var. *citrata*), em função de quatro concentrações de potássio (276, 414, 552 e 690 mg.L$^{-1}$) em soluções hidropônicas. O experimento foi conduzido no sistema hidropônico NFT (Nutrient Film Technique). As folhas foram amostradas e pesadas para determinação da massa fresca e uma alíquota utilizada para extração do óleo, em Clevenger. A análise da constituição química do óleo foi realizada em cromatógrafo gasoso acoplado a espectrômetro de massa. A concentração estimada para máxima produção de massa fresca de folhas correspondeu a 384 mg.L$^{-1}$ de K. A maior concentração de K estudada proporcionou aumento no teor e no rendimento de óleo essencial por planta, porém diminuiu a quantidade de linalol e acetato de linalila presentes no óleo. Nas condições em que o experimento foi conduzido, para obtenção de rendimento de folhas adequado ao maior rendimento de óleo essencial por planta e ao acúmulo de linalol e acetato de linalila, recomenda-se a concentração de 414 mg.L$^{-1}$ de K na solução hidropônica para o cultivo de hortelã-limão.

**Palavras-chave:** *Hortelã, Lamiaceae, cultivo hidropônico, linalol, acetato de linalila, planta medicinal.*

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## INTRODUCTION

The genus *Mentha* (Lamiaceae) includes 25 species and some hybrids with essential oils rich in monoterpenes, which are accumulated in glandular trichomas, especially in leaves and flowers. To this morphologic variability corresponds a wide chemical diversity which is reflected in a varied number of essential oils commercially obtained (Kokkini 1992).

*Mentha* x *piperita* var. *citrata* (Ehrh.) Briq., known as lemon mint or bergamot mint, is erroneously nominated *Mentha citrata* (Harley and Brigthon 1977). This species produces a type of essential oil which contains 84-90% linalool and linalyl acetate, characteristic acyclic components, in contrast to other species of the genus *Mentha* which contain menthol, menthone, carvone, limonene and pulegone as majoritary cyclic components (Murray and Lincoln 1970). Commercially, *Mentha* essential oils and their constituents are widely applied in the food, cosmetics, fragances, tobacco and medicine industries. Essential oils world production is estimated to be from 110,000 to 120,000 t/year (Kothari 2005).

From this amount, 22,000 t come from *Mentha* species, and 20,000 for the obtainment of oils rich in menthol and menthone, 2,000 for oil rich in carvone and 200 for oil rich in linalool and linalyl acetate (Sant Sanganeria 2005). Linalool is one of the monoterpenes more often used in perfumes and it is estimated that it is present in 60-90% of the cosmetics available in the market (Cal and Kryzyzaniak 2006). High contents of linalool and linalyl acetate in the essential oil are important under the economical point of view. These scents take part in the composition of cosmetic products, such as facial creams, body lotions, fragrance in creams, deodorants, perfumes, shampoos, bath products, gels, soaps and hair sprays. They may also be used in non-cosmetic products such as detergents and cleaning products (Letizia et al. 2003a; Leticia et al. 2003b) and in medicines.

As it happens for other economically important characteristics, mineral nutrients are also fundamental for plant growth and essential oil production and may be provided to soil cultivated plants as well as to hydroponic cultivated ones. The water enriched with nutrients of the hydroponic solutions, combined to the controlled environment of the greenhouses, allows a faster growth in relation to soil cultivation, shortening the productive cycle and increasing productivity (Santos 2000). Potassium (K) is among the essential nutrients to the plants and, in spite of being abundant in the tissues, does not make part of any organic component. Potassium interferes in several physiological processes like enzymatic activation, opening and closing of stomata, photosynthesis, cellular extension, translocation of photosynthates to growing regions, resistance to diseases and better efficiency in the use of water (Marschner 1995).

The wide utilization of essential oils and their constituents have motivated some agronomic studies of *Mentha* species. In hydroponic cultivation of *Mentha arvensis*, Paulus et al. (2004) obtained an oil content of 0.60% in nutritive solution containing 299 $mg.L^{-1}$ K, while Maia et al. (2001) obtained the best content (1.45%) in a solution with 468 $mg.L^{-1}$ K, demonstrating that the nutritive solutions for the species should be more concentrated in K. Garlet et al. (2007a) and Garlet et al. (2007b), working with *M.* x *gracilis* concluded that the increase in K concentration in the hydroponic solutions negatively affected the growth and phytomass accumulation in *Mentha* plants, but proportionated increase in essential oil production per plant. The authors suggest concentrations of 276 and 414 $mg.L^{-1}$ K to favor plant growth and oil content in the studied species.

This research aimed to evaluate the production of fresh and dry mass, the content and quality of *Mentha* x *piperita* var. *citrata* (Ehrh.) Briq. essential oil testing four potassium concentrations in hydroponic solutions.

## MATERIAL AND METHODS

### Plant material and growing conditions for the experiment

Lemon mint (*Mentha* x *piperita* var. *citrata* (Ehrh.) Briq.) was used in this study. The experiment was carried out at the experimental area of the Phytotechny Department of the Universidade Federal de Santa Maria (UFSM), Rio Grande do Sul State, Brazil, using the nutrient film technique (NFT). Plants were grown in a 250 $m^2$ greenhouse, using a polyvinyl chloride plastic (PVC) 200 µm thickness to cover the greenhouse and to close the laterals and doors.

Lemon mint plantlets were produced from matrixes cultivated in soil (Garlet et al. 2007a). Exsiccate of the species is found deposited at the Herbarium of the University of Cruz Alta, RS (UNICRUZ), under number 1079, after determination performed by Dr. Ray Harley, of the Royal Botanic Gardens, Kew, England. A complete randomized block design with five plots was used, using three plants per plot. Four K concentrations (276, 414, 552 and 690 $mg.L^{-1}$) in the hydroponic solutions were studied, and were calculated based on previous studies with *Mentha arvensis* L. by Paulus et al. (2004), and the cultivation methodology described by Garlet et al. (2007b).

**Harvesting of lemon mint**

Even though it is recommended that the harvesting of *Mentha* species should be done at the beginning of flowering in order to obtain higher amounts of the essential oil content (Duriyaprapan et al. 1986) that is concentrated in the glandular trichomas of leaves and floral calyxes (Lawrence 1992). The harvesting of lemon mint had to be performed 56 days after transplanting, on December, before flowering, because the intense vegetative growth caused root interlacement which occupied the greater part of the cultivation channels volume of the NFT system, each plant reaching 116 cm height and total aerial fresh matter of 700 g. The collected plants were separated in roots and aerial part (i.e., stems, leaves and flowers). The flowers were added to the leaves due to the presence of oil. Roots were not evaluated because root interlacement did not allow correct separation of plants. Plants were then placed in paper bags with forced air ventilation at 65$^{o}$C until constant mass be reached, being weighted soon after.

**Oil composition and chemical content determination**

For oil composition and chemical content determination, four replicates of 100 g of leaves samples were hydro-distilled in Clevenger apparatus (Simões and Spitzer 2003) for a 2-hour period. The oil obtained was separated of the water and dried with anhydrous sodium sulphate ($Na_2SO_4$), being then weighted for the determination of estimated yield and content per plant.

Essential oil chemical composition analysis were performed using a gas chromatograph fitted to a mass spectrophotometer (CG-EM Shimadzu, QP-5000), at the Pharmacy College of the Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul State, Brazil. A DB-5 molten capillary column (25 m long x 0.25 mm internal diameter and 0.25 µm film thickness) was used; helium as carrier gas, 1 $ml.min^{-1}$ flux with split. Injector and detector temperatures were of 220$^{o}$C and 250$^{o}$C, respectively. The temperature of the column was programmed to vary from 60$^{o}$C to 300$^{o}$C at 3$^{o}$C $min^{-1}$, and mass spectra were obtained from 30 to 400 *m/z*. Identification of constituents was performed comparing their respective mass spectra and retention indexes with authentic samples and literature data (Adams 2001) and also by comparing with mass spectra registered in data bank like NIST 12 and NIST 62 (National Institute of Standards and Technology).

The results obtained were submitted to analysis of variance (ANOVA) and interpreted through regression analysis using F test ($P<0.01$ and $P<0.05$) to evaluate the polynomial equations.

## RESULTS AND DISCUSSION

Potassium concentrations significantly influenced fresh and dry matter of leaves, stems and aerial part ($P<0.05$) 56 days after transplanting. Equations were adjusted to the quadratic model. Analyzing the Figure 1 it is possible to observe that the greater production of fresh and dry mass of leaves (328.5 and 61.5 $g.plant^{-1}$) were obtained with K concentration of 414 $mg.L^{-1}$. The same effect was verified for stems fresh and dry mass (368.96 and 66.32 $g.plant^{-1}$) and aerial part (696.33 and 127 $g.plant^{-1}$), respectively.

For the obtainment of maximum fresh mass of leaves (329 $g.plant^{-1}$) and dry mass (61.8 $g.plant^{-1}$), the estimated K concentration was 384 $mg.L^{-1}$ and 364.7 $mg.L^{-1}$, respectively (equations of the Figure 1a and 1b).

On the other hand, essential oil content (%) increased when K was added to the hydroponic solution, reaching 1.2 g for each 100 g of fresh leaves at the concentration of 690 $mg.L^{-1}$ (Figure 2). In spite of the 690 $mg.L^{-1}$ K have caused plant growth reduction due to the smaller accumulation of fresh mass of leaves (276.8 $g.plant^{-1}$), there was a percent increase in essential oil yield of 35.5% in relation to the smaller K concentration (276 $mg.L^{-1}$), reaching 3.32 g of oil $plant^{-1}$ (Table 1).

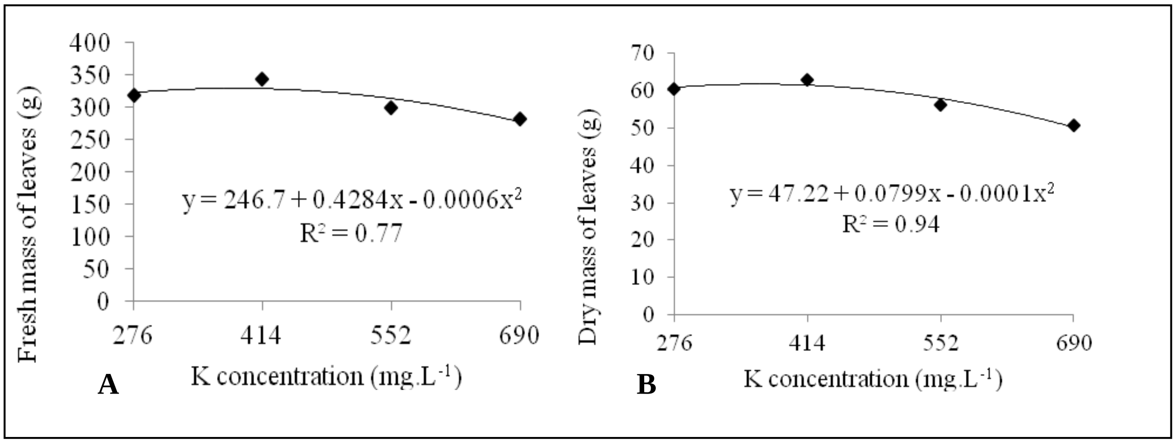


**Figure 1.** Fresh mass of leaves (A) and dry mass of leaves (B) of *Mentha x piperita var. citrata* cultivated in different K concentrations in the hydroponic solution.

Chromatographic analysis identified 20 essential oil components, which in average correspond to 96.6% of the total. Quantitative differences ($P<0.01$ and $P<0.05$) were found in the chemical composition of the oil samples examined. The majoritary components found were linalool with 45 to 53.5% and linalyl acetate with 28.1 to 34% (Table 2).

Other components which showed significance were mircene (0.4 – 1.7%), limonene (0.2 – 0.9%), 1,8-cineol (0.1 – 0.4%), (Z)-β-ocimene (0.4 – 1.6%), (E)-β-ocimene (0.3 – 1.2%), linalyl formate (0.4 – 0.9%), germacrene D (0.8 – 1.6%) and viridiflorol (1,2 – 2.9%) (Table 2). Linalool and linalyl acetate contents in the oil were smaller (%) in the plants submitted to greater K. However, for the components mircene, limonene, 1,8-cineol, (Z)-β-ocimene, (E)-β-ocimene, linalyl formate, germacrene D and viridiflorol, as K concentration increased there was also an increase in their contents.

**Table 1**. Fresh mass yield of leaves, essential oil content and yield of *Mentha* x *piperita* var. *citrata* cultivated under different K concentrations in the hydroponic solution.

| Concentration ($mg.L^{-1}$) | Fresh mass of leaves ($g.plant^{-1}$) | Essential oil | |
|---|---|---|---|
| | | Content (%) | Yield ($g.plant^{-1}$) |
| 276 | 322.5 | 0.76 | 2.45 |
| 414 | 328.5 | 0.91 | 2.99 |
| 552 | 313.3 | 1.05 | 3.29 |
| 690 | 276.8 | 1.20 | 3.32 |
| CV (%) | 8.86 | 7.90 | 7.90 |

Maximum accumulated linalool and linalyl acetate quantity, associated to the fresh mass of leaves ($g.plant^{-1}$), amount (%) and essential oil yield ($g.plant^{-1}$) may be obtained in K concentrations of 414 and 552 $mg.L^{-1}$, being equal to 2.5 $g.plant^{-1}$, a higher value in relation to those calculated for 276 and 690 $mg.L^{-1}$ K concentrations, which, respectively, were 2.1 and 2.4 $g.plant^{-1}$.

The results of this study are in agreement with Garlet et al. (2007b) in works with *Mentha arvensis* f. *piperascens* Holmes, who found that the K dosage estimated for maximum fresh leaves yield was equal to 412 $mg.L^{-1}$. The essential oil and menthol contents, on the other hand, increased with increased K concentration in the hydroponic solution. According with Garlet et al. (2007b), the K dosage of 552 $mg.L^{-1}$ proportionated greater oil yield in $g.plant^{-1}$, but the best chemical composition in menthol content was obtained with the K dosage of 690 $mg.L^{-1}$ and this dosage is, thus, recommended for the cultivation of *M. arvensis* f. *piperascens* in hydropony.

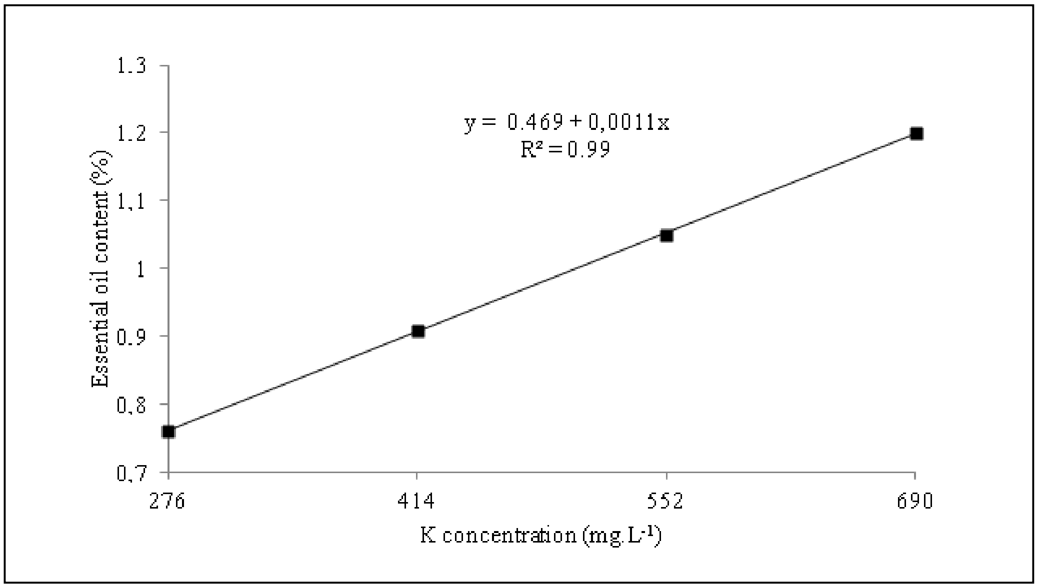


**Figure** 2. Essential oil content of *Mentha* x *piperita* var. *citrata* cultivated under four K concentrations in the nutritive solution.

In a work with *Mentha* x *gracilis* Sole, Garlet et al. (2007c) observed that even though leaves have produced greater oil content in the K dosage of 690 A high oil content itself may not be of agricultural interest since plants that produce too much oil but show low production of leaves will result in low oil yield per area. Tuomi et al. (1991) state that the concentration of secondary metabolites used for vegetal defense tend to present an inverse concentration to growing taxes and, according to Croteau et al. (2000), there exists a direct relation between photosynthates like glyceraldeid-3-phosphate or piruvate and the biosynthesis of the terpenoids. Plants translocate substances of the primary metabolism, which could generate sugar, proteins and fat and that provide energy for the growth, to produce secondary metabolites, like the terpenoids, as an answer to external factors which in this study was the culture exposition to growing K concentrations. These results suggest that K affected the activity of enzymes responsible for the biosynthesis of these terpenes constituents of the *Mentha* x *piperita* var. *citrata* essential oil. Studying *Mentha arvensis*, Maia et al. (2001) cite that nutrient availability in the solution may induce the enzymatic activity of the constituents of the oil and increase menthol content and the essential oil quality.

mg.$L^{-1}$, leaf production was reduced, resulting in a smaller oil yield per plant (2.56 g.$plant^{-1}$) and its equivalent per hectare (256 kg.$ha^{-1}$).

Using a modified Sarruge solution, the authors obtained the greatest menthol content (82.7%) in the solution with higher (468 mg.$L^{-1}$) K concentration. Valmorbida (2003), however, testing solution nº 2 of Hoagland and Arnon and varying K level, through the reduction of 50 to 75% in its concentration, did not observe effect on the chemical constitution of *Mentha* x *piperita* essential oil.

Since leaves correspond to the active site of essential oil synthesis and accumulation in *Mentha* species (Lawrence 1992; Turner et al. 2000), increases in its content and quality are economically interesting aspects. The conditions of potassium concentration increase to which *Mentha* x *piperita* var. *citrata* were submitted may have provoked stimulus in enzymatic activities, altering the composition of oils and suggesting that potassium is involved in the synthesis of these aromatic compounds, since the ion is activator of several enzymes, synthetases, oxyrreduthases**,** deshydrogenases, transferases, kinases and aldolases being the outstanding ones (Marschner 1995).

**Table 2.** Essential oil chemical composition (%) of *Mentha* x *piperita* var. *citrata* cultivated in nutrient solution with different potassium concentrations and respective regression equations.

| Component (%) | K concentration (mg.L$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 276 | 414 | 552 | 690 | Equation | $R^2$ | CV (%) |
| Mircene | 0.4 | 0.5 | 1.2 | 1.7 | y = -0.70 + 0.0034x | 0.95** | 31 |
| Limonene | 0.2 | 0.3 | 0.6 | 0.9 | y = -0.273+ 0.00162x | 0.95** | 14 |
| 1.8-cineol | 0.1 | 0.2 | 0.2 | 0.4 | y = -0.07 + 0.00063x | 0.89** | 21 |
| (Z)-β-ocimene | 0.4 | 0.5 | 0.9 | 1.6 | y = -0.60 + 0.003x | 0.89** | 10 |
| (E)-β-ocimene | 0.3 | 0.4 | 0.6 | 1.2 | y = -0.39 + 0.0021x | 0.83** | 8 |
| γ-terpineno | tr | tr | tr | tr | - | - | - |
| Terpinolene | 0.1 | 0.1 | 0.2 | 0.3 | y = 0.17 | - | 44 |
| Linalool | 53.5 | 51.2 | 45.0 | 45 | y = 59.753 - 0.02297x | 0.89* | 5 |
| N-nonanol | 0.4 | 0.4 | 0.4 | 0.5 | y = 0.41 | - | 26 |
| α-terpineol | 3.6 | 3.6 | 4.2 | 4.7 | y = 4.02 | - | 17 |
| Linalyl formate | 0.4 | 0.5 | 0.7 | 0.9 | y = 0.003 + 0.0013x | 0.97* | 27 |
| Linalyl acetate | 34 | 33 | 31 | 28.1 | y = 38.1 - 0.0136x | 0.93** | 26 |
| Neril acetate | 1.3 | 1.1 | 2.3 | 1.8 | y = 1.65 | ns | 54 |
| β-cariofilene | 1.8 | 1.6 | 2.6 | 2.6 | y = 2.16 | ns | 27 |
| α-humulene | 0.1 | 0.1 | 0.1 | 0.1 | y = 0.11 | - | 27 |
| Germacrene D | 1.0 | 0.8 | 1.5 | 1.6 | y = 0.29 + 0.0019x | 0.71* | 28 |
| Elemol | 0.4 | 0.4 | 0.3 | 0.6 | y = 0.42 | ns | 30 |
| Viridiflorol | 1.2 | 1.7 | 2.9 | 2.9 | y = 0.036 + 0.0044x | 0.90* | 32 |
| γ-eudesmol | 0.1 | 0.1 | 0.2 | 0.1 | y = 0.12 | ns | 40 |
| β-eudesmol | 0.1 | 0.1 | 0.4 | 0.4 | y = 0.23 | ns | 39 |
| Total identified | 99.29 | 96.5 | 95.37 | 95.29 | | | |

**, * = significant by test F, with P<0.01 e P<0.05.respectively; ns = not significant). Tr = traces (<0.1%)

Potassium concentrations in the hydroponic solutions altered the production of fresh mass of leaves, the content and the chemical composition of *Mentha* x *piperita* var. *citrata* essential oil. The estimated K concentration for maximum yield of fresh leaves corresponds to 384 mg.L$^{-1}$. The maximum K (690 mg.L$^{-1}$) proportionates increase in total essential content and yield per plant, but decreases linalool and linalyl acetate quantity.

## CONCLUSION

Under the conditions this experiment was carried out, for the obtainment of an adequate amount of leaves to yield greater amounts of essential oil and linalool and linalyl acetate contents, the K concentration of 414 mg.L$^{-1}$ is recommended in the hydroponic solution for lemon mint cultivation.

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