

Nitrogen supply influences agronomic performance, essential oil yield, and phytochemical composition of basil (*Ocimum basilicum* L.) grown under hydroponic conditions

Influência do nitrogênio no desempenho agrônômico, produtividade e qualidade fitoquímica do manjeriço (*Ocimum basilicum* L.) em cultivo hidropônico

Deise Silva Castro Pimentel^{1*}; Lucas Guilherme Hahn Kehl²; Maira Christina Marques Fonseca³; Herminia Emilia Prieto Martinez³; Beatriz Fernandes de Seia Gonçalves⁴; Edson Fagne dos Santos⁵; Endy Lopes Kailer⁶; Hair Santiago Lozano Puente⁷; Jaqueline Maria de Nascimento⁸; Adilson Sartoratto⁹

Highlights

Nitrogen supply enhanced basil growth and biomass under hydroponic conditions.

The highest N dose (12 mmol L⁻¹) maximized essential oil yield.

The highest N dose increased 1,8-cineole concentration, whereas the highest eugenol concentration was observed at 8 mmol L⁻¹.

Low N supply (4 mmol L⁻¹) acted as elicitor, increasing germacrene D accumulation.

Nitrogen supply altered both agronomic performance and essential oil composition.

Abstract

Basil (*Ocimum basilicum* L.) is an aromatic and medicinal plant widely cultivated for its culinary, seasoning, and therapeutic applications, making it an economically important crop. Among its high-

¹ Agricultural Extension Specialist II, Empresa de Assistência Técnica e Extensão Rural do Estado de Minas Gerais, EMATER-MG, Belo Horizonte, MG, Brazil. E-mail: deise1pimentel@yahoo.com.br

² Doctoral Student in the Graduate Program in Crop Science, Department of Agronomy, Universidade Federal de Viçosa, UFV, Viçosa, MG, Brazil. E-mail: lucaskehl@gmail.com

³ Profa. Dra., Department of Agronomy, UFV, Viçosa, MG, Brazil. E-mail: maira.fonseca@ufv.br; herminia@ufv.br

⁴ Master in Crop Science, Graduate Program in Agronomy, UFV, Viçosa, MG, Brazil. E-mail: beatriz.seia@gmail.com

⁵ Doctor in Crop Science, Graduate Program in Agronomy, UFV, Viçosa, MG, Brazil. E-mail: edson.fagne@ufv.br

⁶ Master's Student in Crop Science, Department of Agronomy, UFV, Viçosa, MG, Brazil. E-mail: endy@ksu.edu

⁷ Doctoral Student in the Graduate Program in Microbiology, UFV, Viçosa, MG, Brazil. E-mail: hairlozano@javeriana.edu.co

⁸ Doctor in Agricultural Microbiology, UFV, Viçosa, MG, Brazil. E-mail: jaquelinemaria92@hotmail.com

⁹ Doctor in Chemistry, Universidade Estadual de Campinas, UNICAMP, Campinas, SP, Brazil. E-mail: adilson@cpqba.unicamp.br

* Author for correspondence

value products, basil essential oil (EO) is widely used to enhance the aroma and flavor of processed foods and beverages and for its pharmacological properties. Nitrogen (N) is one of the primary nutrients regulating plant growth, biomass accumulation, and secondary metabolism, thereby influencing EO yield and chemical composition. This study evaluated the effects of different N concentrations in the nutrient solution on the agronomic performance, EO yield, and phytochemical composition of hydroponically grown basil. Basil seedlings (~12 cm tall) were cultivated in a greenhouse using a completely randomized design with five N concentrations (4.0, 6.0, 8.0, 10.0, and 12.0 mmol L⁻¹) and three replicates. Plants received Hoagland and Arnon (1950) nutrient solution, and the pH was maintained between 5.8 and 6.2. Increasing N supply enhanced leaf and root biomass, root volume, and tissue N concentration. The highest N concentration (12 mmol L⁻¹) produced the greatest EO yield and increased the relative abundance of 1,8-cineole and eugenol, whereas the lowest N concentration (4 mmol L⁻¹) promoted greater germacrene D accumulation.

Key words: Nutrient solution. Greenhouse. Secondary metabolites. Biomass.

Resumo

O manjeriço (*Ocimum basilicum* L.) é uma planta aromática e medicinal amplamente cultivada devido às suas propriedades culinárias, condimentares e terapêuticas, que conferem expressiva importância econômica. Dentre seus produtos de maior valor, destaca-se o óleo essencial (OE), amplamente utilizado para intensificar aroma e sabor de alimentos e bebidas processadas, além de apresentar reconhecido potencial farmacológico. Assim como em outras espécies cultivadas, o nitrogênio (N) constitui um dos nutrientes mais influentes no crescimento vegetal, na produção de biomassa e no metabolismo secundário, afetando diretamente a produtividade e o perfil químico do OE. O objetivo deste trabalho foi avaliar os efeitos de diferentes doses de N em solução nutritiva sobre características biométricas, rendimento e composição fitoquímica do OE de manjeriço cultivado em sistema hidropônico. Mudanças com altura média de 12 cm foram cultivadas em casa de vegetação, em delineamento inteiramente casualizado, com cinco doses de N (4,0; 6,0; 8,0; 10,0 e 12,0 mmol L⁻¹) e três repetições. Os macro e micronutrientes foram fornecidos de acordo com a solução nutritiva de Hoagland e Arnon (1950), referência clássica ainda amplamente utilizada em experimentos de nutrição de plantas e cultivo hidropônico (Marschner, 2012), mantendo-se o pH entre 5,8 e 6,2. O aumento da disponibilidade de N promoveu maior acúmulo de massa foliar e radicular, volume de raízes e teor de N nos tecidos. A dose de 12 mmol L⁻¹ resultou no maior rendimento de OE e maiores teores de 1,8-cineol e eugenol, enquanto a dose de 4 mmol L⁻¹ elevou a concentração de germacrene D.

Palavras-chave: Solução nutritiva. Casa de vegetação. Metabólitos secundários. Biomassa.

Introduction

Basil (*Ocimum basilicum* L.), a member of the family Lamiaceae, is a perennial plant native to tropical Asia that is well adapted to warm and mild climates (Carmo et al., 2019) and widely cultivated for its culinary,

medicinal, and aromatic properties (Azizah et al., 2023)

Its leaves are commonly used as a culinary herb in salads, meat dishes, and beverages. In addition to its medicinal applications, basil essential oil (EO) is used in

the production of fragrances, repellents, and insecticides, with the leaves representing the principal commercial product (Carmo et al., 2019).

Optimal plant growth depends on an adequate supply of essential macro- and micronutrients, which regulate numerous physiological processes throughout the plant life cycle (Marschner, 2012). Adequate nutrient availability is also fundamental for biomass production and the synthesis of bioactive compounds of commercial interest (May et al., 2008).

Nitrogen (N) is considered the macronutrient most likely to limit crop growth and productivity. In addition to promoting biomass accumulation, N plays a central role in plant metabolism as a structural component of proteins, enzymes, chlorophyll, and other essential molecules involved in energy transfer and photosynthesis, such as ATP, NADH, and NADPH (Taiz et al., 2017). Basil responds strongly to nitrogen supply, with adequate N availability increasing leaf biomass (Palaretti et al., 2015). Similarly, in *Ocimum gratissimum* L., low N concentrations reduced EO production, whereas adequate N supply enhanced oil yield. Vilanova et al. (2018) attributed this response to increased electron transport and photosynthetic activity, which improve ATP production and carbon substrate availability for isoprene synthesis, the precursor of terpenoid biosynthesis in essential oils.

This study evaluated the effects of different N concentrations in the nutrient solution on the agronomic performance, EO yield, and phytochemical composition of hydroponically grown basil (*Ocimum basilicum* L.).

Material and Methods

Experimental conditions

The experiment was conducted over a 45-day period in the greenhouse of the Department of Plant Science, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil (20°45'14" S, 42°52'53" W; 648.74 m above sea level). The greenhouse was naturally ventilated, covered with transparent glass, enclosed with anti-aphid screens, and had a ceiling height of 2.4 m.

Basil plants were cultivated in pots containing nutrient solution with five N concentrations in a completely randomized design with three replicates, each comprising two plants. The modified Hoagland and Arnon (1950) nutrient solution contained the following macronutrients (mmol L⁻¹): P-H₂PO₄: 1.5; K, 6.0; Ca, 4.0; Mg, 2.0; and SO₄²⁻, 4.0, and the following micronutrients (μmol L⁻¹): B, 46.0; Cu, 0.3; Fe, 90.0; Mn, 12.6; Zn, 1.3; Mo, 0.1. Nitrogen concentrations were 4.0, 6.0, 8.0, 10.0, and 12.0 mmol.L⁻¹.

Seedlings were obtained from cuttings collected from a single mother plant and rooted in washed, sterilized sand. After 30 days, they were transplanted into 20 L containers (24.5 × 57.5 × 18 cm; width x length x height) containing 16 L of nutrient solution. Continuous aeration was provided by compressed air injection throughout the experimental period (Martinez & Clemente, 2011).

The pH and electrical conductivity (EC) of the nutrient solution were monitored daily. The pH was maintained between 5.8 and 6.2, whereas EC ranged from 0.7 to 0.8 dS m⁻¹. EC was used to monitor nutrient depletion and determine when nutrient replacement was

required. Because EC never declined by more than 30% of its initial value, only water lost through evapotranspiration was replenished during the experiment.

Biometric evaluations

Plants were harvested 45 days after transplantation, and the following variables were determined: plant height and primary root length, measured with a tape measure; root volume, determined by water displacement using a graduated cylinder (Basso, 1999); leaf and root fresh mass, measured on a precision balance (0.01 g); leaf dry mass, estimated from the dry matter percentage of a fresh leaf sample of known weight after drying at 65 °C; and root dry mass, determined after oven drying at 65 °C in a forced-air oven until constant weight. The dried leaves were ground in a Willey mill and stored in paper bags for subsequent chemical analyses.

Leaf nitrogen analysis

Plant material was subjected to sulfuric digestion, and total leaf N concentration was determined using the micro-Kjeldahl method (Kjeldahl, 1883).

Phytochemical evaluations

Chlorophyll content was estimated indirectly using a SPAD-502 chlorophyll meter (Minolta®, Japan). Three measurements were obtained from the basal, middle, and apical leaves of each plant, and the mean SPAD value for each replicate was used for analysis.

Before essential oil extraction, basil leaves were dried in Kraft paper bags at 40 °C in a forced-air oven until constant weight. Air temperature was monitored continuously during the drying process. Dried leaves were then divided into 50 g samples, separated in 40 µm polyethylene bags, and stored at room temperature until the extraction.

Essential oil was extracted by hydrodistillation in a Clevenger-type apparatus coupled to a 1,000 mL round-bottom flask. Heating was maintained at the minimum temperature required to sustain boiling, and extraction proceeded for 2 h. Each extraction used 50 g of dried leaves and 500 mL of distilled water.

Essential oil yield was determined gravimetrically as the difference between the weights of the empty collection bottle and bottle containing the extracted oil, measured on a 0.001 g analytical balance. Oil samples were stored at 4 °C in amber glass bottles with screw caps until chromatographic analysis.

Basil essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS) using an Agilent 6890 GC system equipped with an HP-5975 mass-selective detector and an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Samples were injected in splitless mode, with the injector maintained at 220 °C. The oven temperature was initially set at 60 °C and increased at 3 °C min⁻¹ to a final temperature of 240 °C, while the detector was maintained at 250 °C. Helium was used as the carrier gas at 1 mL min⁻¹. Essential oil samples were diluted in ethyl acetate to 20 mg mL⁻¹ before analysis. Compounds were identified by comparing the experimentally determined retention indices (RI), obtained using C8–C24 hydrocarbon

standards, with values reported in the NIST-11 mass spectral library and published literature.

Data management and statistical analysis

The experimental data obtained were organized in a spreadsheet and analyzed using the RStudio integrated development environment (Allaire, 2012). Data were first subjected to analysis of variance (ANOVA), and when the effects of N concentrations were significant ($p < 0.05$), regression models were fitted. The most appropriate model was selected based on the significance of the model parameters (t-test, $p < 0.05$), the coefficient of determination (R^2), and the goodness of fit to the observed data.

Results and Discussion

Chlorophyll index, plant height, and root length

Increasing N concentrations did not significantly affect the chlorophyll index, which averaged 29.37. Plant height and primary root length were also unaffected, with average values of 71 cm and 57 cm, respectively.

Leaf fresh and dry mass

Leaf fresh and dry mass increased linearly as N concentration in the nutrient solution increased (Figure 1). Nitrogen is the nutrient most frequently limiting plant growth, particularly in leafy crops. This linear increase in leaf biomass reflects the fundamental role of N in plant growth and development. Nitrogen is a constituent of amino acids and, consequently, of proteins and enzymes involved in plant metabolism, thereby promoting biomass accumulation (Marschner, 2012).

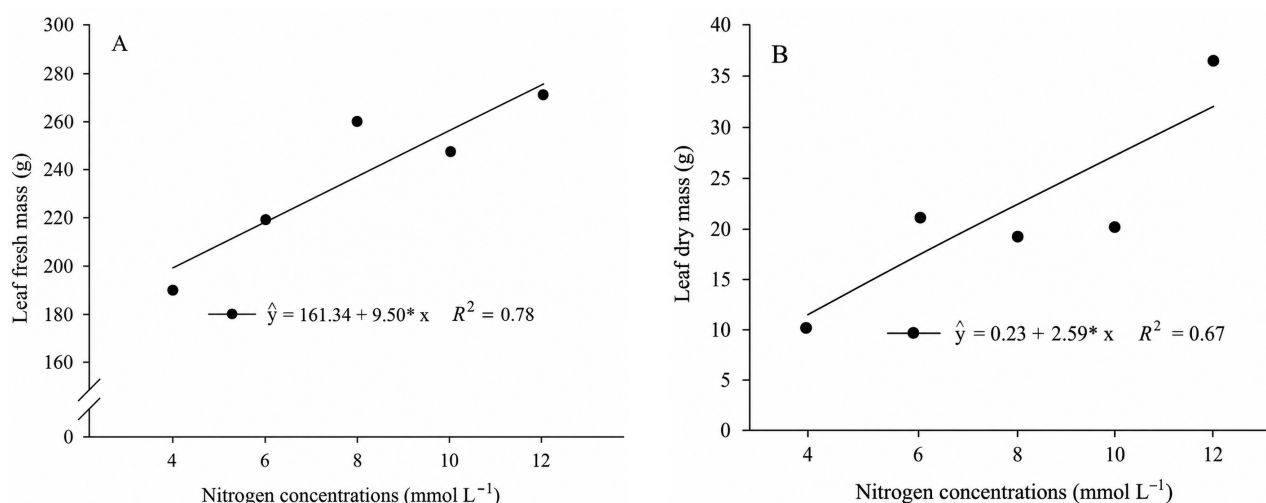


Figure 1. Leaf fresh (A) and dry (B) mass of basil as a function of N concentrations in hydroponic cultivation.

The present findings are consistent with previous studies demonstrating the positive effect of N on biomass accumulation in aromatic species grown under different production systems. Khater et al. (2021) reported significant increases in basil biomass under higher N availability in soilless cultivation, highlighting the importance of N supply for vegetative growth and leaf production. Similarly, Safaei et al. (2024) found that increasing N concentrations enhanced growth and biomass accumulation in hydroponically grown lemon balm. Comparable responses have also been reported for oregano. Ninou et al. (2021) showed that N fertilization increased biomass production and affected essential oil yield, whereas Khan et al. (2024) demonstrated that optimized N management promoted greater vegetative growth and shoot biomass accumulation. Collectively, these findings reinforce the central role of N supply in regulating biomass production in aromatic species, particularly under controlled cultivation systems.

Root fresh and dry mass and root volume

Root fresh and dry mass and root volume increased linearly with increasing N concentrations in the nutrient solution (Figure 2). Although excessive N supply can promote preferential shoot growth at the expense of root development, the N concentrations evaluated in the present study stimulated the growth of both plant organs. Similar responses have been reported for hydroponically grown basil. Kiferle et al. (2013) found that increasing N availability enhanced plant growth, whereas Khater et al. (2021) reported greater root development and improved root health in basil grown under soilless conditions. Together, these findings indicate that, under adequate aeration and nutrient availability, Increasing N supply can promote both shoot growth and root system development.

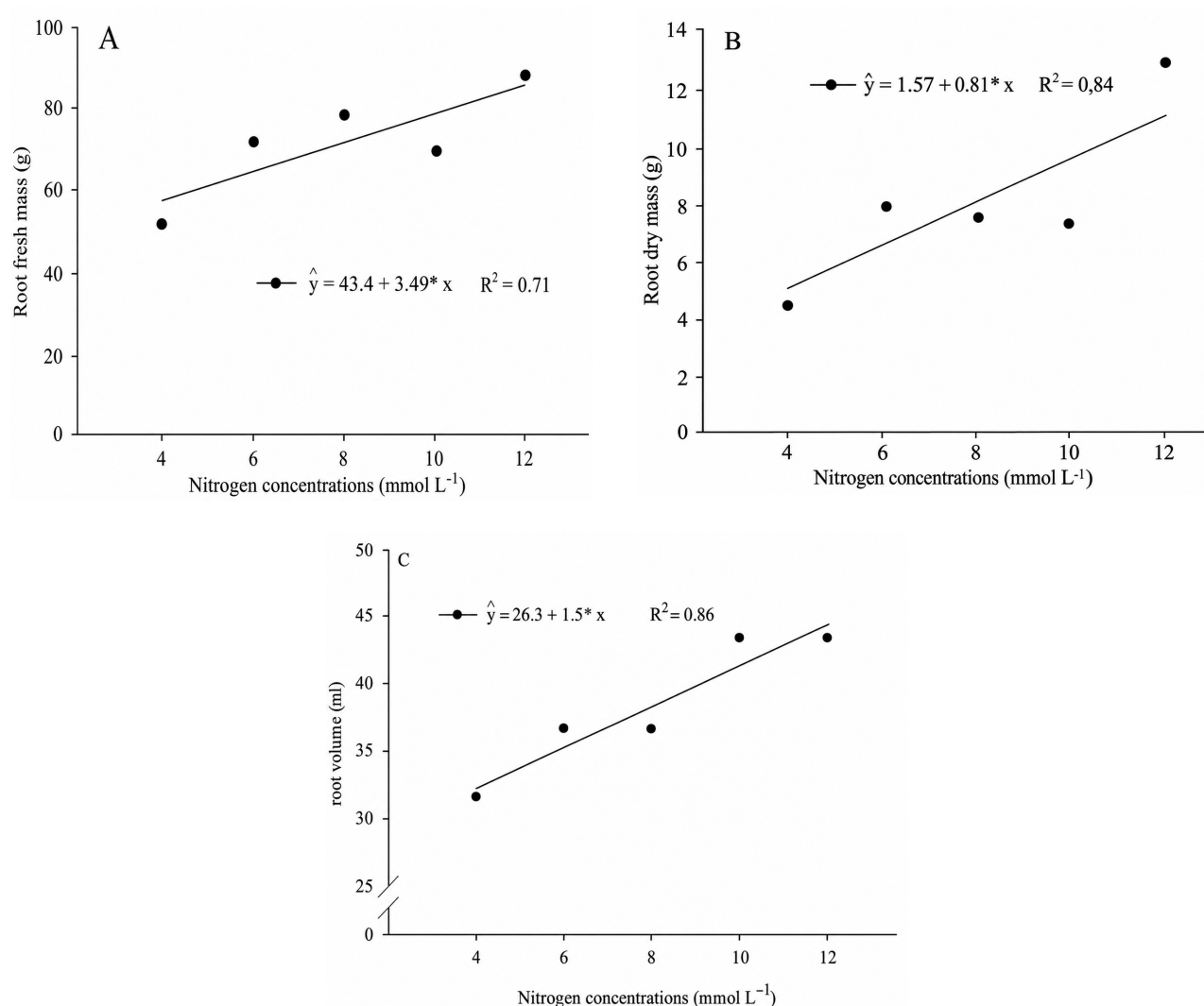


Figure 2. Root fresh mass (A), root dry mass (B), and root volume (C) of basil as a function of N concentration in hydroponic cultivation.

The roots exhibited the characteristic conical architecture commonly observed in hydroponic systems with adequate aeration (Resh, 2013). No symptoms of root rot were observed, and the roots remained white throughout the experiment, indicating healthy root development.

Leaf nitrogen concentration

Leaf N increased linearly with increasing N concentrations in the nutrient solution as observed for most of the evaluated variables. However, the relatively low coefficient of determination ($R^2 = 0.591$) indicates greater variability in the data than

that observed for the other variables. This variability is evident from the dispersion of the values for the 6 and 8 mmol L⁻¹ treatments above and below the fitted regression curve, respectively (Figure 3). This pattern suggests that much of the available N in the nutrient solution was assimilated by the plants, since increases in leaf N concentration were accompanied by greater leaf dry mass accumulation.

The highest N concentration evaluated (12 mmol L⁻¹) resulted in the greatest leaf N concentration. Moreover, variations in leaf N concentration followed the same overall trend as the agronomic variables evaluated, with the highest leaf N concentration corresponding to the greatest

responses in plant growth and biomass production.

N assimilation is regulated by the interaction between environmental signals, carbon metabolism, N availability, and the metabolic demand for N-containing compounds, as well as by the availability of carbon skeletons, energy, and reducing power required for assimilation (Marschner, 2012). Under the greenhouse conditions of the present study, the absence of water deficit in the hydroponic system may have allowed stomata to remain open even at relatively high temperatures, thereby maintaining CO₂ uptake, favoring RuBisCO activity, and contributing to N assimilation.

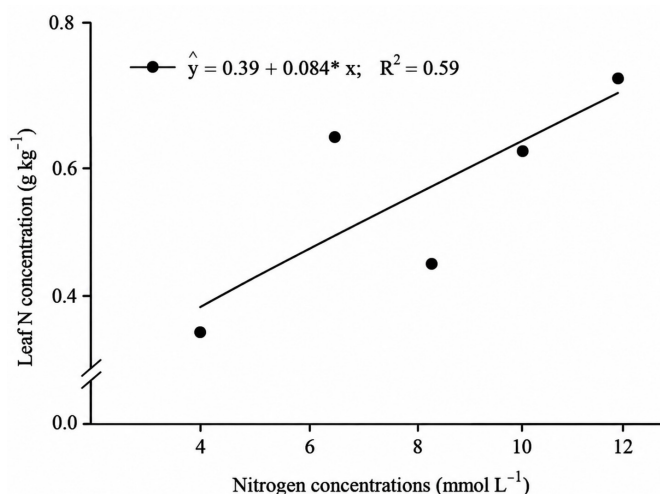


Figure 3. Leaf N concentration as a function of N concentrations in the nutrient solution.

Essential oil yield from basil leaves

N concentration significantly affected the yield of EO extracted from basil leaves (Figure 4). Essential oil yield showed a quadratic response to increasing N concentrations, reaching a minimum at the estimated concentration of 6.75 mmol L^{-1} and a maximum at 12 mmol L^{-1} . The greater EO yield observed at higher N concentrations may be associated with increased biomass accumulation, since N plays a central role in plant metabolism and promotes growth when supplied under conditions favorable for uptake and assimilation (Taiz et al., 2017). These findings agree with those of Sifola and Barbieri (2006), who reported that nitrogen fertilization increased leaf fresh as well as EO concentration and yield in basil. Similarly, Nurzyńska-Wierdak et al. (2012) found that the sweet basil cultivar Genua Star exhibited more vigorous growth and greater EO accumulation in response to nitrogen fertilization.

The predominant constituents identified in basil EO belonged to the monoterpenes and sesquiterpene classes, and their relative proportions varied according to N availability. Monoterpenes, such as 1,8-cineole, increased under higher N concentrations, whereas the sesquiterpene germacrene D was more abundant under lower N availability. These differences may reflect changes in carbon allocation and in the regulation of terpene biosynthesis under contrasting nutritional conditions

(Mahmoud & Croteau, 2002). Monoterpenes are synthesized predominantly through the methylerythritol phosphate (MEP) pathway in plastids, which is closely associated with photosynthetic metabolism and primary growth. Accordingly, greater N availability may stimulate photosynthetic activity, protein synthesis, and enzymatic metabolism, thereby favoring monoterpene biosynthesis and increasing EO yield (Taiz et al., 2017).

By contrast, sesquiterpenes are synthesized primarily through the mevalonate (MVA) pathway in the cytosol and are generally associated with plant defense and adaptive responses to stress (Mahmoud & Croteau, 2002). The greater abundance of germacrene D under low N availability may therefore reflect a shift in carbon allocation toward secondary metabolism under nutrient-limited conditions.

Although the highest N concentration evaluated (12 mmol L^{-1}) produced the greatest EO yield, N concentrations above this level could result in even greater yields and further changes in EO composition. However, excessive N availability may also promote luxury consumption and excessive biomass accumulation through increased production of structural compounds per unit leaf area. Under these conditions, greater N supply produces thicker leaves, altering specific leaf area and leaf area ratio without necessarily resulting in proportional increases in EO concentration (Taiz et al., 2017).

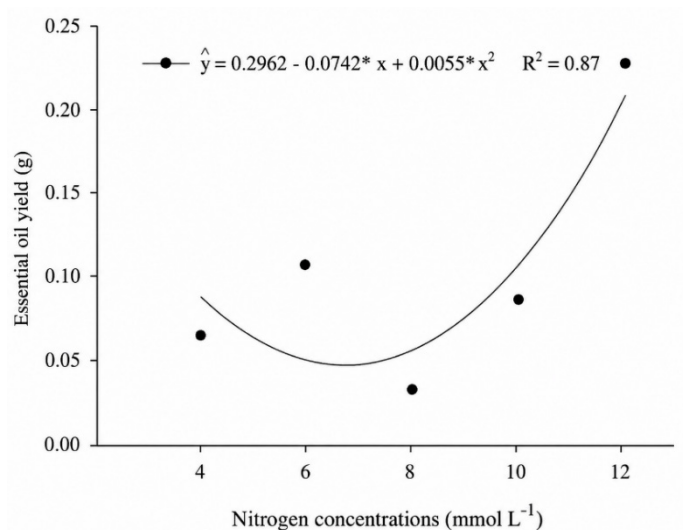


Figure 4. Essential oil yield extracted from basil leaves as a function of N concentrations.

Chemical constituents of basil essential oil

Twenty-eight constituents were identified in the EO extracted from basil leaves (Table 1). Monoterpenes accounted for 57.14% of the identified constituents, whereas sesquiterpenes represented 42.85%. The highest N concentration (12 mmol L⁻¹) resulted in the highest relative abundance of 1,8-cineole (15.49%) and the lowest relative abundance of germacrene

D (14.06%). By contrast, the lowest N concentration (4 mmol L⁻¹) resulted in the lowest eugenol content (2.68%) and the highest relative abundance of germacrene D (23.46%), epi- α -cadinol (19.39%), camphor (16.34%) and α -trans-bergamotene (13.62%). The 10 mmol L⁻¹ treatment also resulted in high relative abundance of germacrene D (24.39%), epi- α -cadinol (17.99%), and α -trans-bergamotene (13.85%).

Table 1

Chemical composition of basil essential oil as a function of N concentration in hydroponic cultivation

Component	Relative abundance (%)				
	Concentrations (mmol L ⁻¹)				
	4	6	8	10	12
α-Pinene	---	---	---	---	0.39
Camphene	---	---	---	---	0.32
Sabinene	---	0.13	---	---	0.53
β-Pinene	---	0.31	0.17	---	1.10
β-Myrcene	---	0.18	---	---	0.63
Limonene	---	0.60	0.39	---	0.97
1,8-cineole (eucalyptol)	7.14	8.55	8.90	2.42	15.49
γ-Terpinene	---	0.24	0.48	0.21	0.53
Fenchone	---	0.53	0.61	0.22	1.00
Linalool	---	0.75	1.26	0.65	1.40
Camphor	16.34	9.98	13.07	6.55	12.95
Trans-β-Terpineol	---	0.29	0.50	---	0.63
Terpin-4-ol	---	0.31	0.53	0.21	0.69
α-Terpineol	3.55	2.39	3.65	2.05	3.74
Eugenol	2.68	9.69	11.98	2.86	9.10
Piperitenone oxide	---	2.94	1.43	---	---
α-Copaene	---	0.50	0.49	0.47	0.51
Trans-caryophyllene	4.43	4.24	3.84	5.07	3.86
α-trans-Bergamotene	13.62	10.62	9.91	13.85	8.52
α-Humulene	1.91	1.82	1.70	2.39	1.63
Cis-muurola-4(14),5-diene	---	0.75	0.73	0.79	0.70
Germacrene D	23.46	18.77	16.10	24.39	14.06
Bicyclogermacrene	---	1.56	1.44	2.22	1.40
γ-Cadinene	5.45	4.34	4.22	6.33	3.48
Cis-calamenene	---	0.51	0.43	0.63	0.40
δ-Cadinene	---	0.82	0.88	1.03	0.76
1,10-di-epi-cubenol	---	1.24	1.18	1.79	1.16
Epi-α-cadinol	19.39	12.24	10.58	17.99	8.59

Among the principal constituents most frequently reported in basil research are eugenol, linalool, and 1,8-cineole. Variation in EO composition has been attributed primarily to intraspecific hybridization but is also influenced by genetic characteristics and environmental and agronomic factors, including climatic conditions, geographic origin, cultivation system, physiological stage at harvest, and storage conditions (Telci et al., 2006; Klimánková et al., 2008; Piras et al., 2018).

In the present study, 1,8-cineole reached its lowest relative abundance at 10 mmol L⁻¹, whereas higher levels were reached at the remaining N concentrations. Germacrene D was the predominant EO constituent, with the highest relative abundances recorded at 4 and 10 mmol L⁻¹ (23.46% and 24.39%, respectively), declining markedly at the other N concentrations. Germacrene A and germacrene D are the two principal members of the germacrene group of sesquiterpenes (Parsaeimehr et al., 2014). The biosynthesis of germacrene D appears to be strongly influenced by plant stress. Senji and Mandoulakani (2018) reported increased germacrene D content in cold-treated *Ocimum basilicum* L., partly through the upregulation of germacrene D synthase (GDS) gene expression. Similarly, Daneshian et al. (2009) reported changes in the relative abundances of germacrene D, 1,8-cineole, linalool, and naphthalene in response to increasing N concentrations.

From a practical perspective, N concentrations of 4 and 10 mmol L⁻¹ are not recommended when the objective is to maximize the relative abundance of the

medicinally important constituents eugenol and 1,8-cineol, as both concentrations resulted in lower levels of these compounds.

Conclusions

Nitrogen markedly influenced the agronomic performance and essential oil characteristics of basil (*Ocimum basilicum* L.) grown hydroponically in an aerated static nutrient solution. Increasing N concentrations promoted biomass accumulation, whereas 12 mmol L⁻¹ produced the greatest leaf fresh and dry mass and essential oil yield.

N availability also altered the chemical composition of the essential oil.

The highest N concentration evaluated (12 mmol L⁻¹) maximized the relative abundance of 1,8-cineole, whereas the highest relative abundance of eugenol was observed at 8 mmol L⁻¹, whereas the lowest concentration (4 mmol L⁻¹) favored germacrene D accumulation.

Acknowledgments

The authors thank the Federal University of Viçosa (UFV) for its institutional support and research facilities. They also acknowledge the Department of Crop Science at UFV for its technical and scientific support. This study was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES), the Research Support Foundation of the State of Minas Gerais (FAPEMIG), and the National Council for Scientific and Technological Development (CNPq) through research funding and scholarships.

References

- Parsaeimehr, A.; Chen, Y.-F.; Sargsyan, E. Bioactive molecules of herbal extracts with anti-infective and wound healing properties. In: Kon, K.; Rai, M. (eds.). Microbiology for surgical infections: diagnosis, prognosis and treatment. Amsterdam: Academic Press, 2014. p. 205-220. DOI: 10.1016/B978-0-12-411629-0.00012-X.
- Allaire, J.J. (2012). RStudio: integrated development environment for R. Boston, MA: Rstudio, 2012
- Azizah, N. S., Irawan, B., Kusmoro, J., Safriansyah, W., Farabi, K., Oktavia, D., Doni, F. & Miranti, M. (2023). Sweet basil (*Ocimum basilicum* L.): a review of its botany, phytochemistry, pharmacological activities, and biotechnological development. *Plants*, 12(24), 4148. doi: 10.3390/plants12244148
- Basso, S. M. S. (1999). *Caracterização morfológica e fixação biológica de nitrogênio de espécies de Adesmia DC e Lotus L.* Tese de doutorado, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil.
- Carmo, C. O. do; Silva, F. da; Silva, R. M. da; Soares, A. C. F. Utilização de compostos orgânicos inoculados com Actinobactéria na adubação de manjerição (*Ocimum basilicum* L.). *MAGISTRA*, Cruz das Almas, v. 30, p. 18-27, 2019. Disponível em: <https://periodicos.ufrb.edu.br/index.php/magistra/article/view/4027>. Acesso em: 4 set. 2026.
- Daneshian, A., Gurbuz, B., Cosge, B., & Ipek, A. (2009). Chemical components of essential oils from basil (*Ocimum basilicum* L.) grown at different nitrogen levels. *International Journal of Natural and Engineering Sciences*, 3(3), 9-13. doi: 10.22092/jmpb.2013.108589
- Hoagland, D. R., & Arnon, D. I. (1950). *The water culture method for growing plants without soil*. California Agricultural Experiment Station. (Circular).
- Khan, A., Kumar, D., Suryavanshi, P., Padalia, R. C., Venkatesha, K. T., Kholiya, S., Verma, P. P. S., & Singh, S. (2024). Spacing and nitrogen application affect Indian oregano (*Origanum vulgare* L.) biomass, essential oil yield and composition. *Industrial Crops and Products*, 218, 119010. doi: 10.1016/j.indcrop.2024.119010
- Khater, E. S., Bahnasawy, A., Abass, W., Morsy, O., El-Ghobashy, H., Shaban, Y., & Egela, M. (2021). Production of basil (*Ocimum basilicum* L.) under different soilless cultures. *Scientific Reports*, 11, 12754. <https://doi.org/10.1038/s41598-021-91986-7>
- Kiferle, C., Maggini, R., & Pardossi, A. (2013). Influence of nitrogen nutrition on growth and accumulation of rosmarinic acid in sweet basil (*Ocimum basilicum* L.) grown in hydroponic culture. *Australian Journal of Crop Science*, 7(3), 321-327.
- Kjeldahl, J. (1883). Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. *Zeitschrift für Analytische Chemie*, 22(1), 366-382. <https://doi.org/10.1007/BF01338151>

- Klimánková, E., Holadová, K., Hajšlová, J., Čajka, T., Poustka, J., & Koudela, M. (2008). Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. *Food Chemistry*, 107(1), 464-472. doi: 10.1016/j.foodchem.2007.07.062
- Mahmoud, S. S., & Croteau, R. B. (2002). Strategies for transgenic manipulation of monoterpene biosynthesis in plants. *Trends in Plant Science*, 7(8), 366-373. doi: 10.1016/S1360-1385(02)02303-8
- Marschner, H. (2012). *Marschner's mineral nutrition of higher plants* (3rd ed.). Academic Press.
- Martinez, H. E. P., & Clemente, J. M. (2011). *O uso do cultivo hidropônico de plantas em pesquisas*. Imprensa Universitária, UFV.
- May, A., Bovi, O. A., Maia, N. B., Barata, L. E. S., Souza, R. C. Z., Souza, E. M. R., Moraes, A. R. A., & Pinheiro, M. Q. (2008). Basil plants growth and essential oil yield in a production system with successive cuts. *Bragantia*, 67(2), 385-389. <https://doi.org/10.1590/S0006-87052008000200014>
- Ninou, E., Mylonas, I., Ntatsi, G., Kougioumoutzis, K., & Paraskevopoulou, A. T. (2021). Nitrogen effects on the essential oil and biomass production of field grown Greek oregano populations. *Agronomy*, 11(9), 1722. doi: 10.3390/agronomy11091722
- Nurzyńska Wierdak, R., Bogucka Kocka, A., Kowalski, R., & Borowski, B. (2012). Changes in the chemical composition of the essential oil of sweet basil (*Ocimum basilicum* L.) depending on the plant growth stage. *Chemija*, 23(3), 216-222.
- Palaretti, L. F., Dalri, A. B., Dantas, G. F., Faria, R. T., Santos, W. F., & Santos, M. G. (2015). Produtividade do manjeriço (*Ocimum basilicum* L.) fertirrigado utilizando vinhaça concentrada. *Revista Brasileira de Agricultura Irrigada*, 9(5), 326-334. doi: 10.7127/RBAI.V9N400234
- Piras, A., Gonçalves, M. J., Alves, J., Falconieri, D., Porcedda, S., Maxia, A., & Salgueiro, L. (2018). *Ocimum tenuiflorum* L. and *Ocimum basilicum* L., two spices of Lamiaceae family with bioactive essential oils. *Industrial Crops and Products*, 113, 89-97. doi: <https://doi.org/10.1016/j.indcrop.2018.01.024>
- Resh, H. M. (2013). *Hydroponic food production: a definitive guidebook for the advanced home gardener and the commercial hydroponic grower* (7th ed.). CRC Press.
- Safaei, F., Alirezalu, A., Noruzi, P., & Alirezalu, K. (2024). Phytochemical and morpho-physiological response of *Melissa officinalis* L. to different NH_4^+ to NO_3^- ratios under hydroponic cultivation. *BMC Plant Biology*, 24(1), 968. Doi: 10.1186/s12870-024-05693-2
- Senji, B. M., & Mandoulakani, B. A. (2018). The impact of cold stress on genes expression pattern of mono and sesquiterpene biosynthesis and their contents in *Ocimum basilicum* L. *Phytochemistry*, 156, 250-256. doi: 10.1016/j.phytochem.2018.09.002
- Sifola, M. I., & Barbieri, G. (2006). Growth, yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. *Scientia Horticulturae*, 108(4), 408-413. doi: 10.1016/j.scienta.2006.02.002

- Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2017). *Fisiologia e desenvolvimento vegetal* (6th ed.). Artmed.
- Telci, I., Bayram, E., Yilmaz, G., & Avci, B. (2006). Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). *Biochemical Systematics and Ecology*, 34(6), 489-497. doi: 10.1016/j.bse.2006.01.009
- Vilanova, C. M., Coelho, K. P., Luz, T. R. S. A., Silveira, D. P. B., Coutinho, D. F., & Moura, E. G. (2018). Effect of different water application rates and nitrogen fertilisation on growth and essential oil of clove basil (*Ocimum gratissimum* L.). *Industrial Crops and Products*, 125, 186-191. doi: 10.1016/j.indcrop.2018.08.047

