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Effect of phosphorus application rate on *Mentha spicata* L. grown in deep flow technique (DFT)

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ABSTRACT

The present study evaluated the impact of phosphorus application rate on plant growth and physiological parameters, antioxidant activity, chemical composition, and essential oil yield and composition of hydroponically grown spearmint plants. Increased P levels resulted in high dry matter content of the aerial part. Antioxidant activity of spearmint leaves was significantly higher at the highest P levels. Although essential oil yield was not affected, essential oil composition varied among the studied P levels, especially carvone content. Total and individual organic acids content was higher when 50 mg/L P were added in the nutrient solution. Rosmarinic acid was the main detected phenolic compound, while the highest total phenolic compounds and rosmarinic acid content was observed at 50 and 70 mg/L of P, respectively. In conclusion, phosphorus application rate may affect spearmint growth and development, as well as chemical composition and essential oil composition.

1. Introduction

Phosphorus (P) uptake from plants has been suggested to determine photosynthetic potential, while any deficiencies are associated with reduced carbon fixation in the chloroplasts. Furthermore, high P application rates have been shown to decrease *Matricaria chamomilla* L. essential oil yield (Emongor, Chweya, Keya, & Munavu, 1990), whereas they are associated with increased oil yield of *Salvia officinalis* L. (Nell et al., 2009). Moreover, interactions between minerals have been usually found to be stronger than the action each individual mineral may have. Research on marjoram (*Origanum majorana* L.) has reported that increasing phosphorus application rates up to 3 mM resulted in an increase of total volatile oil yield by 50% (Trivino & Johnson, 2000). Moreover, in the study of Ramezani, Rezaei, and Sotoudehnia (2009) phosphorus was applied on basil plants (*Ocimum basilicum* L.) by foliar spray at two growth stages, resulting in a significant increase of essential oil yield, without however affecting fresh and dry weight of the aerial biomass. Considering that phosphorus is generally applied in soil with basal dressing prior to seeding or planting, the effectiveness of phosphorus fertilization on plant growth and essential oil content for soilless growing systems needs further examination.

At present, an increasing interest has been noted both in industrial and scientific research for using compounds and extracts of medicinal and aromatic plants as alternatives to synthetic antioxidants, due to their powerful antimicrobial and antioxidant properties. According to Kivilompolo and Hyötyläinen (2007), spearmint leaves are a rich source of rosmarinic, chlorogenic and caffeic acids. Rita, Pereira, Barros, Santos-Buelga, and Ferreira (2016) suggested that antioxidant activity of infusions from apical leaves of spearmint was due to phenolic compounds content and rosmarinic acid in particular, while Gonçalves et al. (2017) also attributed antioxidant potential of spearmint leaves to other phenolic compounds apart from rosmarinic acid, as well to non-phenolic compounds with synergistic effects.

In soilless production systems the nutrient are supplied through the nutrient solutions enabling rapid growth and biomass production while nutrient solution composition is fully controlled and adjusted according to plant requirements (Garlet & Santos, 2008). Float cultivation systems are successfully used for the commercial production of various leafy vegetables such as endive, lettuce, radish, rocket, spinach and so forth. These production systems are also very promising for aromatic plants and herbs and micro-greens production. The aim of the present study was to investigate the effect of five different phosphorus application rates (30–70 mg/L of nutrient solution) on agronomic performance, mineral composition, and antioxidant properties, as well as on essential

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oil, organic acids and phenolic compounds composition of *M. spicata* plants grown in a deep flow hydroponic system.

2. Materials and methods

2.1. Plant material and growing conditions

The current study was carried out during the spring–summer period at the experimental greenhouse of the Cyprus University of Technology, Limassol, Cyprus. Air temperature ranged between 17.7 and 29.0 °C during the experimental period. Plant material and growing conditions have been described by the authors in previous experiments regarding the effect of potassium on spearmint growth and chemical composition (Chrysargyris, Xylia, Botsaris, & Tzortzakis, 2017). Briefly, spearmint plants were grown from cuttings (obtained from the Cypriot National Centre of Aromatic Plants) which were transplanted in plastic 1-L containers (one plant in each container) when they reached 10 cm in height. Containers were sorted according to randomized complete blocks design with each replication consisting of a group of 6 pots, while plant density was arranged at 16 plants/m^2. Nutrient solution composition throughout the experimental period is presented in Supplementary Material Table S1, while nutrient solution consumption and P content in nutrient solution after uptake throughout the growing period are presented in Supplementary material (Figs. S1 and S2, respectively). By measuring the amount of nutrient solution that was added to each container throughout the growing period in order to keep the volume in each container constant (1 L), consumption could be calculated. The impact of P levels was examined by applying five levels of 30, 40, 50, 60 or 70 mg/L of P (P30, P40, P50, P60 or P70, respectively), while N and K content in nutrient solution was kept at 200 mg/L and 375 mg/L, respectively, based on the results of previous studies by our team (Chrysargyris, Panayiotou, & Tzortzakis, 2016; Chrysargyris et al., 2017). For each P level there were three replications of six plants (18 plants in total for each treatment). The plants were treated with the above described nutrient solution for five weeks. Nutrient solution was checked every week for nutrients replenishment, while pH and electrical conductivity (EC) were adjusted every second day at 5.8 and EC = 2.1 mS/cm, respectively.

2.2. Plant growth

Ninety spearmint plants were grown for five weeks and until anthesis took place, treated with the above described P levels (treatments). Detailed measurements for plant growth assessment (aerial part and root fresh and dry plant weight, the number of leaves, plant height, root length, and stem thickness) were obtained from six individual plants for each P level according to the methods described by Chrysargyris et al. (2017). Briefly, weight was measured with a laboratory scale, plant height and root length were measured at the furthest point with a measuring tape, and stem thickness was measured with a caliper.

2.3. Physiological parameters

2.3.1. Chlorophyll determination

Chlorophylls content (Chlorophyll *a*, *b* and total chlorophylls) was determined according to the method previously described by Richardson et al. (2002). Briefly, stomatal conductance of leaves was measured with the aid of a dynamic porometer (Delta-T AP4; Delta-T Devices-Cambridge, UK), while maximum F_v/F_m photochemical quantum yields of PSII were measured with a Chlorophyll Fluorometer (OptiSci OS-30p; Opti-Sciences, Hudson, NH) after incubating leaves for 20 min in dark conditions before recording of measurements.

2.3.2. Activities of antioxidant enzymes

Antioxidant enzymes activity (APX, CAT and SOD) was determined according to the methods previously described by the authors. Briefly, extraction of previously homogenized fresh leaf samples was carried out with the use of ice cold extraction buffer (1 mM ethylenediaminetetraacetic acid (EDTA), 1% (*w/v*) polyvinylpyrrolidone (PVPP), 1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.05% Triton X-100 in 50 mM potassium-phosphate buffer (pH = 7.0)) (Jiang & Zhang, 2002). Results for APX and CAT were expressed as enzyme units per mg of protein (1 unit = 1 mM of H_2O_2 reduction/min), while SOD activity was estimated as the amount of enzyme that resulted in 50% inhibition of the NBT photoreduction rate.

2.3.3. Determination of H_2O_2 content and lipid peroxidation

H_2O_2 content was determined based on the method of Loreto and Velikova (2001), while lipid peroxidation was assessed according to method of De Azevedo Neto, Prisco, Enéas-Filho, Abreu, and Gomes-Filho (2006). H_2O_2 content was expressed in μmol per g of fresh weight, while lipid peroxidation was estimated as nmol of MDA per g of fresh weight.

2.3.4. Antioxidant activity assays

The antioxidant capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) and ferric reducing ability of plasma (FRAP) assays according to Wojdyło, Oszmiański, and Czemerys (2007) with some modifications (Chrysargyris et al., 2016). Antioxidant activity was estimated as Trolox equivalents (positive control) per g fresh weight.

2.4. Plant and nutrient ion concentration analysis

Minerals content (potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and boron (B)] was assayed by coupled plasma atomic emission spectrometry (ICP-AES; PSFO 2.0 (Leeman Labs Inc., Hudson, NH)) (Chrysargyris et al., 2016). N content was determined with Kjeldahl digestion method (BUCHI, Digest automat K-439 and Distillation Kjeldahl K-360).

2.5. Essential oil extraction and gas chromatography/mass spectrometry analysis

Spearmint aerial parts were collected after five weeks of cultivation. The fresh samples were air-dried at 42 °C in a forced-air drying oven. The dried tissues were chopped prior to analysis and essential oil was extracted with a Clevenger apparatus. Each extraction lasted for approximately 3 h, while each treatment was replicated three times. For essential oil composition analysis, GC/MS was implemented (Shimadzu GC2010 interfaced with a Shimadzu GC/MS QP2010; Shimadzu Corporation, Kyoto, Japan). Essential oil components were identified by comparing their retention indices and mass spectra with authentic standards when available. For those compounds where no authentic standards were available, tentative identification was implemented by comparing mass spectra of components with spectral data of the built-in Mass Spectra Library NIST08 of the GC–MS system and the literature.

2.6. Organic acids determination

Organic acids were determined by HPLC-DAD (Shimadzu 20A series; Shimadzu Corporation, Kyoto, Japan) operating under the conditions described by Barros, Pereira, and Ferreira (2013). The results were expressed as mg per 100 g fresh weight.

2.7. Phenolic compounds identification

Composition of phenolic compounds of the aerial plant parts was determined by HPLC-DAD-ESI/MS (Dionex Ultimate 3000, Thermo Scientific, San Jose, CA) according to the method previously described