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Seasonal variations of antioxidants and other agronomic features in soilless production of selected fresh aromatic herbs

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ABSTRACT

The antioxidant activity, herbal fresh yield, water and nitrogen productivity were studied in selected aromatic herbs of the *Lamiaceae* family, namely basil (*Ocimum basilicum*), mint (*Mentha viridis*), balm (*Melissa officinalis*) and thyme (*Thymus vulgaris*), grown in soilless conditions in a typical Mediterranean climate. Seasonal variations of the bioactive substances of the produce were defined. In all crops, total antioxidant activity assessed by (i) ferric reducing antioxidant power/FRAP (μM ascorbic acid equiv/g FW) and (ii) 1,1-diphenyl-2-picrylhydrazyl/DPPH (mg ascorbic acid equiv/g FW), total phenolics (mg gallic acid equiv/g FW) and ascorbic acid content (mg/g FW) were greater in summer (avg. 82.3, 7.01, 8.59 and 0.75, respectively) and lower in winter (avg. 45.4, 2.93, 4.89 and 0.38, respectively), whereas values in spring and autumn were in between (avg. 62.9, 4.8, 7.3 and 0.53, respectively). Natural antioxidants were higher in thyme followed by balm and mint and lower in basil and total antioxidant activity was highly correlated in all cases with phenolics and ascorbic acid. The mean daily evapotranspiration of all crops ranged between 1.8–2.5 mm over the study period. The herbal fresh yield (kg m^{-2}) was greatest in mint (avg. 22.8) followed by basil (avg. 19.3), balm (avg. 15.7) and lowest in thyme (avg. 10.2). Yield per unit of water consumed (kg tn^{-1}) was greatest in mint and basil (avg. 25.5) followed by balm (avg. 21.3) and lowest in thyme (avg. 15.6). Accordingly, yield per unit of nitrogen uptake (kg kg^{-1} N) was highest in mint and basil (avg. 124.6), followed by balm (avg. 103.9) and lowest in thyme (avg. 76.3). These results may serve as a tool for the better management of greenhouse horticulture schemes under Mediterranean climatic conditions in terms of food-crops rich in antioxidants.

1. Introduction

Growing fresh herbs gained a growing interest in recent years mainly as a part of an existing product line of a greenhouse already in business. Interestingly, aromatic, medicinal and culinary harvested production in Europe has increased from 133 in 2008 to 195 thousand tons in 2016 (Eurostat, 2017). Moreover, the market for flavoring products (spices and herbs) is making a dynamic comeback the last years in the United States (International Trade Center, 2017). In particular, culinary aromatic herbs are part of a specialized market due to the association of their antioxidant activity with health beneficial effects (Zheng and Wang, 2001; Opara and Chohan, 2014). The most frequently reported health effects are mainly attributed to antioxidant, antimicrobial, and antiviral properties of aromatic herbs (Sun et al., 2002; Vallverdu-Queralt et al., 2014). Natural antioxidants in edible plant parts contain vitamins, phenolics, and carotenoids (Kaur and Kapoor, 2001; Thaipong et al., 2006; Llorach et al., 2008), which act as reactive oxygen species quenchers in the human body (Blokhina et al., 2003; Zhao et al., 2007).

On the other hand, consumer demand for fresh-cut herbs and ready-to-eat products increases throughout the years. Thus, to ensure a continuous and guaranteed supply of high quality herbs, cultivation under protected environmental conditions is suggested. Indeed, the use of greenhouses could lead to extended harvesting period while providing a product of superior quality compared to low-cost imports. Many species of medicinal and aromatic plants were reported to have added values when consumed fresh (Al-Karaki and Othman, 2009). In this perspective, soilless cultivation may be used to enhance yield and quality by increasing water and nutrient use efficiency in the concept of a more precise agriculture in protected environments (Raviv and Lieth, 2008; Sonneveld and Voogt, 2009).

Recent studies have clearly demonstrated that genotype and environmental conditions may alter the antioxidant composition of edible and non-edible plant parts (Blokhina et al., 2003; Shan et al., 2005). Seasonality and particularly light conditions are well known to significantly affect plants antioxidant content and ultimately the quality of

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the product (Toor et al., 2006; Conte et al., 2008). For example, Chang et al (2008) reported that solar irradiance alters the growth of basil and its content of volatile oils while Hussain et al. (2008) found that essential oils and antioxidant properties depends on seasonal variations. Moreover, Kokkini et al. (1997) reported seasonal variations in Greek oregano and Hudaib et al. (2002) found thyme plants cultivated in Italy to be richest in phenols in June/July while the minimum values recorded in November/December. Accordingly, Müller-Riebau et al. (1997) reported maximum values of terpenic constituents, in aromatic plants grown in Turkey, during June/July.

Although antioxidant content and composition in food crops is a key-quality characteristic and it has been thoroughly studied (Wojdylo et al., 2007, Hussain et al., 2008; Vallverdu-Queralt et al., 2014), there is not enough information regarding the effect of growing season on the accumulation of antioxidants in fresh culinary herbs cultivated under greenhouse hydroponic conditions. There is a strong evidence that solar ultraviolet light induces the accumulation of phenolic substances predominantly in epidermal cells of the plant body (Toor et al., 2006). Therefore, it is hypothesized that seasonal changes in solar radiation and temperatures may affect the antioxidant components of greenhouse grown selected aromatic herbs when their harvested season is extended. Furthermore, in recent years, under the pressure of climatic variations and environmental awareness, water- and nutrient-efficient crops with high dietary value are of great interest for greenhouse growers of the Mediterranean basin (FAO, 2013; IPCC, 2014). In this study we define plant water needs, water and nitrogen uptake efficiency in selected aromatic herbs, since in the eastern Mediterranean region it is almost always the water supply that limits yield (Christou et al., 2017).

In view of the above background, this study was designed to evaluate the seasonal variations in major antioxidant components (phenolics, ascorbic acid and carotenoids) and the total antioxidant activity (estimated by two complementary methods i.e., FRAP and DPPH) of fresh basil, mint, balm and thyme under greenhouse hydroponics in a Mediterranean climatic region. A second major objective of this study was to evaluate fresh-cut yield, water and nitrogen use efficiency of the studied crops under greenhouse experimental conditions.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment took place under natural light conditions in a greenhouse with dimensions of 27 (L) × 21 (W) m at the Agricultural Research Institute of Cyprus (lat. 34°94′N, long. 33°19′E, altitude 40 m). Greenhouse was N-S oriented, PE-covered (standard greenhouse film) and with an automatic climate control system. Four aromatic herbs of the *Lamiaceae* family, namely basil (*Ocimum basilicum*), mint (*Mentha viridis*), balm (*Melissa officinalis*) and thyme (*Thymus vulgaris*), were grown in soilless culture using cocopeat (Pelemix, Israel) substrate growbacks (100 cm × 40 cm × 12 cm), fitted in 24 Polygal-troughs (Mapal plastics, Israel) of 22 m long in a double-row system. Two Polygal-troughs per replication (20 plants per linear meter) and three replications were arranged in the greenhouse giving a final crop density of 25 plants m^{-2}. The average temperature and relative humidity during the experimental period (from November, 2012 to October 2013) were maintained between 15–30 °C and 60–85%, respectively. Average outside solar radiation was 18.8 MJ m^{-2}.

2.2. Agronomic features

A nutrient solution (NS) of the following composition (mmol L^{-1}): 6.0 K, 4.15 Ca, 2.2 Mg, 13.6 N-NO_3, 1.0 N-NH_4, 1.25 P-H_2PO_4, 2.24 S-SO_4, and trace elements (μmol L^{-1}): 20 Fe, 10 Mn, 4 Zn, 0.75 Cu, 25 B and 0.5 Mo, was applied to provide plants with essential nutrients. This NS corresponded to an electrical conductivity (EC) of 2.2 dS m^{-1}. Plants were supplied with a known amount of nutrient solution and the drainage (~25% run-off) was measured. The difference of these two amounts of solution corresponded to the water consumption by the plants, since losses from the system were negligible. The irrigation schedule was controlled by Dagan drain-water monitoring system (Galgon, Kfar-Blum, Israel). In addition to irrigation, the Dagan was capable of controlling and monitoring both pH and EC levels in irrigation and drainage water (e.g. water dosage was increased if EC level was too high or drainage quantity was insufficient).

Water use efficiency (WUE) was defined as the fresh yield per unit of water consumed (kg tn^{-1}) and nitrogen use efficiency (NUE) as the fresh yield per unit of nitrogen uptake (kg kg^{-1} N). Nitrogen uptake (NO_3 + NH_4) was defined indirectly from differences in nitrogen solution composition at drip irrigation solution and in the drainage in relation with the amount of nutrient solution consumed by the crop (Neocleous and Savvas, 2017). Nitrate (NO_3^-) and ammonium (NH_4^+) concentrations in solution samples were measured by UV/VIS spectroscopy at 210 and 653 nm, respectively.

Herbal transplants were established in the soilless system from November, 2012 to October, 2013. Sequential plantings of basil crops were needed, following local practices. Harvested fresh tops (15 cm) of the plants of each replicate were weighted to determine fresh yield. Plant samples (i.e., fresh tops) used for quality analysis were harvested before flowering at four harvesting peaks (early-December, late-March, late-June and mid-October), immediately frozen, placed in polyethylene bags and stored at −30 °C prior to analyses.

2.3. Determination of herbal antioxidants

Edible aerial parts of each replication were macerated in a blender. For total phenolics and ferric reducing antioxidant power (FRAP assay) tissue extraction was performed by acidified acetone (Kähkönen et al. 2001; Asami et al. 2003). The content of total phenolics was measured using the Folin-Ciocalteu's procedure by diluting 0.5 mL of plant extract to 2.5 mL diluted 1/10 Folin-Ciocalteu's reagent and 2 mL 7.5% sodium carbonate aqueous solution and measuring the absorbance at 760 nm (Scalbert et al., 1989). Gallic acid was used as standard and the results were expressed as mg of gallic acid equivalent (GAE)/g FW. For the determination of antioxidant capacity, two complementary methods were applied (Harris and Brannan 2009): (1) the reducing potential was determined by FRAP assay (Benzie and Strain 1996, 1999) using ascorbic acid (AA) as standard to express the absorbance (593 nm) of test samples (100 μL extract and 3 mL FRAP solution; Pantelidis et al., 2007) as μmol AA/g FW, and (2) radical scavenging activity was determined using the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical (Brand-Williams et al., 1995). The samples were homogenized with methanol and the absorbance of the reaction mixtures (20 μL extract and 2980 μL of 100 μM DPPH methanolic solution; Koukounaras et al., 2007) was measured at 517 nm. The standard curve was developed using ascorbic acid (AA) and DPPH values were expressed as mg AA equivalents antioxidant capacity (AEAC)/g FW. Total AA in plant material was determined by homogenization of suitable quantity of leaves with trichloroacetic acid solution according to Reflectoquant® ascorbic acid test protocol (Merck, Darmstadt, Germany) and then analyze the pretreated sample solution using the RQflex plus reflectometer (Merck). For chlorophyll and carotenoid determination, leaves were extracted with 80% acetone and the absorbance of extracts was measured spectrophotometrically at 470, 648 and 664 nm (Lichtenthaler, 1987). Results were expressed in μg/g FW. Chemicals were of analytical grade and purchased from Merck (Germany) and Sigma-Aldrich (Germany). Assays were performed using an automated UV/VIS spectrophotometer (Helios Zeta, Thermo Scientific, USA).

2.4. Statistical analysis

Experimental layout consisted of a randomized complete block design with three replicates for each treatment. Analysis of variance