

Optimization of potassium fertilization/nutrition for growth, physiological development, essential oil composition and antioxidant activity of *Lavandula angustifolia* Mill.

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Abstract

Lavandula angustifolia (Mill.) is a medicinal plant of great importance with a variety of applications in perfume and pharmaceutical industries or landscaping, but only limited information is available about its response to potassium supplementation. This experiment was conducted in order to determine the effects of potassium (K: 275-300-325-350-375 mg L⁻¹) levels on the morphological and biochemical characteristics of lavender grown hydroponically. Results showed that K levels affected plant growth, mainly the root development while no differences were found for leaf chlorophyll content and stomatal conductance. Essential oil yield increased in the 300 mg L⁻¹ in comparison to 275 or 325-375 mg L⁻¹ of K application. The middle ranged K levels (300-325-350 mg L⁻¹) enhanced leaf content in total phenols, flavonoids and antioxidant activity (DPPH, FRAP). Lipid peroxidation content (MDA) employed as damage index, was lower in the 325 mg L⁻¹ of K treatment. No differences were observed in antioxidant enzymes (SOD, CAT, APX) activity and H₂O₂ content among K applications. The main constituents of leaves essential oil (1,8-cineole, borneol, camphor, α -terpineol, myrtenal) and mineral accumulation were affected by K treatments. Lavender grown in 300 mg L⁻¹ of K was appropriate for the essential oil uses/production while the 325 mg L⁻¹ of K were more appropriate for lavender cultivation for fresh and dry matter uses.

Keywords: Antioxidants, essential oil, *Lavandula angustifolia*, minerals, soilless culture

1. Introduction

Common or English lavender (*Lavandula angustifolia*), an important evergreen perennial shrub, is used medicinally, in the perfumery, balms, salves, soaps, cosmetics and extensively used as model plant for isoprenoid biosynthesis studies (Biswas *et al.*, 2009). Essential oil (EO) of lavender has sedative, carminative, antiseptic, anti-inflammatory, analgesic and antimicrobial (antifungal and bactericidal) properties due to the high content in terpenes (Biesiada *et al.*, 2008). Lavender contains polyphenols, natural pigments (anthocyanins, carotenoids) and chemicals with great antioxidant role in human health; however, there is lack of knowledge in literature on the antioxidant properties of lavender (Miliauskas *et al.*, 2004). The typical aroma of lavender is derived mainly by linalool, linalyl acetate, 1,8-cineole, o-cymene, borneol, and camphor compounds (Biswas *et al.*, 2009). Lavender species are usually cultivated in many world regions as ornamental and medicinal plants. Moreover, lavender is native to the Mediterranean basin, in semi-arid regions, and grows in natural sites of lower parts of mountains.

Several variables are responsible for the chemical and biological diversity that medicinal and aromatic plants may have, including the growing area and crop cultural practices, microclimate, vegetative or reproductive stage as well as genetic variation (Miliauskas *et al.*, 2004). The effect of mineral application by means of fertilizers, on lavender plant antioxidant activity as well as essential oil yield and constituents is poorly known. Due to the increasing demand for aromatic/medicinal crop for commercial purposes, appropriate cultivation practices and mineral application are necessary. On that point, modern and precisely growing techniques such as hydroponics (growing crops without soil) may benefit crop production (Klados and Tzortzakakis, 2014). The application of fertilizers and the mineral uptake/accumulation are the two most

important factors that increase plant yield and productivity (Almeida *et al.*, 2015). The essential oil production in aromatic plants may be affected positively or negatively by the form, the type and the amount of the fertilizers (Yadegari, 2015). Minerals such as nitrogen (N), phosphorous (P), and potassium (K) may affect the growth and essential oil synthesis in aromatic plants and are used by plants to build many organic compounds such as amino acids, proteins, enzymes and nucleic acids. These minerals affect the function and levels of enzymes involved in the terpenoid biosynthesis (Hafsi *et al.*, 2014). The monovalent cations, such as K, in enzyme activation, plays a role to aid substrate binding by lowering energy barriers in the ground and/or transition states rather than being the agents of causing catalysis (Page and Di Cera, 2006). Potassium is considered a plant essential mineral that can be usually found in high concentration in tissues, in particular in the meristem and in the phloem, while Hafsi *et al.* (2014) reviewed that K uptake by the plant roots is accomplished by at least two distinct kinetic systems, high and low affinity K⁺ transporters. Nurzynska-Wierdak (2013) reviewed that K deficiency may disrupt the N metabolism, exhibit changes in N:K ratio and/or in the nitrogen fractions proportions as well as in the accumulation of harmful amino substances and ammonium ions in the plant. Chrysargyris *et al.* (2016) reported that N and P level efficiency might be related with the appropriate K levels in lavender plants, highlighting the needs for further study to that direction. Potassium is an important element in plant metabolism, promoting carbohydrates, fats and protein synthesis, increasing crop yield and improving fresh produce quality. Moreover, K is enabling plants efficacy to resist pests and diseases as well as K is acted as enzymes cofactor, including enzymes related to the essential oil synthesis (Cakmak, 2005; Hafsi

et al., 2014). The application of K affected plant growth and essential oil yield of lemongrass (*Cymbopogon flexuosus*), dittany (*Origanum dictamnus*), basil (*Ocimum basilicum*) and rosemary (*Rosmarinus officinalis*) plants (Economakis, 1993; Puttanna *et al.*, 2010).

Nowadays, the use of chemical fertilizers has been confined mainly to the application of N and P in soil, due to basic fertigation/applications before annual crop establishment, including vegetables, aromatic and ornamental crops –in $\text{NH}_4\text{-N}$ form for N- and less attention has been paid to K, often applied as supplementary mineral, according to the crop needs. The objective of the present study was to examine the performance of K application on physiological and biochemical level, considering previous findings on efficacy of N and P levels (Chrysargyris *et al.*, 2016) on lavender plant.

2. Materials and Methods

2.1. Plant material and growth conditions

The current study took place at the hydroponic greenhouse infrastructure of the experimental farm, at the Cyprus University of Technology, Cyprus, during two spring-summer seasons of 2013 and 2014. The effect of K levels into nutrient solution was examined, considering five concentration of 275, 300, 325, 350 and 375 mg L^{-1} with 200 mg L^{-1} N and 50 mg L^{-1} P based on preliminary studies and/or previous reports (Chrysargyris *et al.*, 2016). Each K treatment consisted of 6 replications (2-3 plants in each replication; 15 plants in total for each treatment). Air temperature during this period was fluctuated among 18 and 30 °C.

Lavender (*L. angustifolia* Mill.) plants were purchased from the Cypriot National Centre of Aromatic Plants in trays at the stage of 3-4 leaves and 4-5 cm height. Seedlings were transplanted into pots (1

per pot) with perlite (5 L per pot). Pots arranged in singles row on a density of 0.06 plants per m^2 .

Plants grown in an open soilless culture system, with the excess nutrient solution drained away.

A complete nutrient solution was used (Chrysargyris *et al.*, 2016). Fertigation was applied during daytime (8 times of 1 min @ 30 mL min^{-1} flow rate) using pressure pumps with a drip irrigation system (via emitters; one emitter plant⁻¹). The target pH and electrical conductivity (EC) of the nutrient solution were 5.8 and 2.1 mS cm^{-1} respectively.

2.2. Plant growth and tissue analysis

Lavender plants were grown over one week with a complete nutrient solution application for better root establishment. Following eight weeks of plant growth with five K-level nutrient solutions, six individual plants for each treatment were considered for detail plant growth analysis. Plant height, leaf length, root length, fresh and dry plant weight were determined for upper and root part.

2.3. Leaf stomatal conductance and chlorophylls content

For chlorophylls extraction, leaf tissue (six replications/treatment; each replication consisted of a pool of two plants tissue; leaf disk: 0.1 g) was incubated in heat bath at 65 °C for 30 min, in the dark, with 10 mL dimethyl sulfoxide (DMSO, Sigma Aldrich, Germany). Photosynthetic leaf pigments, chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (t-Chl) content were calculated as follows: Chl a = $0.0127 \times A_{663} - 0.00269 \times A_{645}$; Chl b = $0.0229 \times A_{645} - 0.00468 \times A_{663}$; and t-Chl = $0.0202 \times A_{645} + 0.00802 \times A_{663}$ (Richardson *et al.*, 2002). Stomatal conductance was measured using a ΔT -Porometer

AP4 (Delta-T Devices-Cambridge, UK) according to the manufacturers Instructions.

2.4. Plant mineral element analysis

Leaf (6 replications/treatment) and root (3 replications/treatment) samples (0.2-0.3 g) were analysed for nutrient content as described in Chrysargyris *et al.* (2016). Determination of K, P, Ca, Mg, Fe, Cu, Mn, Zn, Na, and B was done by inductively coupled plasma atomic emission spectrometry [ICP-AES; PSFO 2.0 (Leeman Labs INC., USA) and N by the Kjeldahl (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360) method.

2.5. Leaf essential oil extraction and gas chromatography/mass spectrometry analyses

Three biological samples (pooled of three individual plants/sample) for each treatment, harvested just before flowering, and air-dried lavender leaves (in oven at 42 °C) were chopped and approx. 15-20 g of sample were hydrodistilled for 3 h, using Clevenger apparatus for EO extraction.

The EOs were analyzed by Gas chromatography-Mass Spectrometry (GC/MS) and their constituents were determined as described previously (Chrysargyris *et al.*, 2016).

2.6. Leaf polyphenol extraction and analyses

2.6.1. Preparation of extracts

For polyphenol extraction, six leaves samples (pooled by two individual plants/sample) for each treatment were used. The freshly cut plants (0.5 g) were milled with 10 mL methanol (50% v/v) and extraction was assisted with ultrasound. The samples were centrifuged for 30 min on 4000 g at 4 °C

(Sigma 3-18 K, Sigma Laboratory Centrifuge, Germany). The supernatant was transferred to a 15 mL falcon tube, stored at 4 °C until analysis (within 48 h) for evaluation of total phenolic and flavonoids content and total antioxidant activity by the DPPH and FRAP radical scavenging assay.

2.6.2. Total phenolic content

The total phenolic content of the methanol (50% v/v) extracts was determined by using Folin-Ciocalteu reagent (Merck), according to the procedure described by Tzortzakis *et al.* (2007). The absorbance at 755 nm was measured versus the prepared blank. Each measurement was repeated in triplicate and the total phenolic content was expressed as μmol of gallic acid equivalents per gram of fresh weight, through a calibration curve with gallic acid.

2.6.3. Total flavonoid and DPPH and FRAP radical scavenging assay

The total flavonoid content was determined according to aluminium chloride colorimetric method as modified in Chrysargyris *et al.* (2016). The absorbance was measured at 510 nm. The total flavonoid concentrations are expressed as rutin equivalents (mg rutin g^{-1} of fresh tissue). The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and the ferric reducing antioxidant power (FRAP) assay performed of the plant extracts, as described previously (Chrysargyris *et al.*, 2016). Results were expressed as mg trolox g^{-1} of fresh weight.

2.7. Damage index: Determination of content of H_2O_2 and lipid peroxidation

The content of H_2O_2 was determined using the method given by Loreto and Velikova (2001). Leaf tissue of

0.2 g was ground in ice cold 0.1% trichloroacetic acid (TCA) and centrifuged at 15000 g for 15 min. Then, 0.5 mL of the supernatant were mixed with 0.5 mL of 10 mM potassium-phosphate buffer (pH=7.0) and 1 mL of 1 M potassium iodide. H_2O_2 concentration was evaluated using a standard calibration curve plotted in the range from 5 to 1000 μM . The absorbance of samples and standards was read at 390 nm and results were expressed as $\mu mol H_2O_2 g^{-1}$ fresh weight.

Lipid peroxidation was measured in terms of malondialdehyde content (MDA) as described by Azevedo-Neto *et al.* (2006). Leaf tissue of 0.2 g was homogenized in 0.1% TCA and the extract was centrifuged at 15000 g for 10 min. The reaction mixture of 0.5 mL extract and 1.5 mL of 0.5% thioarbituric acid (TBA) in 20% TCA was incubated at 95 °C for 25 min and then cooled on ice bath. The absorbance was determined at 532 nm and corrected for non-specific absorbance at 600 nm. MDA amount was determined using the extinction coefficient of 155 $mM^{-1} cm^{-1}$. Results were expressed as nmol of MDA g^{-1} fresh weight.

2.8. Activities of antioxidant enzymes

Fresh leaf tissue was homogenized in mortar and pestle using ice cold extraction buffer containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1% (w/v) polyvinylpyrrolidone (PVPP), 1 mM phenyl-methylsulfonyl fluoride (PMSF) and 0.05% Triton X-100 in 50 mM potassium-phosphate buffer (pH=7.0). For APX activity extraction buffer was supplemented with 5 mM ascorbic acid. Protein content in the enzyme extracts was determined using bovine serum albumin (BSA) as a standard (Chrysgyris *et al.*, 2016).

Total catalase activity (CAT) (EC 1.11.1.6) and total superoxide dismutase activity (SOD) (EC 1.15.1.1)

were assayed following the methods as described by Jiang and Zhang (2002). For catalase activity the reaction mixture consisted of 50 mM potassium phosphate buffer (pH=7.0), 10 mM H_2O_2 , 10 μL of extract and distilled water until final volume 1.5 mL. Reaction started with the addition of H_2O_2 , and the decrease in absorbance recorded at 240 nm and quantified by its extinction coefficient (39.6 $mM^{-1} cm^{-1}$). The results were expressed as CAT units mg^{-1} of protein (1 unit= 1 mM of H_2O_2 reduction $min^{-1} mg^{-1}$). SOD reaction consisted of 50 mM potassium phosphate buffer (pH=7.5), 13 mM methionine, 75 μM nitro blue tetrazolium (NBT), 0.1 mM EDTA, 2 μM riboflavin and 10 μL of extract. Water was added until volume reached 1.5 mL. Reaction started with the addition of riboflavin and placing tubes with the reaction mixture below a light source of two 15 watt fluorescent lamps for 15 min. Reaction stopped by placing the tubes in the dark. Mixtures without enzyme extract developed maximal color (control) and non-irradiated mixture used as blank. The absorbance was determined at 560 nm and activity was expressed as units mg^{-1} of protein. One unit of enzyme activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT photo-reduction rate.

The activity of ascorbate peroxidase (APX) (EC 1.11.1.11) was determined according to Zhu *et al.* (2004), by the decrease in absorbance of ascorbate at 290 nm. The assay mixture contained 50 mM potassium phosphate buffer (pH=7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA and enzyme extract. Reaction started with the addition of 25 μL of 30 mM H_2O_2 , to a final volume of 3 mL. The APX activity was calculated using the extinction coefficient 2.8 $mM^{-1} cm^{-1}$. One enzyme unit is the amount for the decomposition of 1 μmol of substrate per minute. Results were expressed as units APX mg^{-1} of protein.

2.9. Statistical methods

The whole experiment was carried out twice, with similar outcomes (EO was analysed only in the 1st year), and therefore the 1st year experiment was further analyzed/presented. Data were statistically analysed using analysis of variance (ANOVA) by IBM SPSS v.22 for Windows, and presented as treatment mean \pm SE of six biological measurements.

Percentage values were log-transformed prior to subjecting data to ANOVA. The chemical compounds and the relationship among nutrient concentrations were determined by Linear Discriminate analysis (LDA) performed on the percentages of all identified compounds for all K concentrations using the SPSS program. Duncan's multiple range tests were calculated for the significant data at $P < 0.05$.

3. Results

3.1. Growth parameters

Table 1 presents the effect of K application on plant growth related variables. Leaf length and root fresh weight were significantly ($P < 0.05$) increased in 325 mg L⁻¹ K application compared with lower (275-300 mg L⁻¹) or higher (350-375 mg L⁻¹) K concentrations. The highest ratio of upper plant biomass: root was in 300 mg L⁻¹ K due to the low root fresh weight of that treatment. The opposite findings were evidenced for the 325 mg L⁻¹ K treatment. The K concentrations into nutrient solution did not affect plant height (averaged in 18.7 cm), fresh and dry biomass (averaged in 15.4 and 4.4 g plant⁻¹ respectively). Root length was increased (up to 41 %) in 325-350 mg L⁻¹ compared

Table 1. Influence of different potassium (K) levels on lavender plant height (cm), leaf length (cm), biomass fresh weight (FW; g plant⁻¹), biomass dry matter (DM; %), root fresh weight (FW; g plant⁻¹), root dry matter (DM; %), biomass:root ration and root length (cm).

K	K275	K300	K325	K350	K375
Plant height	18.16 \pm 1.01a ^Y	18.33 \pm 0.42a	20.31 \pm 0.70a	17.83 \pm 0.70a	19.16 \pm 1.13a
Leaf length	5.44 \pm 0.07b	4.90 \pm 0.10c	6.24\pm0.10a	4.88 \pm 0.09c	4.99 \pm 0.17c
Biomass FW	17.02 \pm 2.57a	13.06 \pm 1.54a	15.89 \pm 1.05a	14.69 \pm 1.38a	17.21 \pm 2.09a
Biomass DM	28.49 \pm 1.45a	30.43 \pm 0.75a	28.66 \pm 0.61a	29.80 \pm 0.91a	27.92 \pm 0.48a
Root FW	8.96 \pm 1.33b	3.14 \pm 0.96c	19.07\pm1.39a	5.07 \pm 0.72c	6.34 \pm 1.55bc
Root DM	10.80 \pm 1.01b	27.25\pm3.27a	12.02 \pm 0.27b	14.94 \pm 1.19b	15.60 \pm 0.90b
Biomass : root	2.05 \pm 0.43bc	5.33\pm0.85a	0.82 \pm 0.08c	3.10 \pm 0.37b	3.13 \pm 0.40b
Root length	15.58 \pm 1.02b	17.66 \pm 1.73ab	21.90\pm1.68a	20.08\pm0.91a	17.66 \pm 1.51ab

^Y Each value is means \pm SE (n=6). Values in rows followed by the same letter are not significantly different, $P < 0.05$. K275, K300, K325, K350 and K375 indicate potassium concentration in mg L⁻¹, respectively.

with the 275 mg L⁻¹ K application. Considering regression analysis among K level into nutrient solution and lavender biomass produced, it was revealed significant negative correlation ($Y = -0.0001x + 0.0915$; $R^2 = 0.70$; $P = 0.03$) as K levels increased from 275 to 375 mg L⁻¹.

3.2. Physiological parameters

In the present study, neither the content of chlorophylls (Chl a, Chl b and total Chl) nor the stomatal conductivity were affected by different K concentrations into the nutrient solution (Table 2).

Table 2. Influence of different K levels on leaf stomatal conductivity (cm s^{-1}), chlorophylls (Chl a, Chl b, Total Chl) content (mg g^{-1} fresh weight) and essential oil (EO) yield (%) of lavender plants.

K	K275	K300	K325	K350	K375
Stomatal conductivity	2.13±0.78a ^Y	2.07±0.35a	1.31±0.22a	1.97±0.29a	1.51±0.53a
Chl a	1.07±0.05a	1.11±0.04a	1.10±0.19a	1.08±0.04a	1.11±0.06a
Chl b	0.33±0.01a	0.34±0.01a	0.33±0.01a	0.34±0.01a	0.34±0.02a
Total Chl	1.40±0.07a	1.45±0.06a	1.43±0.02a	1.42±0.05a	1.45±0.08a
EO	0.93±0.05b	1.65±0.03a	0.89±0.16b	0.97±0.19b	1.00±0.21b

^YEach value is means ± SE (n=6 for stomatal conductivity and Chls; n=3 for EO). Values in rows followed by the same letter are not significantly different, $P < 0.05$. K275, K300, K325, K350 and K375 indicate potassium concentration in mg L^{-1} , respectively.

Thus, the Chl a content ranged from 1.07 to 1.11 mg g^{-1} fresh weight, the Chl b content ranged from 0.33 to 0.34 mg g^{-1} fresh weight and the total Chl content ranged from 1.40 to 1.45 mg g^{-1} fresh weight. Leaf stomatal conductivity ranged from 1.31 to 2.13 cm s^{-1} for the different K applications.

Intermediate potassium concentrations (300-350 mg L^{-1}) affected the antioxidant activity of the lavender plants (Figure 1). Total phenols and DPPH radical scavenging activity were increased (up to 34% and 55% respectively) in 300-350 mg L^{-1} compared with the 275 mg L^{-1} K concentration. The FRAP radical scavenging activity as well as the total flavonoids content were increased in 300, 325 and 350 mg L^{-1} K applications comparing with the lowest (275 mg L^{-1}) and the highest (375 mg L^{-1}) K concentrations.

The lowest MDA content was found in 325 mg L^{-1} of K application while the greater one was found in 275 mg L^{-1} of K application (Figure 2). Neither H_2O_2 content nor the activities of antioxidant enzymes (SOD, CAT, APX) were significantly affected by K concentration.

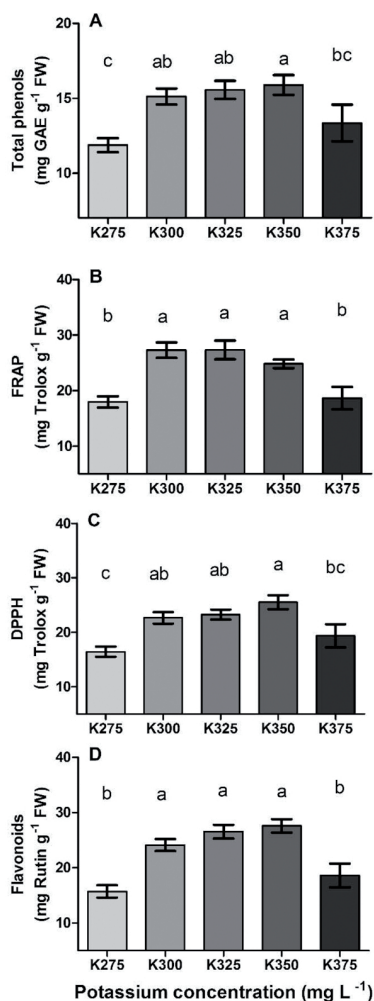


Figure 1. Effects of different potassium (K) levels on the content of total phenols and flavonoids and antioxidant activity in lavender. (A) total phenols, (B) FRAP, (C) DPPH and (D) flavonoids. Significant differences ($P < 0.05$) among treatments are indicated by different letters. Error bars show SE (n=6).

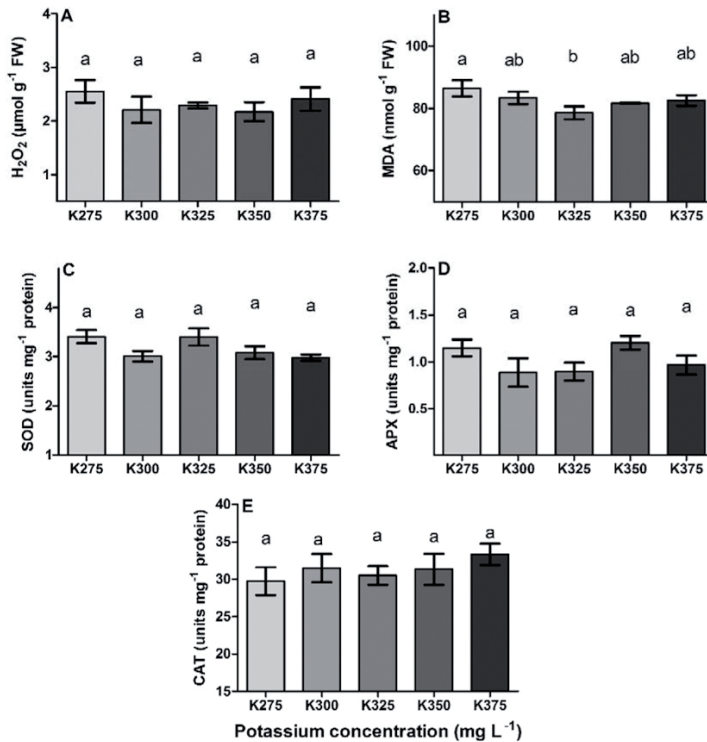


Figure 2. Effects of different potassium (K) levels on the damage index and antioxidant enzymes activities in lavender plant. (A) H₂O₂, (B) Lipid peroxidation (MDA), (C) SOD, (D) APX, and (E) CAT. Significant differences (P<0.05) among treatments are indicated by different letters. Error bars show SE (n=6).

3.3. Leaf and root nutrient content

Increasing K concentration affected macronutrients and micronutrient in both leaves and roots (Tables 3 and 4). Plant tissue mineral content ranged from 17.46 to 19.19 g kg⁻¹ for N; 15.48 to 18.18 g kg⁻¹ for K; 1.37 to 1.68 g kg⁻¹ for P. The greater content for N (including NO₃ and NH₄ form, data not presented) and K were observed in 375 mg L⁻¹ K. Moreover, the greater content for Ca, Mg, P and Mn and the lowest Al (known for possible toxic effects in high concentrations) content were observed in 325 mg L⁻¹ K treatment. Lower K concentration at 275 mg L⁻¹ into

nutrient solution resulted in plant higher Fe, Zn and B content. The Cu content was not affected by the different K concentrations into the nutrient solution. The highest Na content was observed at 300 mg L⁻¹ K which is lower than the optimum K concentration found for the above mentioned cations. Regression analysis among K levels into nutrient solution and lavender tissue mineral content revealed a significant positive correlation as K increased from 275 to 375 mg L⁻¹ for N (P= 0.0089) and K (P= 0.0014) while significant negative correlation was found for Al (P< 0.0001), B (P= 0.013), Fe (P= 0.0008), Zn (P= 0.0329) and Mg (P= 0.0026).

Table 3. Effects of different potassium (K) level on lavender leaf analysis (macronutrient and micronutrient)

K	K275	K300	K325	K350	K375
N (g kg ⁻¹)	17.46±0.55b ^Y	17.60±0.39b	18.62±0.43ab	18.06±0.45ab	19.19±0.36a
K (g kg ⁻¹)	16.33±0.45bc	15.48±0.36c	16.44±0.54bc	16.83±0.15b	18.18±0.44a
Ca (g kg ⁻¹)	7.33±0.10bc	7.57±0.27ab	8.05±0.17a	7.63±0.27ab	6.82±0.17c
P (g kg ⁻¹)	1.45±0.08ab	1.37±0.05b	1.68±0.08a	1.55±0.09ab	1.56±0.02ab
Mg (g kg ⁻¹)	2.14±0.05bc	2.26±0.09ab	2.18±0.04a	2.07±0.07ab	1.87±0.03c
Na (g kg ⁻¹)	0.66±0.07ab	0.80±0.02a	0.75±0.06ab	0.69±0.06ab	0.58±0.01b
Al (mg kg ⁻¹)	121.83±3.52a	101.50±4.88b	76.61±2.79c	90.91±6.08b	74.03±3.74c
Fe (mg kg ⁻¹)	171.33±14.15a	155.16±7.32ab	138.91±11.30abc	119.50±6.29c	127.16±12.07bc
Mn (mg kg ⁻¹)	21.48±2.39b	26.06±2.50ab	29.52±1.22a	21.98±2.24b	21.71±1.70b
Zn (mg kg ⁻¹)	5.33±0.35a	5.05±0.54ab	3.84±0.23bc	3.43±0.54c	4.29±0.22abc
Cu (mg kg ⁻¹)	3.98±0.28a	3.70±0.24a	3.81±0.23a	3.86±0.36a	3.87±0.33a
B (mg kg ⁻¹)	35.15±1.13a	33.13±0.98ab	32.61±0.76ab	32.45±1.77ab	30.45±1.35b

^Y Each value is means ± SE (n=6). Values in rows followed by the same letter are not significantly different, P<0.05.

K275, K300, K325, K350 and K375 indicate potassium concentration in mg L⁻¹, respectively.

Table 4. Effects of different potassium (K) level on lavender root analysis (macronutrient and micronutrient)

K	K275	K300	K325	K350	K375
N (g kg ⁻¹)	19.51±0.12b ^Y	21.15±1.57b	25.83±1.55a	18.42±0.60b	19.93±0.08b
K (g kg ⁻¹)	13.02±1.20b	15.46±1.03b	22.65±0.67a	13.40±0.92b	13.85±1.81b
Ca (g kg ⁻¹)	13.83±1.77a	11.37±1.36a	9.59±0.53a	14.06±1.70a	11.48±1.62a
P (g kg ⁻¹)	2.90±0.12a	3.34±0.14a	2.85±0.35a	3.27±0.20a	2.77±0.02a
Mg (g kg ⁻¹)	3.57±0.16b	3.64±0.04b	4.43±0.30a	3.45±0.08b	3.45±0.02b
Na (g kg ⁻¹)	4.98±0.61b	4.97±0.34b	7.70±0.80a	3.65±0.28b	3.75±0.43b
Al (mg kg ⁻¹)	191.00±30.08a	134.33±30.71a	151.31±22.80a	224.66±61.41a	162.00±33.00a
Fe (mg kg ⁻¹)	487.66±47.47a	337.00±43.14a	323.00±31.16a	467.00±97.81a	390.00±73.90a
Mn (mg kg ⁻¹)	120.00±7.00c	121.66±15.19c	144.61±17.54bc	185.00±9.45a	181.50±5.48ab
Zn (mg kg ⁻¹)	29.70±0.60a	26.73±3.79a	22.23±4.01a	27.33±3.09a	24.56±1.81a
Cu (mg kg ⁻¹)	78.23±4.87a	81.21±3.07a	70.01±3.11a	71.62±6.81a	70.86±5.10a
B (mg kg ⁻¹)	28.26±1.67b	22.33±1.26bc	36.61±4.07a	22.60±1.50bc	18.10±0.05c

^Y Each value is means ± SE (n=3). Values in rows followed by the same letter are not significantly different, P<0.05.

K275, K300, K325, K350 and K375 indicate potassium concentration in mg L⁻¹, respectively.

3.4. Essential oil yield and constituents

Lavender EO yield significantly (P< 0.05) increased in 300 mg L⁻¹ of K application, while no differences on essential oil yield were found for the 275, 325, 350 and 375 mg L⁻¹ K treatments (Table 2). The effects of different K levels on EO chemical composition are presented in Table 5.

Thirty nine components were identified in the EOs of lavender plants underwent at different treatments that represented 99.32-99.66% of the oils. It can be noticed that hydrocarbon compounds ranged from 9.01 to 10.31% while oxygenated (monoterpenes and sesquiterpenes) compounds were ranged from 85.78 to 88.40 and 1.40 to 2.78%, respectively.

Table 5. Effects of different potassium (K) level on lavender leave chemical composition (%) of essential oils

Compound	RI	K concentrations				
		K275	K300	K325	K350	K375
α -Pinene	933	2.05±0.11 a	2.11±0.08 a	2.10±0.02 a	2.04±0.09 a	2.28±0.18 a
Camphene	948	0.43±0.03 a	0.43±0.03 a	0.50±0.00 a	0.44±0.07 a	0.44±0.05 a
Thuja-2.4 (10)- diene	954	0.04±0.00 b	0.04±0.00 b	0.09±0.02 a	0.05±0.00 b	0.04±0.00 b
Sabinene	973	0.84±0.04 ab	0.78±0.03 b	0.98±0.03 a	0.82±0.01 b	0.88±0.09 ab
β -Pinene	977	3.47±0.22 a	3.25±0.16 a	3.55±0.00 a	3.32±0.10 a	3.65±0.33 a
β -Myrcene	991	0.37±0.02 a	0.43±0.02 a	0.43±0.00 a	0.46±0.07 a	0.47±0.01 a
α -Terpinene	1017	0.14±0.01 ab	0.12±0.01 b	0.20±0.02 a	0.12±0.02 ab	0.13±0.02 ab
o-Cymene	1024	0.16±0.00 b	0.14±0.00 bc	0.21±0.00 a	0.16±0.01 b	0.12±0.01 c
Limonene	1028	1.65±0.13 ab	1.37±0.00 b	1.69±0.07 ab	1.43±0.01 b	1.80±0.19 a
1.8-Cineole	1031	59.72±1.17 b	63.00±1.53a	58.54±0.59 b	62.95±1.45 a	62.22±0.17 ab
γ -Terpinene	1058	0.31±0.01 ab	0.25±0.00 b	0.40±0.03 a	0.26±0.03 b	0.30±0.04 ab
<i>cis</i> -Sabinene hydrate	1067	0.43±0.06 a	0.48±0.02 a	0.67±0.12 a	0.56±0.10 a	0.51±0.04 a
<i>p</i> -Mentha-2,4(8)-diene	1089	0.06±0.00 b	0.06±0.00 b	0.12±0.01 a	0.07±0.01 b	0.06±0.00 b
Linalool	1100	0.17±0.00 a	0.16±0.01 a	0.23±0.04 a	0.17±0.02 a	0.17±0.00 a
Fenchol	1114	0.03±0.00 b	0.03±0.00 b	0.09±0.01 a	0.03±0.00 b	0.04±0.00 b
α -Campholenal	1127	0.26±0.00 b	0.22±0.01 b	0.34±0.02 a	0.22±0.00 b	0.22±0.02 b
<i>trans</i> -Pinocarveol	1139	0.97±0.07 ab	1.00±0.03ab	1.17±0.01 a	0.93±0.07 b	0.92±0.07 b
<i>cis</i> -Verbenol	1141	0.05±0.03 b	0.08±0.01 ab	0.11±0.00 a	0.06±0.01 ab	0.03±0.01 b
Camphor	1145	5.73±0.94 b	5.77±0.30 b	8.02±0.11 a	8.18±0.45 a	5.55±0.07 b
Pinocarvone	1163	0.90±0.00 b	0.83±0.01 b	1.01±0.04 a	0.83±0.00 b	0.83±0.01 b
Borneol	1166	11.85±0.61 a	11.02±0.95 a	9.09±0.37 b	8.56±0.05 b	11.06±0.14 a
Terpinen-4-ol	1178	0.61±0.02 a	0.65±0.01 a	0.66±0.02 a	0.53±0.01 b	0.61±0.01 a
<i>p</i> -Cymen-8-ol	1185	0.11±0.01 a	0.14±0.01 a	0.11±0.00 a	0.10±0.02 a	0.11±0.03 a
Cryptone	1187	0.27±0.01 a	0.38±0.00 a	0.22±0.02 a	0.23±0.05 a	0.29±0.09 a
α -Terpineol	1191	2.11±0.13 a	2.12±0.18 a	2.22±0.02 a	2.02±0.05 a	2.31±0.20 a
Myrtenal	1197	2.15±0.09 ab	1.86±0.09 c	2.38±0.03 a	1.90±0.08 bc	1.88±0.05 c
Verbenone	1211	0.05±0.01 a	0.11±0.00 a	0.05±0.02 a	0.06±0.02 a	0.07±0.05 a
<i>trans</i> -Carveol	1219	0.21±0.03 a	0.23±0.02 a	0.26±0.01 a	0.18±0.04 a	0.17±0.05 a
Bornyl formate	1229	0.13±0.03 a	0.12±0.00 a	0.11±0.01 a	0.05±0.00 b	0.10±0.01 a
Cumin aldehyde	1241	0.26±0.01 ab	0.21±0.00 bc	0.28±0.02 a	0.20±0.00 c	0.20±0.00 c
Carvone	1244	0.23±0.00 abc	0.24±0.00 ab	0.27±0.05 a	0.22±0.01 bc	0.20±0.02 c
<i>p</i> -Cymen-7-ol	1291	0.14±0.01 ab	0.12±0.01ab	0.17±0.00 a	0.10±0.03 ab	0.08±0.03 b
<i>p</i> -Mentha-1,4-dien-7-ol	1329	0.11±0.00 a	0.08±0.01 a	0.11±0.00 a	0.07±0.02 a	0.05±0.02 a
Coumarin	1443	0.05±0.00 a	0.07±0.01 a	0.08±0.02 a	0.05±0.02 a	0.07±0.02 a
Caryophyllene oxide	1587	0.52±0.03 a	0.26±0.02 b	0.48±0.02 a	0.32±0.02 b	0.27±0.00 b
tau-Cadinol	1642	0.64±0.03 a	0.33±0.04 c	0.56±0.03 ab	0.44±0.07 bc	0.35±0.01 c
Bisabolol oxide II	1656	0.30±0.03 a	0.14±0.00 b	0.27±0.01 a	0.15±0.03 b	0.11±0.02 b
α -Bisabolol	1685	1.32±0.08 a	0.66±0.06 b	1.08±0.04 ab	0.97±0.18 ab	0.78±0.03 b
Muurool-5-en-4-one	1689	0.47±0.03 a	0.23±0.02 c	0.39±0.02 ab	0.26±0.06 bc	0.20±0.03 c
Monoterpenes hydrocarbons		9.54±0.52 a	9.01±0.28 a	10.31±0.03 a	9.20±0.25 a	10.19±0.95 a
Sesquiterpenes hydrocarbons		0.00	0.00	0.00	0.00	0.00
Oxygenated monoterpenes		86.10±0.80 b	88.40±0.09 a	85.78±0.31 b	87.93±0.39 a	87.27±0.67 ab
Oxygenated sesquiterpenes		2.78±0.16 a	1.40±0.13 c	2.41±0.11 ab	1.89±0.32 bc	1.53±0.07 c
Others		0.92±0.07 a	0.80±0.01 a	0.82±0.07 a	0.60±0.13 a	0.66±0.05 a
Total		99.34±0.4 a	99.63±0.04 a	99.32±0.17 a	99.66±0.14 a	99.51±0.07 a

Each value is means \pm SE (n=3). Values in rows followed by the same letter are not significantly different, P<0.05.

K275, K300, K325, K350 and K375 indicate potassium concentration in mg L⁻¹, respectively.

The major components were 1,8-cineole (alcohol; 58.54-63.00%), borneol (alcohol; 8.56-11.85%), camphor (ketone; 5.55-8.18%), β -pinene (monoterpene hydrocarbon; 3.25-3.65%), α -terpineol (alcohol; 2.02-2.31%), myrtenal (aldehyde; 1.86-2.38%), α -pinene (monoterpene hydrocarbon; 2.04-2.28%), limonene (monoterpene hydrocarbon; 1.37-1.80%), α -bisabolol (alcohol; 0.66-1.32%) and trans-pinocarveol (alcohol; 0.92-1.17%). Other components were present in amounts less than 1% in most treatments. Examining the K effects on oil constituents, 1,8-cineole reached to its maximal percentage (63.00%) as a result of K 300 mg L⁻¹ application. Camphor reached the greatest (8.18%) value at 350 mg L⁻¹ K and the lowest (5.55%) value at 375 mg L⁻¹ K. Myrtenal greatest percentage was reached at 325 mg L⁻¹ K, while the lowest value was obtained at the 300 mg L⁻¹ K. Borneol content was increased in 275, 300 and 375 mg L⁻¹ K applications. Other components which differed

significantly among K levels were *trans*-pinocarveol (0.92-1.17%), limonene (1.37-1.80%) and α -bisabolol (0.66-1.32%). No differences were found in α -terpineol (averaged in 2.14%), β -pinene (averaged in 3.45) and α -pinene (averaged in 2.11%). The linear discriminant analysis was used in order to identify possible relationships between EO constituents and K concentration (Figure 3). The LDA, performed on average contents of all compounds for each K concentration, showed that the first two principal axes represented 85.2% of the total variation, with 10 EO compounds were identified as having effects. The first axis (65.5% of the total variation) was mainly correlated with α -terpinene, cis-sabinenehydrate and o-cymene. The second axis represented 19.5% of the total variation, with the α -pinene, limonene, camphene, β -pinene, thuja-2,4(10)-diene, β -myrcene and sabinene consisting the main compounds contributing to its definition.

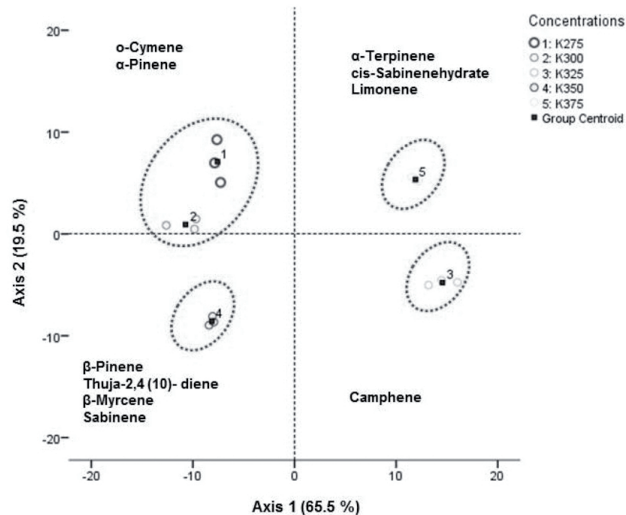


Figure 3. Linear discriminant analysis (LDA) for the lavender essential oil compounds under different potassium (K) levels. Projection of the average contents of the essential oil compounds onto the first two principal axes (+ and – indicate positive and negative correlations with axes, respectively). Coding numbers referred to K concentrations.

According to the LDA, four concentration groups in relation to the nutrient solution could be distinguished situated at the periphery of the plot. The first group represented by concentration 375 mg L⁻¹ K, situated at the positive side of axis 1 and 2. The second group represented by 325 mg L⁻¹ K, situated at the positive side of axis 1 and at the negative side of axis 2. The third group represented by 350 mg L⁻¹ K, situated at the negative sites of both axis 1 and 2. The fourth group represented by 275 mg L⁻¹ K and 300 mg L⁻¹ K, situated at the negative side of axis 1 and the positive side of axis 2.

4. Discussion

The aim of this study was to optimize the K fertigation/nutrition in different levels for the *L. angustifolia* growth, physiological development, mineral content, antioxidant activity and essential oil composition. Previous experimentation on effects of N and P levels on lavender revealed the necessity of examining the optimum K application, related specially to the effects of N:K ratio (Chrysargyris *et al.*, 2016). Therefore, K levels at this study were required to be kept in quite narrow levels, in order to meet the appropriate N:K ratio. Potassium levels usually ranged of 300-350 mg L⁻¹ and more rarely above 400 mg L⁻¹ based on nutritional demands of the crops (Savvas and Passam, 2002). Potassium shortage during plant growth leads to a decrease in chlorophyll content and photosynthetic rate (Gerardeaux *et al.*, 2010), enzymes activation, poor growth and reduced yield (Kanai *et al.*, 2007). The results of this study showed that K had no pronounced effect on most plant growth related parameters (except of leaf length) while considerable effects were observed in root development (root length and fresh weight) for the 325 mg L⁻¹ K application, and as such represented the most favorable treatment for its growth and development.

Leaf length increased possible due to the appropriate sugar signaling as suggested by Gerardeaux *et al.* (2010), however, a more precise examination is necessary before drawing definitive conclusions. In cotton and peanuts, Xiao-Li *et al.* (2008) and Almeida *et al.* (2015) indicated that K⁺ accumulation was positively correlated with both root length and root surface area, increasing biomass and grain yields, respectively. In general, an improved root biomass would also lead to enhanced shoot biomass, however this trend was not clearly evidenced in the present study possibly due to the short period of plant development or to the narrow ranged K concentrations (275-375 mg L⁻¹ K) that were examined in order to prevent any K shortage during crop cultivation.

The content of chlorophylls, the chloroplast ultrastructure and the stomatal conductance are the main factors in the leaf photosynthetic rate (Zhao *et al.*, 2001). In the present study, the content of chlorophylls (Chl a, Chl b and total Chl) and the stomatal conductance were not affected by the K levels, which definitely is not a negative issue, as it is implying similar photosynthetic rates among the examined K levels. The negative impact of K⁺ deficiency on photosynthesis, transpiration and stomatal conductance is well documented (Kanai *et al.*, 2007). Similar photosynthetic rates in the present study may be attributed to the fact that nitrogen concentration was 200 mg L⁻¹ into the nutrient solution whereas, different K application affected the nitrogen:potassium ratio ranging from 1:1.4 (N:K) at 275 mg L⁻¹ K up to 1:1.8 (N:K) at 375 mg L⁻¹ K. Savvas and Passam (2002) reported that the appropriate N:K ratio for several vegetable and flower crops should vary among 1.5-2.2 and actually include the 300, 325, 350 and 375 mg L⁻¹ applications. K-shortage symptoms may not necessarily be readily visible or observed in the field due to a rapid reallocation of K within the plant, the fundamental importance of K may not be directly appreciated (Ren *et al.*, 2013).

Middle ranged potassium concentrations (300-350 mg L⁻¹ K) affected the antioxidative activity of the lavender plants. Consumers and market/industry require plant material with high antioxidant capacity, providing added value products. Inappropriate K levels may also induce stress responses (Cakmak, 2005) and as a consequence stresses can result in the accumulation of reactive oxygen species (ROS). The accumulation of ROS should be avoided as it may result in cell death due to several oxidative processes such as lipid peroxidation, protein oxidation and DNA damage (Gill and Tuteja, 2010). Plants scavenge ROS by increasing the antioxidant enzymes activities/contents, such as SOD, APX, CAT and glutathione reductase (GR) (Foyer and Noctor, 2011). H₂O₂ can be removed through the ascorbate-glutathione cycle AsA-GSH whereas the APX and the SOD are the key enzymes in this cycle (Pasternak *et al.*, 2005). Antioxidant enzymes (SOD, CAT, peroxidase) activity increased in K-deficient bean leaves (Cakmak, 2005) and *Houttuynia cordata* (Xu *et al.*, 2011). Results of the present study showed that 325 mg L⁻¹ of K application decreased the MDA content compared with the 275 mg L⁻¹ of K, maybe by elimination of free radicals (Sharma *et al.*, 2012). MDA increases with environmental stresses and is used for determination of lipid peroxidation or plasmalemma and organelle membranes damage evaluation (Wang *et al.*, 2013). In general, oxidative stress was not evidenced in the present study as neither H₂O₂ production nor the activities of SOD, CAT and APX enzymes got significantly affected by K concentration. Shin *et al.* (2005) demonstrated that H₂O₂ is implicated (accumulated) in the regulation of plant response to K⁺ starvation, which was not the case in the present study, as K⁺ starvation was not evidenced, due to the well-balanced nutrient solution on K levels and K:N ratio indeed.

Increasing K concentration affected macronutrients and micronutrient of both leaves and roots. In general,

lower (i.e. 275 mg L⁻¹ K) levels into nutrient solution affected mainly micronutrients such as Fe, Zn, Al and B content, medium (325 mg L⁻¹ K) level affected Ca, Mg, P and Mn content while higher (375 mg L⁻¹ K) potassium level affected more the N and K content. In roots, the application of 325 mg L⁻¹ K increased the content of N, K, Mg, Na, and B, while the 350 mg L⁻¹ of potassium was mainly affected the Mn content in the roots. Potassium application may resulted in increased N level which actually depressed the Ca and Mg content, due to cation antagonisms (interactive effect), in 375 mg L⁻¹ K treatments, and this was also found in wheat shoots but not in roots, although K did not affect the rate of Mg influx (Hafsi *et al.*, 2014). Although the increased K levels resulted in higher contents of leaf potassium as compared to lower K levels, the higher leaf potassium did not improve the plant biomass, being in accordance with previous studies (Xu *et al.*, 2011). Potassium interacts with almost all essential elements. A synergistic role of K with either N or P has been already noted (Nurzynska-Wierdak, 2013; Cecilio Filho *et al.*, 2015).

Although the 375 mg L⁻¹ of K application resulted in higher contents of potassium content in leaves but not in roots as compared to the 325 mg L⁻¹, there were no any benefits on plant growth/development and essential oil yield. Enhancement in rate of metabolic processes with higher dose of K may also result in increased demand and utilization of other plant nutrients such as N and/or P, being of prime importance. Thus, mineral overdoses may not only result in no plant growth benefits but also negatively affected environmental issues and human health. On the other hand, K deficiency inhibits nitrogen assimilation (Hafsi *et al.*, 2014).

Essential oil yield and constituents in aromatic plants may be supported beneficially by the appropriate fertilization program during crop cultivation. Increasing the potassium rate (i.e. K:N) significantly differentiated

the proportions of the particular components in the investigated lavender oils. In the present study, essential oil yield was increased in 300 mg L⁻¹ of K application, while potassium:nitrogen rate affected the basil oil yield (Nurzynska-Wierdak, 2013). Similarly to our findings, the high K level decreased the essential oil content of bracts and leaves of *Origanum dictamnus* L. plants (Economakis, 1993). It has been reported in *L. officinalis* that 1,8-cineole, borneol and camphor were the predominant components of leaf volatile oil while 1,8-cineole, borneol, linalool and camphor were the major component of inflorescence oil (Chrysargyris *et al.*, 2016). Essential oil quality decreases with increasing of camphor ratios (Biswas *et al.*, 2009), consisting the 275, 300 and 375 mg L⁻¹ K treatment as the most appropriate one. Borneol is easily oxidized to the camphor (ketone) and this was evidence for the 325 and 350 mg L⁻¹ K application. Moreover, the application of 300 mg L⁻¹ of K resulted in increased 1,8-cineole and borneol content. The 1,8-cineole, also known as eucalyptol, due to the pleasant spicy aroma and taste is used extensively in cosmetics, fragrances and flavorings as well as it is used as an insecticide and insect repellent (Sfara *et al.*, 2009).

In plants, the plastidial formation of monoterpenes (C₁₀) are synthesized from the plastid-derived geranyl diphosphate (GDP), and diterpenes (C₂₀) are synthesized from geranyl-geranyl diphosphate. In contrast, the cytoplasmic formation of sesquiterpenes (C₁₅) is synthesized from farnesyl diphosphate (FDP). Based on our findings, monoterpenes were predominated compared with the sesquiterpenes, indicating the 1-deoxy-D-xylulose-5-phosphate (DXP) pathway activation which is localized in plastids, compared with the classical mevalonic acid (MVA) pathway – operates in the cytosol and produces precursor for the biosynthesis of sesquiterpenes (which were in low percentages in the present study) and triterpenes, being in accordance with previous findings in lavender (Lane *et al.*, 2010).

It is worthwhile to mention that several aromatic species, including species by Lamiaceae family, are being harvested typically before or during the flowering period, before seed set, resulting in low regeneration and continues population drop. This fact increases the needs for species cultivation to ensure their conservation under sustainable usage strategy. Indeed, wild medicinal species cultivation is not so simple, as it requires a good interpretation to their adaptation in different microclimate and/or cultivation practices with direct impacts on their chemical composition and/or bioactivity. Successful cultivation and marketing of medicinal and aromatic plants requires reproducibility of bioactive compounds, and on that task, medicinal plants cultivated in hydroponics provide stable and controlled-manner bioactivity. Suitable cultivation practices are needed while controlled mineral uptake either in soils or through hydroponics is one effective way for favorable plant growth and appropriate EO biosynthesis.

5. Conclusions

We examined the effects of potassium rates on hydroponically grown lavender plants, under controlled nutrition status and the middle K levels 300-350 mg L⁻¹ benefited antioxidant activity of lavender. Considering greater essential oil yield and lower camphor percentage, the 300 mg L⁻¹ K treatment could be appropriate for lavender cultivation and production for essential oil uses. Indeed, considering the content for Ca, P, Mg and Mn, lower Al content, the 325 mg L⁻¹ K treatment could be appropriate for lavender cultivation and production for fresh and dry matter uses.

Careful nutrient management is essential to balance high growth in biomass with the production of high quality oil - two essential parameters for profitable production, environmental and human safe.

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