Agronomic performance, essential oils and hydrodistillation wastewaters of *Lavandula angustifolia* grown on biochar-based substrates.

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ABSTRACT

In order to find an efficient alternative to peat as substrate component for horticultural industry, a study was accomplished to assess the influence on *Lavandula angustifolia* potted plants grown in a peat-based substrate amended with increasing amount of conifer wood biochar: 0, 25, 50, 75 and 100% (by volume). Higher values of plant height, leaf area, leaves and flowers production were recorded in potted lavenders grown in substrates with a biochar content ranging from 25% to 75%; at a visual-quality evaluation no significant differences were observed among substrates on the general aesthetic effect of the plants. The aforesaid five different substrates tested did not significantly affect the leaf dry weight and the root-to-shoot ratio. The biochar amendment of peat-based substrates, even at high rates (75% or 100%), did not influence lavender essential oil (EO) quality, its chemical profile and antioxidant activity. The radical scavenging activities of EO and hydro-distilled wastewaters were also detected. Main results from our study seem to suggest that a conifers wood biochar may be efficiently used as substrate alternative to peat to grow *Lavandula angustifolia* plants for EO production.

KEYWORDS

Biochar, growing media, peat reduction, Lavender, essential oils, hydro-distilled wastewaters, polyphenols, antioxidant activity.

Introduction

The genus Lavandula belongs to the Labiatae (Lamiaceae) family including 39 species, numerous hybrids and 400 varieties and is one of the most well-known essential oil crop in the world (Kivrak, 2018). The natural geographical distribution of lavenders ranges from the Canary Islands to Capo Verde, including the Mediterranean area, North Africa, the Arabian Peninsula, and India (Tuttolomondo et al., 2015). The most common cultivated species in the Mediterranean basin is *Lavandula angustifolia* Miller (aka common or English lavender, syn. *L. vera* or *L. officinalis*), an evergreen perennial shrub which is one of the most famous aromatic and medicinal plants (Basch et al., 2004; Pistelli et al., 2017). Nowadays, lavender essential oil (EO), characterized by a high linalool/linalyl acetate and low camphor content, is among the most requested and used in food industry (as flavoring agent for beverages, ice creams, candies and chewing gums), in cosmetics and fragrance industries (as preservative additive for soaps, perfumes, colognes and skin lotions) (Biswas et al., 2009; Da Porto et al., 2009; Kunicka-Styczyńska et al., 2015). Bulgaria and France are the main lavender oil producers in the world (52% and 26%, respectively), reaching together two thirds of total world lavender production (Giray, 2018). In 2017, Bulgaria had a production area of about 4500 ha and produced 200 tons of lavender oil. The volatile compounds of lavender EO have been recently employed in aromatherapy against anxiety, depression, fatigue, hypertension and stress (Lopez et al., 2017).

Lavender EO may be used in dermatology against dermatitis, eczema and psoriasis because it has sedative, antiseptic, anti-inflammatory, analgesic properties and as a potential natural biopesticide for its antimicrobial (antifungal and bactericidal) action

due to the high content in terpenes, polyphenols and natural pigments with high antioxidant activity (Cavanagh and Wilkinson, 2002; Chrysargyris et al., 2016a).

Many of these beneficial properties are due to the EO composition (Pistelli et al., 2017) which is most likely related to growing and pedoclimatic conditions (Figueiredo et al., 2008). Numerous variables may be responsible for the biochemical diversity that aromatic plants show, including the crop cultural practices (Biesiada et al., 2008; Chrysargyris et al., 2016b). Some authors suggest that the physico-chemical characteristics of soil/substrate may be considered a determinant factor in the secondary metabolite biosynthesis of aromatic plants as well as the cultivation techniques (Najar et al., 2019), though few studies were up to now conducted on the effect of different substrates on lavenders crop response (Papafotiu et al., 2000; Kotsiris et al., 2012; Agullo et al., 2013). Production of Lavender plants generally occurs in nurseries as containerized plants, with peat as the main growing substrate component (Najar et al., 2019). Young potted plants (3-6 month-old) are usually purchased for ornamental uses and landscaping, adult plants (1-1.5 year-old) may be grown to start a cultivation aimed to EO production (Pistelli et al., 2017; Giray, 2018).

Peat is the most used organic growing media for horticultural potted plants being commonly considered as a high-quality substrate for producing vegetables and ornamentals (Kern et al., 2017). Up to now, peat has been readily available, easily processed and high performing. Though the numerous and undoubted advantages of peat as growing substrate (low pH and salinity, good water holding capacity and air volume, low amount of pathogens and weeds, easy handling and blending), the continuous extraction of the peat for agricultural uses has resulted in an increasing deterioration of northern hemisphere peatland ecosystems with a contemporary increase of greenhouse

gases production (Zulfiqar et al., 2019). The environmental concern about peat mining for horticulture use is also linked to one of most important environmental services provided by peatlands represented by their carbon sink function (Alvarez et al., 2018). The recent limitation of peat extraction from peatlands, due to a higher environmental concern, has resulted in high demand and increasing prices of this material, which has led to a constant search for alternatives (Fascella, 2015).

Biochar is the solid, carbon rich co-product of incomplete, anaerobic combustion of organic biomass by bio-energy industrial processes known as pyrolysis or gasification (Roccuzzo et al., 2018; oppure Ferlito et al., 2020). These processes are based on the thermochemical decomposition of organic material of plant or animal origin (municipal and agricultural and forestry wastes, sewage sludge) at high temperatures with the absence of oxygen. Physical and chemical properties of biochar may vary according to the thermal conversion process method, temperature and the biomass source (Gaskin et al., 2008; Trazzi et al., 2016). Agricultural interest on biochar is focused on its potential to decrease global net CO_2 emission by an increased carbon storage in soils and on the numerous positive effects as increasing water storage, nutrient supply, microbial life and, consequently, plant growth (Alvarez et al., 2018). Other general interest on biochar is due to its use as peat substitute in the preparation of growing media (soilless substrates) for greenhouse horticultural production (Alvarez et al., 2017; Fascella, 2015; Gasco et al., 2018). However, if few papers on the effect of biochar as substrate component on the performance of potted ornamentals have been published (Tian et al., 2012; Zhang et al., 2014; Dispenza et al., 2016; Fascella et al., 2017), no information are available in the scientific literature regarding the use of biochar on aromatic plants grown in containers. Therefore, the main aim of the current study was to evaluate the influence of different

substrates, containing decreasing percentages of peat and increasing content of conifer wood biochar, on growth and quality of *Lavandula angustifolia* potted plants in view of its possible future use as growing media alternative to peat. The impact of the above mentioned substrates on chemical profiles and antioxidant activities of essential oils and hydro-distillation wastewaters were also investigated.

Materials and methods

Plant materials and growing conditions

The study was accomplished during the 2018 growing seasons (from January to September) in an unheated (28°C day/14°C night) single-span greenhouse (200 m^2) with steel structure and polyethylene cover, located at the Research Centre for Plant Protection and Certification of Palermo (38° 5′ N, 13° 30′ E), NW Sicily. One year-old plants of *Lavandula angustifolia* Mill. were transplanted on 15 January into plastic containers of 22 cm diameter (5 L) using different mixtures (v:v) of sphagnum peat (Baltic Peat, Varena, Lithuania) and conifers wood biochar obtained from chipped trunks and large branches of woody plant trees (*Abies alba*, *Larix decidua*, *Picea excelsa*, *Pinus nigra* and *P. sylvestris*) pyrolysed at 500°C for 4h. Biochar C content was 49.5 g/kg, N content ranged between 0.20 to 0.25%, ash content varied between 25 and 27% of total dry matter. Five peat-based substrates containing increasing percentages of conifers wood biochar at 0% (CB0), 25% biochar (CB25), 50% (CB50), 75% (CB75) and 100% (CB100) (by volume) were used in the study after careful mixing of peat (pH 4.4, 0–3 mm sized) and biochar (pelletized, sieved with a 5 mm-mesh), addition of 2 L of water and air-drying the mixtures.

The resulting five treatments (the growing substrates) were arranged in a completely randomized blocks design with three replicates per treatment and 20 plants for replicate, reaching to a total of 300 plants.

Water and nutrients were supplied to plants by a drip fertigation system (1 dripper/plant, flow rate 2 L/h) controlled by a computer. The composition (mg/L) of the nutrient solution was 180 N total, 50 P, 200 K, 120 Ca, 30 Mg, 1.2 Fe, 0.2 Cu, 0.2 Zn, 0.3 Mn, 0.2 B, with a pH of 6.0 and an electrical conductivity (EC) of 2.0 dS/m. Irrigation management of all treatments was achieved by means of electronic low-tension tensiometers (LT-Irrometer, Riverside, CA, USA) which control irrigation based on substrate matric potential. Five tensiometers were placed in each block (15 per treatment) at the midpoint of the containers (8 cm depth). The tensiometers were connected to the fertigation computer which controlled the beginning and the end of each delivery, corresponding respectively to high (-5 kPa) and low (-1 kPa) tension points for most of the growing substrates. Irrigation frequency ranged between 2 and 8 deliveries per day with a duration of 1-2 min each. Single irrigation ended when leachate was equal to 15% of nutrient solution supplied; this approach was adopted for avoiding salts accumulation in the substrates.

Physical and chemical characteristics of the growing substrates

For the measurement of the physical characteristics of the five growing substrates at the beginning of the experiment, five samples per each treatment were water-saturated into a doubled ring and equilibrated on a sand box at a water pressure head of -10 cm. Subsequently, main properties were measured from the wet and dry weights of samples in the lower ring. The water content at 5 kPa (corresponding to a 50 cm-high water column

required to reach this tension value) was determined by drying the samples at 105°C for 24h and measuring pressure from the middle of the lower ring. Total porosity was assessed according to the formula: 1.1 - (Bulk density/particle density) (Fascella et al., 2017) where bulk density was calculated as the ratio between samples dry mass and ring volume and particle density was determined from the organic matter and ash density (1550 and 2650 kg m^{-3}, respectively) of the samples.

Regarding to the initial chemical characteristics of the five substrates, the pH was measured on five samples per treatment with a pH-meter (GLP 21, Crison, Barcelona, Spain) in a settling suspension of 60 g sample and 300 ml of deionized water (1:5; v:v), after shaking at 22°C for 60 min. The EC was measured on the same water extract used for the pH determination using an EC-meter (HI 4321, Hanna Instruments, Padua, Italy). The total concentration of phosphate (PO_4^{3-}) was determined after dry combustion of the samples by means of an elemental analyzer (Carlo Erba Instruments, Milan, Italy). For the determination of the total concentration of potassium (K), calcium (Ca), and magnesium (Mg), 200 mg of dry sample were acid-digested in a microwave oven (Mars 5, CEM, Matthews, NC, USA), then filtered, diluted and analyzed by an ion chromatography system (Dionex ICS-6000, Thermo Fisher Scientific, Waltham, MA, USA).

Growth and biomass production

In order to evaluate the effect of the substrates on plant growth and biomass production, fifteen plants for each treatment were randomly selected at the end of the experiment (September 2018) and successively divided into leaves, stems and roots. The different plant organs were first weighed, and then dried to constant weight in a forced-air oven (at

80 ºC for 3 days). Shoot dry biomass, expressed in g/plant, was equal to the sum of leaves and stems. Plant height was measured as the distance from the top of the plant to the surface of the substrate. The number of branches, as well as the number of leaves and flowers, produced by each plant was counted. Leaf area was measured using a digital area meter (WinDIAS 2; DELTA-T DEVICES Ltd., Cambridge, U.K.). Root length was considered as the distance from the base of the trunk and the end of the longest root. Root-to-shoot (R/S) ratio was also calculated by dividing root dry weight by the sum of leaf and stem dry weights. The water use efficiency (WUE), expressed in g/L, was calculated as the ratio of dry weight of the total biomass (g/plant) and the total water use (L/plant).

Essential oil extraction and analysis

Aerial parts (about 50 g in about 500-800 ml of water) of plants at the flowering stage corresponding to the higher balsamic period grown in the different substrates (10 plants per treatment) were subjected to hydrodistillation in a Clevenger-type apparatus for 3 h. The essential oil (EO) from the five samples was recovered and dried over anhydrous sodium sulfate and stored under N_2 in a sealed vial until required. GC-FID (flame ionization detector) and GC/MS (Gas-chromatography-mass spectrometry) analyses were run on a 17-A gas chromatograph and a GCMS-QP5050A (Shimadzu) respectively, in the same analytical conditions and oven temperature already reported (Tuttolomondo et al., 2015). An aliquot of 20 µL of each oil was diluted in 480 µL of CH_2Cl_2 and 1 µL of this solution was injected with the split mode (1:96), percentages of compounds were determined from their peak areas in the GC-FID profiles. For each sample the identity of components was confirmed on the basis of their literature reported GC retention index (relative to C_9-C_{20} n-alkanes on SPB-5 column; Adams, 2007), computer matching of

spectral MS data as already reported (La Bella et al., 2015) and, whenever possible, co-injections with authentic samples.

Hydro-distillation wastewaters analysis

Water coming from EO hydro-distillation was filtered on filter paper (Whatman, cat. n° 1004-930, grade 4) to remove inert materials and then an aliquot were filtered on PTFE 0.45 mm filters (PALL Corporation), and put into 2 mL amber vials for analysis. Polyphenols analysis was carried out on a Ultimate3000 instrument equipped with a photodiode array detector (ThermoScientific, Italy). All chromatographic runs were performed using a reverse-phase column (Gemini C_{18}, 250 x 4.6 mm, 5 μm, Phenomenex, Italy) equipped with a guard column (Gemini C_{18} 4 x 3.0 mm, 5 μm particle size, Phenomenex, Italy). Samples were eluted with a gradient of 5%-90% buffer B (2.5% formic acid in acetonitrile) in buffer A (2.5% formic acid in water) over 50 min after which the system was maintained for 7 min at 100% Buffer B. The solvent flow rate was 1 mL/min. Quantifications were carried out at 280 nm using gallic acid as standard for phenolic acids (R^2=0.999), at 330 nm using caffeic acid (R^2=0.998), *p*-coumaric acid (R^2=0.998) and ferulic acid (R^2=0.999) as standards for cinnamic acids, and at 350 nm using apigenin-7-*O*-glucoside (R^2=0.999) and luteolin-7-*O*-glucoside (R^2=0.999) as standards for flavones. In order to unambiguously identify the chromatographic signals and/or to confirm peak assignments, a series of HPLC/ESI-MS analyses were performed. The HPLC apparatus, solvent system, elution programs used were the same as a described above, whilst ESI mass spectra were acquired as already reported (Napoli et al., 2018).

Antioxidant and radical scavenging activity of EO and hydro-distilled wastewaters

The antioxidant activity of the lavender essential oil and hydro-distillation wastewaters was determined according the Folin-Ciocalteu (FC) method. An aliquot (1 µl/ml or 0.012 ml for EO and hydro-distilled wastewaters, respectively) of the samples was mixed with 0.5 ml of FC reagent and, after keeping for 5 min at room temperature, the mixture was added to 0.45 ml of a 7.5% (w/v) Na_2CO_3 solution and incubated in the dark for 2 h at 20 °C. Then, the absorbance at 765 nm of each sample was measured spectrophotometrically. Chlorogenic acid (CGA) was used for the standard curve both for EO and wastewaters and the antioxidant activity was expressed as µg of CGA equivalents per mL EO or wastewaters. The analysis was performed in triplicate.

The radical scavenging activity of EO and hydro-distilled wastewaters was assessed according to the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method. An aliquot (0.335 ml) of a 0.25 mM DPPH methanolic solution was added to 0.665 ml of each sample at different concentrations (5-10-50-300 µg/ml and 30-60-90-150 µg/ml for EO and wastewaters, respectively), and incubated in the dark at room temperature for 30 min. The antiradical activity of EO and wastewaters was measured spectrophotometrically as a decrease in absorbance at 517 nm and results were expressed as µg dry weight (DW) per ml EO or wastewaters. These values corresponded to the sample concentration necessary to inhibit 50% of the radical. The analysis was conducted in triplicate.

Statistical analysis

Collected data were subjected to a one way-analysis of variance by means of the software package Statistica for Windows 9.0 (Tulsa, OK, USA). For each treatment, significant means were separated with the Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Results and Discussion

Physical and chemical characteristics of the growing substrates

Main physical-chemical properties of the studied growing substrates are reported in Table 1. Increasing the conifers wood biochar content in the growing substrates resulted in an increase of the pH and of the EC. Likewise, the increase of biochar amendment determined an increase of K content of the growing substrates. In contrast, a reduction in the P, Ca and Mg concentration of the substrates was measured according to the increase of biochar amendment (Table 1).

The increase in pH, EC and K content of the tested substrates recorded as the biochar amendment increased is similar to the results of Prasad et al. (2018) who reported that increasing biochar percentages in peat-based substrates increased the pH and K content of the substrates but decreased the P concentration. Our outcomes agree with those from Vaughn et al. (2015) who observed an increase of substrate pH and EC as biochar content increased and suggested that high pH values recorded with higher biochar concentration are probably linked with the K levels measured in the original feedstocks before the pyrolysis process. Also, Altland and Locke (2017) reported that a 15–20% biochar amendment of a peat-based substrate supplied adequate K amount for plants grown in soilless culture. The reduced P concentration recorded in the substrates with higher biochar percentage may be correlated to their higher pH values, compared to the substrates with lower biochar content. Chrysargyris et al. (2019) observed that adding biochars in ratios from 5% to 20% increased the pH of peat-based materials and this reflected the decreased P levels found in the growing media with increasing biochar content.

The physical properties of our growing substrates were also influenced by the amendment with conifers wood biochar. Actually, the increase of biochar content in the substrates resulted in a decrease of the water content at 5 kPa, and in a slight increase of the air content (Table 1).

The decrease of substrate's water content and the increase of air content as the biochar amendment increased is most probably due to the gradual reduction of peat and to the concurrent addition of biochar, which is a highly porous material. According to our results, Gasco et al. (2018) reported that 50% biochar amendment of a peat-based substrate resulted in a 43% increase of air space with respect to the pure peat. Guo et al. (2018) referred that increasing the biochar content of a commercial peat-based mixture resulted in a decrease of the container capacity whereas the pore space increased. Nieto et al. (2016) observed that adding biochar to peat resulted in growing mixtures with lower water holding capacity and higher air space volume than peat. Moreover, according to Conversa et al. (2015) and Zaccheo et al. (2014), the increase of biochar amendment of peat-based substrates corresponded to an increase of air content and to a reduction of water content.

Plant growth and biomass production

The effect of biochar content of the growing substrates on plant growth and quality are reported in Tables 2 and 3. Plant height was higher when *Lavandula angustifolia* plants were grown in substrates with a biochar content ranging from 25% to 75%, lower values were recorded in pure peat. Leaf production was higher when plants were grown in CB25 and CB75, lower number of leaves per plant was measured with 100% biochar (Table 2). Higher leaf area was recorded in plants grown with 25% biochar, lower values were

observed with CB100. Flower production was higher in plants grown with 50% and 75% biochar, but lower with CB25 (Table 2). The biochar content of the substrate did not affect the number of branches produced by the plants in the five treatments. Higher root length was recorded in plants grown with 75% and 100% biochar, plants grown with CB25 produced the shortest roots (Table 2).

The best growing performances (in terms of plant height, leaf area, leaves and flowers production) of potted lavenders grown in substrates amended with a biochar content ranging from 25% to 75% are in line with the results from Dispenza et al. (2016): these authors reported that, in *Euphorbia x lomi* cultivated with different peat/biochar ratios, higher shoots and leaves production and leaf area were recorded in plants grown in 40/60% peat/biochar, with respect to Euphorbias grown in pure peat and in pure biochar. Huang et al. (2019) referred that a mixture containing 60-70% biochar and 5% compost did not negatively affect the growth index, stem, root and total DW of containerized tomato plants. Similarly, Kaudal et al. (2018) reported that a mixture of biosolids and green waste biochar may replace sphagnum peat as growing medium for silverbeet plants at the rate of 60% on volume basis. According to Guo et al. (2018), Poinsettia plants grown in 40% biochar showed a similar growth to those in 0% biochar and up to 80% biochar, plants did not evidence significant changes, except for dry weight, which decreased at highest percentages. Moreover, Margenot et al. (2018) demonstrated that softwood biochar can replace peat (≤70% volume) as soilless substrates and without negatively affecting marigold biomass and flowering. The effect of different growing media on lavender growth and development has been recently observed (Najar et al., 2019) but, obviously, not all substrates are suitable for *L. angustifolia* as reported by

Linderman and Davis (2003) referring that lavender growth was depressed in all coir-amended media compared to the non-amended control (peat).

However, though lavenders grown with different biochar content statistically differed on many morphological parameters, at a visual-quality evaluation our plants did not show significant variations on the general aesthetic effect. This latter outcome is partially confirmed by the absence of difference on the number of branches produced by the plants grown in the five substrates (Table 2).

Regarding to the biomass fresh weight, lavenders grown with 25% biochar produced higher leaf and stem FW, lower values were recorded with CB100 (Table 3). Higher root FW were measured in plants grown with 50% and 75% biochar, respectively; lower weights were recorded with CB0 and CB100. No significant difference was recorded among the five substrates with regard to the leaf dry weight (Table 3). Higher stem DW was recorded in lavenders grown with 25% biochar, lower value was observed with CB100. Root DW was higher with CB50 and CB75, lower weights were measured with CB0 and CB100 (Table 3). Regarding to the root-to-shoot ratio (R/S), an average value of 0.28 was recorded irrespective of the growing substrates (Table 3). With regards to the water use efficiency (WUE), CB100 showed lower value than those recorded in the remaining treatments (Table 3).

The absence of difference among the five substrates on leaf dry weight and R/S ratio seems to confirm the capacity of *L. angustifolia* to normally grow in substrates amended with higher biochar content. According to Matt et al. (2018), for Pinus, Gaillardia and Clarkia plants grown in soilless nursery media at 4 biochar rates (0, 15, 30 and 45%), root and total biomass production were similar. Nieto et al. (2016) reported that lettuce biomass was substantially higher when biochar was added to peat than with

peat alone. Positive growth response of potted lavenders in the biochar-amended growing substrates may be linked to the favorable physical and chemical characteristics of our mixtures, in particular those amended with 25% and 50% biochar, evidencing a pH close to the neutrality, an equilibrate K content, adequate water and air contents together to a high porosity (Table 1).

Essential oil qualitative profiles

The production of high quality EO is one of the main purpose of lavender cultivation. For this reason a comparative chemical analysis of the profiles of the EOs obtained from plants grown on different substrates mixtures were carried out with the scope of highlighting any influence of the substrates to this very important primary product. The essential oil (EO) yield was not affected by the growing substrates as limited differences were recorded among treatments (from 0.75 to 0.85 ml/100 g DW for CB100 and CB25-CB50, respectively). Gas-chromatographic analysis identified 51 components covering more than 92.0% of the total. The phytochemical profile of EO from lavenders grown in different substrates is reported in Table 4 revealing that the *L. angustifolia* samples were 1,8-cineole-camphor chemotype. Chemical profile of the EOs was mainly characterized by a high amount of 1,8-cineole (32.48-35.92%) and camphor (25.88-28.50%) and a low concentration of linalool (2.79-6.26%), which increased by increasing biochar amendment (Table 4). The peculiarity of these samples is the high concentration of α-bisabolol (6.51-8.96%), which slightly decreased by increasing biochar content. Focusing the attention on the chemical classes, the most represented is that of oxygenated monoterpenes (70.04-76.55%), which increase with the increment of biochar amendment,

followed by sesquiterpenes (11.98-16.17%) that, on the contrary, decrease by increasing the biochar percentage in the substrate.

Looking deeply within the five growing substrates, no significant differences, in terms of EOs quality, can be found between the 100% biochar and the other substrates regarding their chemical profiles. The influence of the growing substrate on lavender EO content and composition is reported by other authors (Chrysargyris et al., 2016b; Chilosi et al., 2017). The slight differences on EO chemical profile among the five substrates is in line with the results from Najar et al. (2019) who reported that the volatile organic compounds (VOC) profile of *L. angustifoli*a grown in different peat-based substrates did not change and that the highest amounts of linalool, an important compound for medicinal uses, were obtained from plants cultivated in a peat/compost/demolition aggregates mixture. Similarly to our findings, Pandey et al. (2016) observed that a mixture of soil and biochar derived from Eucalyptus pruning wastes did not affect EO constituents of potted basil plants.

Hydro-distilled wastewaters profiles

The hydrodistillation of aromatic plants produces two types of waste: the solid residue of the plant itself and the water in which the plant is immersed during the process. The second type, in addition to representing a source not yet adequately valued source of biologically active secondary metabolites, it is the result of a hot extraction that can provide information on the secondary metabolism of the plant at least for those thermally stable compounds, without any further solvent use. The polyphenols profile of hydro-distilled wastewaters from lavenders grown in different substrates is reported in Table 5, which shows the presence of a considerable amount of potentially biologically active

polyphenols such as rosmarinic acid, luteolin-7-glucuronide, salvianolic acid A and several cinnamic acids. The quantity of these compounds seemed to be related to the different growing substrates used. The amount of all identified polyphenols in the hydro-distilled wastewaters was lower (2.74% w/w of plant dried weight) when plants were grown in pure peat (Table 5). The percentage of the total identified polyphenols increased by increasing the biochar content of the substrates, reaching a maximum (4.80% w/w of plant DW) at 50% biochar. This biochar amendment corresponded also to the maximum amount of rosmarinic acid (0.97% w/w of plant DW) and caffeic acid derivative (1.21% w/w DW) (Table 5).

Antioxidant and radical scavenging activity of EO and hydro-distilled wastewaters

The antioxidant activity of *Lavandula angustifolia* essential oil, determined according the Folin-Ciocalteu (FC) method, ranged from 3.01 µg/ml to 3.82 µg/ml recorded for plants grown in CB50 and CB100, respectively (Table 6). The antioxidant activity of hydro-distillation wastewaters (FC method) was varied from 2.78 µg/ml to 4.87 µg/ml measured in plants grown in CB0 and CB50, respectively (Table 6). The radical scavenging activity of Lavender EO, measured with the DPPH method, showed lower values (corresponding to higher activity) in CB100 and CB75 (91.3 and 99.5 µg/ml, respectively) whereas higher value (corresponding to lower capacity) was recorded with CB25 (136.2 µg/ml) (Table 6). The radical scavenging activity of the hydro-distilled wastewaters (DPPH method) evidenced lower value with CB50, CB25 and CB75 (from 61.5 to 68.9 µg/ml) while higher value was observed with CB0 (142.8 µg/ml) (Table 6). Both in Lavender EO and in hydro-distilled wastewaters a correlation between the antioxidant activity and the scavenging activity was found: the highest value (3.82 µg/ml) of EO from CB100

corresponded to the minimum value of EC50 (91.3 µg/ml); the highest wastewaters activity of CB50 (4.87 µg/ml) corresponded to the lowest EC50 value (61.5 µg/ml).

Lin et al. (2009) used the DPPH method to study the antioxidant properties of Lavander EO, and, in particular, its capacity to inactivate free radicals with activities similar to lime and marjoram EOs. Others studies aimed to test the ability of Lavender EO to reduce 50% DPPH radicals (Hussain et al., 2011; Viuda-Martos et al., 2011) led to divergent outcomes with values ranging from 289 µg/ml to 48.7 mg/ml.

Pistelli et al. (2017) reported that the EO of *L. angustifolia* cv. Mailette was characterized by a high radical scavenging activity (DPPH) which strongly increased in the second year of cultivation, due to favorable environmental conditions (temperature, photoperiod, light intensity) which can affect the chemical composition of EO and, consequently, its related antiradical capacity. Carrasco et al. (2016) reported that, comparing EOs from Spanish lavenders, *L. angustifolia* showed higher DPPH antiradical activity than *L. latifolia* due to higher content of some molecules present in the first species.

Figures 1 and 2 show the radicals inhibition percentages obtained using different concentrations of EO (from 5 to 300 µg/ml) and hydro-distilled wastewaters (from 30 to 150 µg/ml) in the DPPH method to obtain the relative EC50 (Table 6). The radical scavenging activity (EC50) of the Lavender EO was measured at different sample concentrations and expressed as radicals inhibition percentage, showing higher values in CB100, CB75 and CB0 (63.4% on average), in particular at 50 and 300 µg/ml, whereas in CB25 evidenced the lower activity (50.9%) (Figure 1). The scavenging activity of Lavender hydro-distilled wastewaters was higher when plants were grown with 50% biochar, especially at 90 and 150 µg/ml, followed by those grown with 75% and with

25% biochar; lower antiradical activity was recorded in plants cultivated in pure peat and with 100% biochar (Figure 2). The chemical composition of lavender EO that influenced the antioxidant properties depends on many factors such as genotype, plant development stage, substrate typology, climatic conditions, harvest period, drying techniques and/or extraction methods (Prusinowska et al. 2014). It has been demonstrated that the EO and hydro-distilled wastewaters from lavenders grown on biochar-based substrates showed considerable antioxidant and radical scavenging activity than the control (CB0).

Conclusions

The present study is the first attempt to assess the effect of biochar amendment of peat-based growing substrates on Lavandula agronomic performances, EO yield and quality. The outcomes coming from the current experiment seem to suggest that a biochar obtained from conifers wood pyrolysis may be used as substrate component with peat to grow *Lavandula angustifolia*. This agricultural by-product seems to be suitable for this kind of cultivation even at high rates (75% or 100%) as it did not negatively affect plant growth, EO yield, qualitative characteristics and phytochemical profile, with economic (reduction of peat purchase and consequently of productive costs) and environmental (limitation of peatland depletion, waste recycling) advantages. And the above mentioned benefits could be enhanced whether biochar was produced as near as possible to the aromatic plant farms. Moreover, the high antioxidant and radical scavenging activities of EO and hydro-distilled wastewaters recorded in plants grown with higher biochar content seem to evidence the good potentialities of this waste-derived material as growing substrate for plants with industrial uses.

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Table 1. Chemical (pH, electrical conductivity [EC], nutrients content) and physical (water content, air content, total porosity) characteristics of the growing substrates as affected by conifers wood biochar content.[a]

	CB0	CB25	CB50	CB75	CB100	Significance[b]
pH	5.7 b	6.5 ab	7.1 a	7.8 a	8.4 a	*
EC (dS/m)	2.1 b	5.5 b	11.3 a	14.6 a	16.5 a	**
P (g/kg dw)	29.3 a	18.0 b	15.3 b	8.5 c	4.2 c	**
K (g/kg dw)	102.5 c	117.1 b	131.2 a	133.7 a	137.5 a	*
Ca (g/kg dw)	105.0 a	62.5 b	46.6 bc	25.2 c	16.8 c	***
Mg (g/kg dw)	37.5 a	18.0 b	16.5 b	9.5 bc	6.0 c	**
Water content (% v:v)	58.7 a	55.5 a	49.7 ab	44.8 b	40.2 b	*
Air content (% v:v)	26.3 b	29.2 ab	32.1 a	35.0 a	39.2 a	*
Total porosity (% v:v)	89.7 a	90.2 a	90.5 a	91.5 a	92.2 a	NS

[a] 100% peat (CB0), 75% peat-25% biochar (CB25), 50% peat-50% biochar (CB50), 25% peat-75% biochar (CB75), 100% biochar (CB100). In any raw different letters are significant at $p<0.05$ (DMR test).

[b] NS, *, **, ***, non-significant or significant at $P\leq0.05$, 0.01 and 0.001, respectively.

Table 2. Effect of biochar content in the growing substrates on plant height, number of leaves, leaf area, number of flowers and branches, and root length of *Lavandula angustifolia* potted plants.[a]

Growing substrates	Plant height (cm)	Leaves (no./plant)	Leaf area (cm^2/plant)	Flowers (no./plant)	Branches (no./plant)	Root length (cm)
CB0	64.2 c	1945.0 b	5557.1 b	13.7 b	5.2 a	71.2 b
CB25	78.5 a	2397.5 a	6147.4 a	10.6 c	4.7 a	54.0 c
CB50	86.5 a	1837.4 b	4966.2 c	15.8 a	4.2 a	66.5 bc
CB75	79.5 a	2240.1 a	4977.8 c	14.3 ab	4.5 a	88.7 a
CB100	71.7 b	1305.2 c	3346.1 d	12.9 bc	3.0 a	82.2 a
Significance[b]	*	**	**	*	NS	*

[a] 100% peat (CB0), 75% peat-25% biochar (CB25), 50% peat-50% biochar (CB50), 25% peat-75% biochar (CB75), 100% biochar (CB100). In any column, different letters are significant at $p<0.05$ (DMR test).

[b] NS, *, **, non-significant or significant at $P\leq0.05$, and 0.01, respectively.

Table 3. Effect of biochar content in the growing substrates on leaves, stem and root biomass fresh and dry weight, root-to-shoot ratio (R/S), and water use efficiency (WUE) of *Lavandula angustifolia* potted plants.[a]

Growing substrates	Fresh weight			Dry weight			R/S	WUE (g/L)
	Leaves (g/plant)	Stem (g/plant)	Root (g/plant)	Leaves (g/plant)	Stem (g/plant)	Root (g/plant)		
CB0	87.9 ab	114.5 b	40.5 b	20.5 a	62.3 a	17.5 b	0.21 a	1.91 a
CB25	108.6 a	162.2 a	49.1 ab	23.3 a	70.1 a	23.0 ab	0.25 a	2.09 a
CB50	82.1 b	125.4 b	60.5 a	20.3 a	62.2 a	29.1 a	0.35 a	1.92 a
CB75	98.9 a	126.2 b	64.0 a	22.6 a	61.9 a	29.0 a	0.34 a	1.87 a
CB100	77.0 b	82.1 c	38.2 b	15.4 a	40.8 b	13.5 b	0.24	1.05 b
Significance[b]	*	**	*	NS	*	*	NS	*

[a] 100% peat (CB0), 75% peat-25% biochar (CB25), 50% peat-50% biochar (CB50), 25% peat-75% biochar (CB75) and 100% biochar (CB100). In any column, different letters are significant at $p<0.05$ (DMR test).
[b] NS, *, **, non-significant or significant at $P\leq0.05$, and 0.01, respectively.

Table 4. Phytochemical profile of *L. angustifolia* essential oil as affected by biochar-amended growing substrates.

			Growing substrate				
#[a]	KI	Class/Compound	CB100	CB75	CB50	CB25	CB0
		Monoterpene hydrocarbons	**4.52**	**4.84**	**4.66**	**5.95**	**5.30**
1	934	α-pinene[b]	1.20 (±0.02)	1.49 (±0.00)	1.35 (±0.00)	1.80 ((±0.00)	1.65 (±0.00)
2	949	camphene[b]	0.53 (±0.01)	0.63 (±0.00)	0.69 (±0.00)	0.74 (±0.00)	0.65 (±0.00)
3	973	sabinene[b]	0.26 (±0.00)	0.33 (±0.00)	0.23 (±0.00)	0.33 (±0.00)	0.26 (±0.00)
4	976	β-pinene	1.72 (±0.08)	1.89 (±0.01)	1.69 (±0.02)	2.21 (±0.01)	2.12 (±0.01)
6	988	myrcene[b]	0.21 (±0.05)	0.16 (±0.01)	0.11 (±0.00)	0.20 (±0.00)	0.12 (±0.00)
8	1014	α-terpinene	0.15 (±0.00)	0.09 (±0.00)	0.24 (±0.02)	0.16 (±0.00)	0.14 (±0.00)
10	1026	*p*-cymene	0.20 (±0.05)	0.12 (±0.00)	0.21 (±0.01)	0.25 (±0.01)	0.15 (±0.00)
12	1060	γ-terpinene	0.25 (±0.00)	0.13 (±0.00)	0.14 (±0.00)	0.26 (±0.00)	0.21 (±0.00)
		Oxygenated monoterpenes	**75.29**	**74.46**	**76.55**	**71.23**	**70.04**
7	990	dehydro-1,8-cineole	0.08 (±0.01)	0.09 (±0.00)	0.10 (±0.00)	0.15 (±0.00)	0.13 (±0.00)
11	1034	1,8-cineole	33.58 (±0.10)	33.96 (±0.16)	35.92 (±0.27)	32.48 (±0.13)	31.98 (±0.05)
13	1069	*cis*-sabinene hydrate	0.14 (±0.00)	0.55 (±0.00)	0.40 (±0.00)	0.18 (±0.00)	0.19 (±0.00)
14	1074	*trans*-linalool oxide	0.14 (±0.00)	0.12 (±0.00)	0.10 (±0.00)	0.07 (±0.00)	0.15 (±0.00)
15	1088	fenchone	-	0.85 (±0.01)	1.00 (±0.00)	0.85 (±0.00)	0.88 (±0.00)
16	1099	linalool	6.26 (±0.19)	3.33 (±0.21)	4.19 (±0.01)	3.97 (±0.01)	2.79 (±0.07)
17	1107	α-pinene oxide	0.04 (±0.00)	0.12 (±0.00)	0.12 (±0.00)	0.07 (±0.00)	0.09 (±0.00)
18	1118	*exo*- fenchol	0.06 (±0.00)	0.09 (±0.00)	0.11 (±0.00)	0.09 (±0.00)	0.08 (±0.00)
19	1127	α-campholenal	0.40 (±0.00)	0.44 (±0.00)	0.48 (±0.00)	0.48 (±0.00)	0.45 (±0.03)
20	1142	*trans*-pinocarveol	0.19 (±0.02)	0.22 (±0.00)	0.26 (±0.04)	0.23 (±0.07)	0.26 (±0.00)
21	1151	camphor	28.50 (±0.17)	28.42 (±0.09)	27.84 (±0.03)	25.88 (±0.02)	26.12 (±0.05)
22	1165	pinocarvone	0.64 (±0.01)	0.74 (±0.01)	0.77 (±0.01)	0.82 (±0.00)	0.78 (±0.00)
23	1170	borneol	1.27 (±0.01)	1.36 (±0.00)	1.28 (±0.01)	1.45 (±0.00)	1.50 (±0.00)
24	1180	terpinen-4-ol	0.61 (±0.01)	0.43 (±0.00)	0.42 (±0.00)	0.63 (±0.00)	0.62 (±0.00)
25	1187	*p*-cymen-8-ol	0.16 (±0.01)	0.57 (±0.00)	0.59 (±0.01)	0.60 (±0.00)	0.73 (±0.00)
26	1197	α-terpineol	0.80 (±0.00)	0.79 (±0.00)	0.59 (±0.00)	0.78 (±0.00)	0.80 (±0.00)
27	1198	myrtenal	0.59 (±0.01)	0.66 (±0.04)	0.69 (±0.02)	0.73 (±0.02)	0.70 (±0.03)
28	1200	myrtenol	0.51 (±0.01)	0.76 (±0.15)	0.72 (±0.01)	0.77 (±0.02)	0.72 (±0.08)
30	1212	verbenone	0.13 (±0.00)	0.16 (±0.00)	0.16 (±0.00)	0.16 (±0.00)	0.19 (±0.00)
31	1222	*trans*-carveol	0.20 (±0.00)	0.27 (±0.00)	0.30 (±0.00)	0.30 (±0.00)	0.33 (±0.00)
33	1248	carvone	0.28 (±0.00)	0.32 (±0.00)	0.32 (±0.00)	0.33 (±0.00)	0.33 (±0.00)
34	1260	piperitenone	0.06 (±0.00)	0.06 (±0.00)	0.05 (±0.00)	0.07 (±0.00)	0.07 (±0.00)
36	1296	*p*-cymen-7-ol	0.65 (±0.00)	0.15 (±0.00)	0.14 (±0.00)	0.14 (±0.00)	0.15 (±0.00)
		Sesquiterpenes	**11.98**	**13.38**	**13.64**	**16.13**	**16.17**
37	1424	β-caryophyllene	0.58 (±0.00)	0.37 (±0.00)	0.21 (±0.00)	0.26 (±0.00)	0.30 (±0.00)
38	1439	α-bergamotene	0.16 (±0.00)	0.11 (±0.00)	0.06 (±0.00)	0.08 (±0.00)	0.08 (±0.00)
39	1458	β-farnesene	0.22 (±0.01)	0.14 (±0.00)	0.07 (±0.00)	0.08 (±0.00)	0.11 (±0.00)
40	1472	dehydro-sesquicineole	0.12 (±0.00)	0.21 (±0.02)	0.23 (±0.01)	0.26 (±0.02)	0.27 (±0.02)
41	1486	germacrene D	0.58 (±0.00)	0.38 (±0.00)	0.20 (±0.01)	0.18 (±0.02)	0.31 (±0.00)
42	1503	bicyclogermacrene	0.17 (±0.01)	0.14 (±0.00)	0.09 (±0.01)	0.11 (±0.02)	0.13 (±0.02)
43	1509	α-bisabolene	0.11 (±0.00)	0.13 (±0.03)	0.10 (±0.03)	0.12 (±0.02)	0.13 (±0.02)
44	1519	γ-cadinene	0.24 (±0.01)	0.25 (±0.00)	0.22 (±0.00)	0.26 (±0.00)	0.24 (±0.00)
45	1527	*trans*-calamenene	0.11 (±0.00)	0.10 (±0.00)	0.08 (±0.00)	0.11 (±0.00)	0.11 (±0.00)

46	1591	caryophyllene oxide	1.32 (±0.01)	1.72 (±0.02)	2.20 (±0.03)	2.26 (±0.02)	2.09 (±0.00)
47	1649	epi-α-cadinol	0.56 (±0.01)	0.73 (±0.03)	0.84 (±0.02)	1.08 (±0.01)	0.79 (±0.00)
48	1659	β-eudesmol	0.08 (±0.03)	0.13 (±0.04)	0.21 (±0.01)	0.25 (±0.01)	0.19 (±0.00)
49	1664	β-bisabolol oxide	1.17 (±0.07)	1.65 (±0.12)	2.21 (±0.01)	2.52 (±0.00)	2.35 (±0.01)
50	1667	14-hydroxy-9-epi-(E)-caryphyllene	0.05 (±0.01)	0.07 (±0.02)	0.14 (±0.00)	0.14 (±0.01)	0.11 (±0.00)
51	1693	α-bisabolene	6.51 (±0.12)	7.25 (±0.09)	6.78 (±0.05)	8.42 (±0.06)	8.96 (±0.01)
		Others	**0.60**	**0.59**	**0.52**	**0.59**	**0.62**
5	979	6-methyl-5-hepten-2-one	0.34 (±0.10)	0.31 (±0.01)	0.20 (±0.05)	0.27 (±0.01)	0.28 (±0.01)
32	1244	cumin aldehyde	0.26 (±0.00)	0.28 (±0.00)	0.32 (±0.00)	0.35 (±0.00)	0.34 (±0.00)

[a] The numbering refers to elution order and values (relative peak area percent) represent averages of 3 determinations;
[b] Co-elution with authentic sample.

Table 5. Identified polyphenols in *Lavandula angustifolia* hydro-distilled wastewaters (% w/w of dried plant material) as affected by growing substrates.

Compound	CB100	CB75	CB50	CB25	CB0
Dihydroxyphenyllacetic acid	0.681 (±0.003)	0.720 (±0.005)	0.610 (±0.011)	0.698 (±0.008)	0.434 (±0.004)
Caftaric acid	0.056 (±0.001)	0.103 (±0.008)	0.111 (±0.000)	0.066 (±0.001)	0.036 (±0.000)
Fertaric acid	0.104 (±0.000)	0.104 (±0.006)	0.077 (±0.000)	0.115 (±0.001)	0.074 (±0.000)
Feruloyl glycoside	0.380 (±0.009)	0.413 (±0.003)	0.405 (±0.001)	0.494 (±0.004)	0.332 (±0.001)
Caffeic acid derivative	0.149 (±0.020)	0.183 (±0.003)	0.174 (±0.001)	0.145 (±0.001)	0.096 (±0.000)
Caffeic acid dimer	0.246 (±0.012)	0.303 (±0.002)	0.344 (±0.012)	0.312 (±0.002)	0.236 (±0.009)
Apigenin derivative	0.087 (±0.000)	0.084 (±0.001)	0.075 (±0.001)	0.098 (±0.001)	0.063 (±0.000)
Amentoflavone	0.053 (±0.000)	0.116 (±0.001)	0.119 (±0.000)	0.063 (±0.000)	0.041 (±0.000)
Caffeic acid derivative	0.903 (±0.002)	0.894 (±0.004)	1.208 (±0.005)	0.789 (±0.008)	0.531 (±0.000)
Luteolin-7-glucuronide	0.526 (±0.002)	0.479 (±0.009)	0.514 (±0.009)	0.542 (±0.010)	0.400 (±0.001)
Rosmarinic acid	0.737 (±0.003)	0.810 (±0.009)	0.971 (±0.001)	0.816 (±0.009)	0.332 (±0.004)
Salvianolic acid A	0.281 (±0.001)	0.192 (±0.001)	0.187 (±0.001)	0.288 (±0.003)	0.167 (±0.001)
	4.203	**4.401**	**4.795**	**4.426**	**2.742**

Table 6. Antioxidant activity (µg/ml) and scavenging activity (EC50, µg/ml) of *Lavandula angustifolia* essential oil and hydro-distilled wastewaters as affected by biochar content in the growing substrates.[a]

Growing substrates	Antioxidant activity		Scavenging activity (EC50)	
	Essential oil	Hydrodistilled wastewaters	Essential oil	Hydrodistilled wastewaters
CB0	3.17 ± 0.04	2.78 ± 0.01	107.6 ± 0.2	142.8 ± 1.6
CB25	3.03 ± 0.02	4.60 ± 0.04	136.2 ± 1.3	65.5 ± 0.1
CB50	3.01 ±0.01	4.87 ± 0.05	113.0 ± 0.8	61.5 ± 0.2
CB75	3.08 ± 0.02	4.54 ± 0.08	99.5 ± 0.4	68.9 ± 0.1
CB100	3.82 ± 0.05	4.04 ± 0.08	91.3 ± 0.2	89.1 ± 0.1

[a] 100% peat (CB0), 75% peat-25% biochar (CB25), 50% peat-50% biochar (CB50), 25% peat-75% biochar (CB75) and 100% biochar (CB100). In any column, means ± standard error.