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Effects of Vermicompost, Compost and Digestate as Commercial Alternative Peat-Based Substrates on Qualitative Parameters of *Salvia officinalis*

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Abstract: Peat is a common substrate used for the cultivation of potted plants. However, the use of peat in horticulture has recently been questioned from an environmental standpoint, since it is a non-renewable resource and plays a major role in atmospheric CO_2 sequestration. The aim of this work was to assess the potentialities of substrates obtained from vermicompost, compost and anaerobic digestion processes to partially substitute peat for sage (*Salvia officinalis* L.) cultivation. Therefore, we planned an experiment to assess the effect of these substrates on essential oil (EO) yield and composition, as well as on leaf nutrients concentration of sage plants. The three substrates were mixed with commercial peat (Radicom) at a ratio of 40% of alternative substrates and 40% of commercial peat. The chemical properties of the alternative substrates did not affect the leaf content of macro and micronutrients, as well as of heavy metals. Moreover, the EO yield and quality was not affected by the substrates and did not differ among them. Results provided evidence that the three alternative substrates can be used to partially substitute peat in soilless cultivation of sage plants. However, due to the higher values of the electrical conductivity of the substrates obtained from composting and anaerobic digestion processes, such substrates must be used with caution.

Keywords: biowaste reuse; substrate heavy metals; sage essential oil; sage heavy metals

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1. Introduction

Soils provide humanity with 98.8% of its food and supply a broad range of services, e.g., carbon sequestration, greenhouse gas (GHG) regulation, flood mitigation, support for sprawling cities. However, soil is a limited resource and rapid human population growth, coupled with its increasing consumption, is placing unprecedented pressure on soils [1].

Soilless cultivation can be a valid alternative in order to reduce the anthropogenic pressure on soils [2]. Actually, the major advantage of soilless culture systems is the uncoupling plant growth from problems associated with soil, such as soilborne pests and diseases, non-arable soil, soil salinity and poor soil fertility [3]. One of the main challenges for soilless cultivation is the choice of the growing medium, since it affects plant physiology, yield and fruit quality [4]. The ideal growing medium has high total porosity, low bulk density and high water holding capacity, in order to facilitate root penetration and increase nutrient availability to the plants [5].

Peat has been used for a long time as a component of potting mixes and has become the most widely used growing medium for containers, as a complete growing medium by itself [6], able to adsorb and release nutrients and water, as well as having a low bulk

density and other properties [7]. However, the use of peat in horticulture has recently been questioned from an environmental standpoint, since peat is a non-renewable resource and plays a major role in atmospheric CO_2 sequestration. However, being peat a fossil and, hence, non-renewable resource, during the last 20 years, its extraction has come under increasing scrutiny for the ecological and sometimes archaeological value of peat bogs throughout the world [8,9]. The demand for sustainable and environmental-friendly growing media as alternatives for peat or inorganic substrates for vegetable and flower production in greenhouses is increasing. At the same time, growing media have to meet the requirements of the nursery managers and farmers with respect to plant growth, crop yield and product quality [10]. Several studies have been carried out in order to find alternative, efficient and sustainable components of growing substrates for the soilless cultivation [11–13]. A valid alternative could be represented by those substrates deriving from the composting process of solid waste. Composting is a biological decomposition process of organic waste under aerobic conditions. Vermicomposting is a form of composting that uses earthworms, in order to speed up the biodegradation process [14]. The compost and vermicompost produced from biowaste can be reused as nutrient-rich fertilisers or growing substrates [15–17]. Although some nitrogen in the form of ammonia volatises during both composting and vermicomposting, these biological decomposition processes belong to a sustainable waste management strategy, which is almost in line with the zero waste concept: the resource flow is circular, so that the resources themselves are conserved and recovered for being reused in other processes. The uses of vermicompost and compost are several: biofertilisation and soil improvement; peat replacement in growing substrates [18–20]; carbon sequestration; maintaining or increasing soil organic matter; reducing greenhouse gases emission [21].

Digestate is, above all, the liquid but also the solid fraction produced together with biogas through the anaerobic digestion (AD) process of biowaste, such as animal husbandry effluents, plant biomass [22], food industry by-products, sewage sludge and organic fraction of municipal solid waste (OFMSW), including food waste [23–29]. Moreover, digestate can be used as a fertiliser, as it determines the increase of organic matter, as well as nitrogen, phosphorous and potassium [17]. Nitrogen from the solid fraction is not readily available to the plants, because of high C:N ratio and a portion of organic nitrogen [30]. The reuse of waste as substrate after composting is in line with the EU 2020 growth strategy, wishing the shift from linear to circular models of production and consumption [31].

Sage is a perennial, herbaceous and aromatic plant, belonging to the family of *Lamiaceae*. It is used as medicinal herb for the presence of essential oil (EO), contained in glands and secreting hairs on its stem and leaves [32,33]. Indeed, according to several studies [34–36], sage EO has therapeutic and medicinal potentials as antimicrobial agents, spasmolytics, hypotensives, antioxidants and anti-inflammatories, as well as for extending sleeping and treating Alzheimer's disease. Therefore, the aim of this study was to evaluate the effect of vermicompost, compost and digestate, mixed with commercial peat, as growing media in soilless cultivation of sage (*Salvia officinalis* L.).

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The suitability of vermicompost, compost and digestate as sustainable growing substrates for sage pot cultivation was evaluated by comparing EO yield and composition, as well as the elemental composition of the leaves of sage plants cultivated in these alternative substrates and commercial peat.

The study was carried out from April to October 2019 at the Research Centre for Plant Protection and Certification of Bagheria (Palermo, Italy), in an open greenhouse with a 30% shading net and a mulching cloth covering the floor. At the beginning of the survey, three-month old plants of sage were transplanted into polyethylene pots having a diameter of 18 cm and a volume of 4 L, filled in with three different substrates.

A vermicompost constituted by particles having a diameter $\leq$ 5 mm and produced by "Red Worm Sicily" company, located in Milazzo (Messina, Italy), inside litters, through a slow digestion process of cattle and horse manure, operated by earthworms of *Eisenia fetida* and *Eisenia andrei* species, was used [15].

The compost was produced inside the composting plant owned by Green Planet, located in Ciminna (Palermo, Italy), by using the OFMSW [15], differentially collected according to door-to-door method.

The solid digestate was produced inside a bioreactor having a power of 600 kW and built up by AB Group agricultural company, located in Vittoria (Ragusa, Italy). In this bioreactor, the AD process of chicken manure, cattle slurry, cheese whey, citrus industry by-product and oil pomace, as well as sorghum, corn and triticale silage, is carried out in order to produce biogas and, then, electrical and thermal energy by means of a combined heat and power plant [15].

Pots were filled in with three growing substrates, mixed with commercial peat. The commercial peat used to prepare the three substrates was composed of a mixture of blond sphagnum peat, black swampland peat and green compost (commercial name Radicom, Vigorplant Italia srl, Fombio, Italy). The three growing substrates (S) were: S1 (40% vermicompost and 60% peat); S2 (40% compost and 60% peat); S3 (40% digestate and 60% peat). Peat was also used as control (100%, S4). After transplanting, the potted plants were moved to the greenhouse (Figure 1). Water was supplied through an automated drip irrigation plant, having a very flow rate, for 10 min every 2–3 days/week in April–May and every 3–4 days/week from June to October, in order to maintain a constant substrate humidity (50% of water holding capacity).

Figure 1. Potted sage plants grown with four different substrates inside the greenhouse.

2.2. Substrate and Plant Analyses

The dry bulk density of the growing substrates was determined by using the mass per unit volume technique [37], slightly modified with several 100 mL hard plastic graduate cylinders. The graduate cylinder was filled in up to 100 mL with the growing media and dropped four times from a height of 100 mm into a hard surface. The cylinders were then filled in again up to 100 mL. Between 4 and 8 measures were done, depending on the heterogeneity of the growing substrates sampled and the repeatability of the results obtained. The water holding capacity was determined by the method described by Brischke and Wegener [38] and slightly modified: 5 g of growing substrates were weighed in funnel with filter paper (Whatman grade 42). Then, the funnel was saturated with water for 48 h and then allowed to drain for 48 h. The samples were weighed, oven dried at 105 °C for 48 h

and then reweighed. The water holding capacity was calculated as the weight difference between the drained mass and the oven dry mass, divided by the oven dried mass.

Reaction and electrical conductivity (EC) of the substrates were determined in water extract (1/10; *w*/*v*) by a pHmeter (FiveEasy, Mettler Toledo Spa, Milan, Italy) and a conductometer (HI5321, Hanna Instruments Italia srl, Padua, Italy).

The bioavailability of macro and micronutrients, as well as of heavy metals, of the four growing substrates was determined at the beginning of the test. Total nitrogen (N) was determined by the Kjeldahl method [39] and available phosphorous by the Olsen method [40]. The bioavailability of macro (except for N and P) and micronutrients, as well as of heavy metals, of the four substrates was determined on the 5.0 mM diethylenetriaminepentaacetic acid (DTPA) extracts analysed by Agilent 7500-ce Inductively coupled plasma mass spectrometry ICP-MS (Agilent Technologies Italia SPA, Milan, Italy) [41].

Seven months after transplanting, destructive analyses were carried out in order to determine the yield and composition (volatile compounds) of the EO, as well as the elemental composition of the sage leaves.

Nitrogen (N) concentration in the sage leaves was determined by the Perkin-Elmer 2400 CHNS/O elemental analyser (Perkin Elmer Italia S.P.A., Milan, Italy).

Elemental composition (except for N and P) in plant samples was determined by Agilent 7500-ce ICP-MS (Agilent Technologies Italia SPA, Milan, Italy) on digested samples with concentrated HNO_3 in a microwave [42].

2.3. Essential Oil Analysis

For EO extraction and analysis, 200 g of leaves were manually cut in small pieces and subjected to water distillation for three hours by means of a Clevenger equipment. The extracted EO was dried by adding anhydrous sodium sulfate and storage at 4 °C in the darkness until the analysis.

EO was analysed by a gas chromatograph—mass spectrometer (Shimadzu GC-MS-QP2010 Ultra), according to Zito et al. [43]. The GC-MS was equipped with a self-injector AOC-20i (Shimadzu, Kyoto, Japan) and a molten silicon dioxide column ZB-5 (5% of phenyl-polysiloxane; length of 30 m, internal diameter of 0.32 mm, film thickness of 0.25 µm, Phnomenex). For each analysis, 1.3 µL of the sample were injected at 280 °C in a ratio 1:1 and the flow of the column (having helium as carrier gas) was fixed at 3 mL min^{-1}. The oven temperature was kept at 60 °C for one minute, then increased by 10 °C min^{-1} until 300 °C and kept for five minutes. The MS interface was working at 300 °C, while the ion source was operational at 200 °C. The mass spectra were taken at 70 eV (in EI manner) from 30 to 450 m z^{-1}. The GC-MS data were processed by means of GC-MS Solution packet, version 4.11 (Shimadzu Corporation, 1999–2013).

2.4. Experimental Design and Statistical Analysis

The test was arranged in a complete randomised design with four replications per substrate. Each replication consisted of 20 potted plants, reaching a total amount of 320 ones. The collected data were subjected to a one way-analysis of variance (substrate as factor), followed by the Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ significance level for means separation. The statistical analysis was carried out by using Statistica (Tulsa, OK, USA) software package.

3. Results

3.1. Substrate and Plant Analyses

The growing substrates had similar bulk density (626–653 kg m^{-3}) and water holding capacity (3.3–3.7 g water g^{-1} dray mass). Substrate pH was neutral and did not show significant difference among them (Table 1). EC ranged from 0.5 to 2.8, increasing according to the following order: S4 < S1 < S3 < S2.

Table 1. Chemical properties of the four substrates.

Properties		Substrates			
		S1	**S2**	**S3**	**S4**
pH		6.66 ± 0.32 ^{a}	7.05 ± 0.22 ^{a}	7.06 ± 0.21 ^{a}	6.82 ± 0.31 ^{a}
EC	dS m^{-1}	1.1 ± 0.3 ^{c}	2.8 ± 0.3 ^{a}	2.0 ± 0.2 ^{b}	0.5 ± 0.1 ^{d}
N	%	1.3 ± 0.1 ^{a}	1.5 ± 0.2 ^{a}	1.1 ± 0.1 ^{b}	1.3 ± 0.1 ^{a}
P	%	0.7 ± 0.1 ^{a}	0.7 ± 0.2 ^{a}	0.4 ± 0.1 ^{b}	0.8 ± 0.3 ^{a}
Na	g kg^{-1}	0.4 ± 0.1 ^{b}	2.6 ± 0.6 ^{a}	1.6 ± 0.6 ^{a}	0.3 ± 0.2 ^{b}
Mg	g kg^{-1}	15.4 ± 3.0 ^{a}	9.8 ± 1.3 ^{b}	12.3 ± 2.2 ^{a,b}	9.4 ± 2.4 ^{b}
Al	g kg^{-1}	9.3 ± 1.3 ^{a}	7.5 ± 2.3 ^{ab}	3.8 ± 1.3 ^{c}	6.9 ± 1.3 ^{b}
K	g kg^{-1}	6.2 ± 3.3 ^{a}	8.6 ± 2.1 ^{a}	10.3 ± 3.7 ^{a}	8.3 ± 1.3 ^{a}
Ca	g kg^{-1}	39.6 ± 4.3 ^{a}	36.5 ± 8.5 ^{a,b}	25.7 ± 2.7 ^{b}	20.2 ± 2.3 ^{c}
Mn	g kg^{-1}	0.8 ± 0.1 ^{a}	0.4 ± 0.2 ^{a}	0.5 ± 0.2 ^{a}	0.5 ± 0.1 ^{a}
Fe	g kg^{-1}	19 ± 3.7 ^{a}	12 ± 1.7 ^{bc}	10 ± 0.8 ^{c}	14 ± 1.9 ^{ab}

Substrates: S1 (40% vermicompost and 60% peat); S2 (40% compost and 60% peat); S3 (40% diges-tate and 60% peat); S4 (100% peat). Different lowercase letters along a row indicate significant differences among substrates at $p < 0.05$.

The pattern of macro and micronutrients did not show any univocal trend among the four substrates and sage leaves (Tables 1–4).

Table 2. Bioavailability of heavy metals and micronutrient in the four substrates (mg kg^{-1} dry matter).

Element	Substrates			
	S1	**S2**	**S3**	**S4**
V	20.3 ± 2.7 ^{a}	10.3 ± 2.9 ^{b,c}	8.5 ± 1.3 ^{c}	12.6 ± 1.3 ^{b}
Cr	20.4 ± 2.5 ^{a}	19.6 ± 3.3 ^{a}	17.3 ± 2.9 ^{a}	21.0 ± 3.5 ^{a}
Co	7.6 ± 1.7 ^{a}	2.0 ± 1.3 ^{b}	1.9 ± 0.9 ^{b}	2.6 ± 1.2 ^{b}
Ni	12.5 ± 1.9 ^{a}	9.0 ± 2.7 ^{a}	10.2 ± 1.8 ^{a}	12.7 ± 2.1 ^{a}
Cu	94.4 ± 10.7 ^{b,c}	119.3 ± 12.7 ^{a,b}	138.5 ± 11.5 ^{a}	84.2 ± 7.2 ^{c}
Zn	476 ± 35.9 ^{a}	295 ± 23.4 ^{b}	122 ± 14.9 ^{c}	93 ± 19.9 ^{c}
As	2.6 ± 0.2 ^{a}	1.9 ± 0.2 ^{b}	2.3 ± 0.4 ^{ab}	3.0 ± 0.4 ^{a}
Se	0.7 ± 0.1 ^{a}	0.4 ± 0.2 ^{a}	0.4 ± 0.1 ^{a}	0.4 ± 0.1 ^{a}
Cd	0.3 ± 0.2 ^{a}	0.9 ± 0.4 ^{a}	0.3 ± 0.2 ^{a}	0.3 ± 0.1 ^{a}
Pb	18.4 ± 1.8 ^{b,c}	28.9 ± 3.9 ^{a}	15.5 ± 2.4 ^{c}	23.1 ± 2.7 ^{a,b}

Substrates: S1 (40% vermicompost and 60% peat); S2 (40% compost and 60% peat); S3 (40% diges-tate and 60% peat); S4 (100% peat). Different lowercase letters along a row indicate significant differences among substrates at $p < 0.05$.

Table 3. Leaf elemental composition (%, mg kg^{-1} or g kg^{-1} of dry matter) of sage plants grown in the four substrates.

	Substrates			
Element	**S1**	**S2**	**S3**	**S4**
N (%)	1.9 ± 0.3 ^{a}	1.4 ± 0.2 ^{b}	1.7 ± 0.3 ^{a,b}	1.6 ± 0.2 ^{a,b}
P (mg kg^{-1})	102 ± 11.1 ^{b}	129 ± 8.9 ^{a,b}	142 ± 10.1 ^{a}	110 ± 12.9 ^{b}
Na (g kg^{-1})	1.5 ± 0.3 ^{a}	2.1 ± 0.5 ^{a}	2.0 ± 0.4 ^{a}	2.0 ± 0.3 ^{a}
Mg (g kg^{-1})	2.6 ± 0.6 ^{b}	3.1 ± 0.5 ^{b}	5.0 ± 0.8 ^{a}	2.8 ± 0.5 ^{b}
K (g kg^{-1})	13.3 ± 0.7 ^{c}	19.3 ± 2.2 ^{a}	14.0 ± 1.1 ^{b,c}	16.0 ± 1.6 ^{a,b}
Ca (g kg^{-1})	11.7 ± 1.8 ^{c}	16.4 ± 1.9 ^{a,b}	18.4 ± 2.6 ^{a}	14.3 ± 0.9 ^{b,c}
Al (mg kg^{-1})	274 ± 42 ^{a}	337 ± 63 ^{a}	314 ± 48 ^{a}	330 ± 22 ^{a}
V (mg kg^{-1})	0.6 ± 0.1 ^{b}	1.4 ± 0.4 ^{a}	0.9 ± 0.2 ^{a}	1.0 ± 0.3 ^{a}
Cr (mg kg^{-1})	0.7 ± 0.1 ^{c}	1.6 ± 0.5 ^{a}	0.9 ± 0.2 ^{b,c}	1.3 ± 0.5 ^{ab}
Mn (mg kg^{-1})	39 ± 6 ^{b}	30 ± 2 ^{c}	60 ± 2 ^{a}	62 ± 9 ^{a}
Fe (mg kg^{-1})	284 ± 35 ^{b}	326 ± 32 ^{a,b}	288 ± 40 ^{a,b}	354 ± 34 ^{a}
Co (mg kg^{-1})	0.1 ± 0.1 ^{b}	0.7 ± 0.5 ^{a}	0.2 ± 0.2 ^{ab}	0.4 ± 0.3 ^{a,b}

Table 3. *Cont.*

	Substrates			
Element	**S1**	**S2**	**S3**	**S4**
Ni (mg kg^{-1})	1.1 ± 1.9^{a}	1.1 ± 0.6^{a}	0.5 ± 0.3^{b}	0.9 ± 0.6^{a}
Cu (mg kg^{-1})	2.0 ± 2.4^{b}	7.2 ± 1.4^{a}	3.8 ± 0.4^{b}	6.7 ± 1.0^{a}
Zn (mg kg^{-1})	119 ± 59^{a}	147 ± 23^{a}	145 ± 30^{a}	110 ± 22^{a}
As (mg kg^{-1})	1.2 ± 0.2^{b}	9.8 ± 6.1^{a}	$2.9 \pm 2.1^{a,b}$	5.9 ± 4.9^{a}
Se (mg kg^{-1})	0.5 ± 0.1^{c}	10.0 ± 3.5^{a}	3.0 ± 2.4^{b}	3.5 ± 2.8^{b}
Cd (mg kg^{-1})	0.1 ± 0.1^{c}	1.2 ± 0.7^{a}	0.4 ± 0.1^{b}	$0.8 \pm 0.7^{a,b}$
Pb (mg kg^{-1})	1.7 ± 0.2^{a}	0.9 ± 0.3^{b}	2.0 ± 1.6^{a}	2.4 ± 0.8^{a}

Substrates: S1 (40% vermicompost and 60% peat); S2 (40% compost and 60% peat); S3 (40% diges-tate and 60% peat); S4 (100% peat). Different lowercase letters along a row indicate significant differences among substrates at $p < 0.05$.

Table 4. Amount (%) of volatile compounds, grouped on the basis of their chemical characteristics and identified in the EO extracted by hydrodistillation from fresh leaves of sage plants cultivated with the four growing substrates.

Compound	**Similarity**	**Retention Time**	**S1**	**S2**	**S3**	**S4**
Monoterpene hydrocarbons	%	min	%	%	%	%
α-pinene	96	5.208	3.54 ± 1.1^{a}	0.05 ± 0.02^{b}	0.39 ± 0.1^{b}	2.66 ± 0.9^{a}
Camphene	93	6.522	$10.36 \pm 1.9^{a,b}$	$10.22 \pm 2.1^{a,b}$	12.24 ± 1.3^{a}	9.26 ± 1.3^{b}
β-pinene	96	7.961	6.87 ± 0.6^{a}	6.68 ± 1.1^{a}	6.37 ± 0.8^{a}	6.46 ± 1.2^{a}
β-myrcene	91	11.055	1.87 ± 0.4^{ab}	1.53 ± 0.4^{b}	1.40 ± 0.8^{b}	2.19 ± 0.5^{a}
Crisantenone	97	25.501	12.43 ± 0.6^{a}	10.76 ± 0.7 b	12.64 ± 0.5^{a}	12.87 ± 0.9^{a}
α-thujone	97	26.676	5.66 ± 0.5^{a}	4.30 ± 0.3 b	5.08 ± 0.2^{a}	5.32 ± 0.3^{a}
Subtotal			40.73 ± 2.1^{a}	33.54 ± 2.2 b	38.12 ± 1.9^{a}	38.76 ± 1.6^{a}
Oxygenated sesquiterpenes						
Palustrol	85	53.605	0.47 ± 0.1^{b}	0.79 ± 0.2^{a}	0.35 ± 0.2^{b}	0.75 ± 0.1^{a}
Ledol	90	58.510	$0.30 \pm 0.2^{a,b}$	0.53 ± 0.1^{a}	0.21 ± 0.1^{b}	0.53 ± 0.1^{a}
Viridiflorol	88	61.131	$0.81 \pm 0.1^{a,b}$	1.36 ± 0.4^{a}	0.61 ± 0.2^{b}	1.22 ± 0.3^{a}
Spathulenol	96	63.245	0.23 ± 0.1^{a}	0.37 ± 0.1^{a}	0.14 ± 0.1^{a}	0.31 ± 0.1^{a}
Subtotal			$1.81 \pm 0.6^{b,c}$	3.05 ± 0.5^{a}	1.31 ± 0.5^{c}	$2.81 \pm 0.4^{a,b}$
Oxygenated monoterpenes						
Eucalyptol	96	12.908	29.2 ± 2.2^{a}	29.79 ± 3.1^{a}	28.55 ± 2.1^{a}	27.67 ± 2.7^{a}
Camphor	90	30.709	21.12 ± 2.4^{a}	23.83 ± 1.9^{a}	24.02 ± 2.1^{a}	21.91 ± 3.6^{a}
4-terpineol	94	36.729	0.51 ± 0.1^{a}	0.48 ± 0.2^{a}	0.37 ± 0.1^{a}	0.54 ± 0.3^{a}
Borneol	85	41.876	2.39 ± 0.7^{a}	2.86 ± 0.5^{a}	2.84 ± 0.4^{a}	2.59 ± 0.8^{a}
4-Caranol	91	42.289	0.16 ± 0.1^{a}	0.38 ± 0.1^{a}	0.15 ± 0.1^{a}	0.09 ± 0.07^{a}
Subtotal			53.38 ± 3.2^{a}	57.34 ± 3.7^{a}	55.93 ± 2.2^{a}	52.8 ± 2.1^{a}
Sesquiterpene hydrocarbons						
α-gurjunene	96	31.759	0.12 ± 0.09^{a}	0.14 ± 0.07^{a}	0.11 ± 0.10^{a}	0.13 ± 0.10^{a}
β-caryophyllene	95	35.639	$1.15 \pm 0.18^{a,b}$	1.60 ± 0.38^{a}	0.89 ± 0.22^{b}	1.46 ± 0.21^{a}
Alloaromadendrene	92	36.271	0.24 ± 0.10^{a}	0.25 ± 0.11^{a}	0.14 ± 0.07^{a}	0.24 ± 0.12^{a}
α-caryophyllene	94	39.743	0.69 ± 0.31^{a}	0.98 ± 0.42^{a}	0.84 ± 0.22^{a}	0.89 ± 0.19^{a}
Subtotal			2.20 ± 0.35^{a}	2.97 ± 0.44^{a}	1.98 ± 0.48^{a}	2.72 ± 0.49^{a}
Other compounds						
Bornyl acetate	96	34.930	1.24 ± 0.1^{a}	1.77 ± 0.1^{a}	1.44 ± 0.1^{a}	1.45 ± 0.1^{a}
Naphthalene	94	44.743	0.17 ± 0.1^{a}	0.21 ± 0.1^{a}	0.11 ± 0.1^{a}	0.28 ± 0.1^{a}
Diethyl phthalate	97	74.304	0.27 ± 0.1^{c}	0.61 ± 0.1^{ab}	0.99 ± 0.1^{a}	0.53 ± 0.1^{b}
Maool	90	90.474	0.19 ± 0.1^{b}	0.51 ± 0.1^{a}	0.12 ± 0.1^{b}	0.62 ± 0.1^{a}
Subtotal			$1.87 \pm 0.21^{b,c}$	3.10 ± 0.52^{a}	2.66 ± 0.16^{b}	$2.88 \pm 0.29^{a,b}$

Substrates: S1 (40% vermicompost and 60% peat); S2 (40% compost and 60% peat); S3 (40% digestate and 60% peat); S4 (100% peat). Different lowercase letters along a row indicate significant differences among substrates at $p < 0.05$.

Nitrogen (N) concentration in the substrates ranged from 1.1 to 1.5% (Table 1): substrate S3 showed the lowest N value, whereas no significant difference was found

among the other substrates. S3 also evidenced the lowest phosphorus (P) concentration, whereas potassium (K) did not show any significant difference among substrates.

As far as the concentration of chromium, nickel, selenium and cadmium, they did not show significant differences among the substrates (Table 2). Substrate S1 evidenced the highest concentration of vanadium, cobalt and zinc, whereas S2 and S3 showed the highest copper concentration (Table 2). Arsenic was higher in S1, S3 and S4, while lead was higher in S2 and S4.

With regard to the leaf macronutrient concentration, the sage plants grown with S1 showed the highest N but the lowest P, K and Ca (Table 3). The plants grown with S2 evidenced the lowest N but also the highest K, whereas those grown with S3 were characterised by the highest P, Mg and Ca (Table 3).

With regard to the leaf heavy metals concentration, the sage plants grown with S2 showed the highest amount of vanadium, chrome, cobalt, arsenic, selenium and cadmium and, together with those grown with S4, the highest concentration of aluminum, iron and copper (Table 3). Leaf manganese was higher in plants grown with S3 and S4. Leaf lead was higher in plants grown with S1, whereas zinc was higher in the leaves of the plants grown with S2 and S3 (Table 3).

3.2. Essential Oil Analysis

The EO extracted from sage leaves had a light yellow colour and a typical smell. The EO yield ranged from 0.22 (S2) to 0.29% (S4), showing no significant difference among the four tested substrates (Figure 2). The 23 volatile compounds identified by GC-MS were grouped based on their chemical composition in monoterpene hydrocarbons, oxygenated sesquiterpenes, oxygenated monoterpenes and sesquiterpene hydrocarbons (Table 4). Moreover, other compounds not included in the previous ones were identified. Monoterpene hydrocarbons and oxygenated monoterpenes were the most abundant identified compounds for all sage leaves, accounting for more than 90% of the total identified compounds. Camphene and crisantenone were the most abundant compounds among the monoterpene hydrocarbons (on average 10.52 and 12.17%, respectively), whereas eucalyptol and camphor were the most abundant compounds among the oxygenated monoterpenes (on average 28.80 and 22.72%, respectively). However, the four compounds extracted from sage leaves did not differ with respect to the eucalyptol and camphor content in the EO (Table 4).

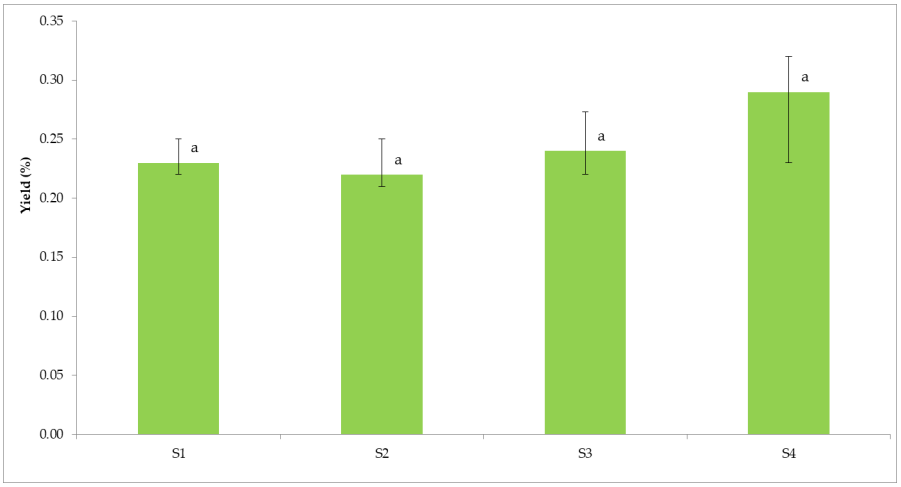

Figure 2. Effect of the growing substrates on sage essential oil (EO) yield (%). Vertical bars (means of the four substrates) with the same letters indicate not significant differences at $p \leq 0.05$ (Duncan's Multiple Range Test—DMRT).

The sage leaves cultivated with S2 substrate showed, at least, 4.58% lower monoterpene hydrocarbons than the other substrates, among which no statistical difference occurred (Table 4). Such lower monoterpene hydrocarbons with S2 substrate was due to a

lower amount of almost all the identified compounds and mainly to a lower content of crisantenone and α-thujone. The four sage leaves tested showed significant differences with respect to the amount of oxygenated sesquiterpenes but they were lower than 2%.

4. Discussion

The substrates tested in this study showed significantly different chemical properties, mainly due to the processes carried out for their production or to the raw materials used for their production [44].

Substrate EC was very variable, showing the highest value for S2; also S3, however, it had a value of 2.0 dS m^{-1}. Such higher values suggested some limitations in using the S2 and S3 substrates for the cultivation of less salt tolerant plants. Indeed, results from the study previously carried out by other authors [15] using the same substrates for sage cultivation suggested that they could reduce the leaf area and the weight of leaves, roots and stems, as well as the root length.

The lowest concentration of total N in the substrate obtained from digestate (S3) could be due to the passage of nitrogen in the liquid phase during separation [45].

Moreover, the lower concentration of available P and Fe (extracted with DTPA solution) is to be ascribed to Fe-phosphate and other minerals (struvite) precipitation [46]. With regard to the effect of AD process on P availability, contrasting results are reported in literature. Indeed, some studies report that this process increases nutrient the availability for plants or that it does not have any direct effect; other studies state that AD decreases P availability [47].

On the other hand, S3, together with S2 (compost from OFMSW), showed from five to ten times, respectively, more Na than S4. This higher concentration of Na in S3 and S2 is due to the raw materials used for their production, mainly deriving from food waste [48], that in turn contributes to increase the EC of these substrates.

The concentration of Ca in S4 was of the same magnitude to that reported by Arkhipov and Bernatonis [49], who found a concentration of Ca in different types of peat ranging from 0.2 to 3.0%. The higher Ca concentration in the other substrates compared to S4 may be due to the raw materials used for their production [50].

Finally, with regard to the concentration of heavy metals, they do not exceed the limits defined by national and international laws, as well as EU regulations (i.e., Italian Legislative Decree 75/2010, European Union Regulation 2019/1009, U.S. Environmental Protection Agency 503 Rule) for the use of biosolids and other waste as growing substrates, thus being potentially usable as substrate for plant cultivation [50]. Macro and micro-elements, as well as heavy metals, in the plant-substrate system undergoes to several interactions among the organic phases, the soil solution and the vegetation. Originating from the solution, the elements excreted by substrate decomposition, by suction, move into the plant roots and then flow through the plant. Within the plants, some elements could accumulate in high amount, although they are not in relevant concentration in substrates [51]. No correlation was found between the same element determined in the substrates and in the sage plant leaves. Such an aspect is not surprising, due to the young age of the sage plants, that probably cannot establish a complete elements feedback with the substrates.

The different macronutrients concentration in sage leaves cultivated in S3 and S4 may be due to the presence of P-minerals in S3. Indeed, as previously described, AD process may lead to the precipitation of Fe-phosphate and other minerals (struvite), that, being then solubilised by root exudates, can be uptaken by plants. [52]. This explains the higher concentration of P, Mg and Ca in sage leaves cultivated in S3 than in S4.

The EO yield of sage plants did not show significant differences among the four different substrates and was lower than that reported by Baydar et al. [53], ranging from 1.43 to 3.24%, and by Arraiza et al. [54], ranging from 0.6 to 1.5%. The lower yield values found in this study, compared to those of Baydar et al. [53] and Arraiza et al. [54], may be due to intrinsic and extrinsic factors, such as substrates used for the cultivation, climate, maturity of the plants at the harvest time during the day and extraction method. Consider-

ing that many of these factors did not differ, among them, the lower EO yield values can be ascribed to the young age (9 months) of the sage plants used in this study compared to the old age of those reported by Baydar et al. [53] and by Arraiza et al. [54].

The most abundant compounds in EO extracted from sage plants cultivated on the four tested substrates were eucalyptol, camphor, α-thujone, β-pinene, crisantenone and camphene, thus agreeing with previous studies [53–55]. These compounds are those required for the aromatic characteristics and nutraceutical uses of the EO extracted from sage plants. The eucalyptol, camphor and α-pinene did not show significant differences among EOs extracted from different plants and were found in concentration similar to those found in previously studies [53–55]. However, the camphor content extracted from sage EO resulted higher than that reported by Arraiza et al. [54]. On the other hand, the α-thujone and crisantenone were lower in S2 compared to S4, whereas camphene was lower in S4 than in S3. Differences among these monoterpene hydrocarbons were, however, below 3%.

5. Conclusions

The search for substrates alternative to peat is of great importance, due to the high environmental concern about massive peat extraction for agricultural uses. On the other hand, to reuse waste, after its transformation, such as vermicomposting, composting and anaerobic digestion processes, is crucial in view of the circular economy and a sustainable use of resources. The results of this study provided evidence that vermicompost, compost and solid digestate can be used as valid alternatives to peat for sage cultivation. Sage plants cultivated on compost, vermicompost and solid digestate did not show significant differences in terms of their chemical composition, as well as yield and composition of essential oils extracted from them. However, the substrates obtained from composting process and from anaerobic digestion did not affect the qualitative parameters of sage plants but have to be used with caution, due to their higher values of electrical conductivity, mainly due to Na and other salts.

Further studies have to be addressed to find the proper ratios among the substrates, different from those tested in this work, in order to maximise the sage EO yield, improve its qualitative composition and reduce heavy metals concentration in plant leaves.

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