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Cotton and cardoon byproducts as potential growing media components for *Cichorium spinosum* L. commercial cultivation

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ABSTRACT

The intensification of horticultural crops cultivation makes urgent the seeking for alternative growth substrates that could substitute non-renewable and/or synthetic growing media, such as peat and rock wool. The aim of the present study was to evaluate the potential use of byproducts from two industrial crops commonly cultivated in the Mediterranean basin, namely cardoon and cotton, as growth substrates for *Cichorium spinosum*, while zeolite addition was also tested as a soil amendment. A pot experiment was carried for two consecutive growing periods and plant growth was evaluated for six growing media compositions, while plant extracts were also evaluated in terms of their phenolic compounds profile, antioxidant and antimicrobial activities. The results of this study showed that cotton byproducts and zeolite may partially substitute peat in growth substrate of *C. spinosum* and high yields comparable to peat may be achieved. Phenolic compounds content and antioxidant activity of leaves' extracts was higher for plants grown in soil which showed severe stress symptoms comparing to the other tested substrate blends. Antimicrobial activity was also affected by growth substrate composition, only in the case of antibacterial properties of leaves' extracts, whereas none of the extracts presented significant antifungal activities. In conclusion, the use of cotton ginning byproducts and zeolite in growth substrate blends may partially substitute conventional substrates as peat in horticultural crops production, resulting in reduction of production cost and lessening of bulky byproducts' management and related environmental burden without compromising yield.

1. Introduction

There is a growing demand for the management of agro-industrial by-products within the concept of sustainable agriculture and circular economy which aims at reducing environmental burden and increasing crops added value (Morales et al., 2017). Cotton (*Gossypium hirsutum* L.) is an important industrial crop producing great amounts of byproducts annually, including cotton stalks and linters (ginning industry waste), while it also contributes substantially to global production of greenhouse gas emissions due to high nutrient and energy requirements (Hedayati et al., 2019). Moreover, although the common practice of burning or leaving remaining cotton stalks in the field reduces the handling and disposal cost of crop byproducts, it further increases environmental burden through gas and particle emissions (Kazemi et al., 2018; Riley et al., 2016). Cardoon (*Cynara cardunculus* L. var. *altilis* DC.) is another industrial crop, increasingly used as an energy crop (Ciancolini et al., 2013; D'Antuono et al., 2018), which produces high amounts of biomass and seeds intended for energy production

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and biofuel (Curt et al., 2002; Petropoulos et al., 2018a; Razza et al., 2016). The use of artichoke waste for the recovery of phenolic compounds and bioenergy production has been suggested by Zuorro et al. (2016), while Petropoulos et al. (2017, 2018a) have reported the bioactive compounds profile of cardoon plant parts for potential use in the pharmaceutical industry. While the byproducts of both cotton and cardoon processing are considered bulky biowaste (De Corato et al., 2018), the increasing demand of non-synthetic, renewable, and locally available growing media for soilless-grown horticultural crops may open new perspectives for the use of these materials as substitute of peat-based growing media (Di Gioia et al., 2017).

Sphagnum peat or peat-based mixes are the most commonly used growth substrates in vegetable and ornamental crops production, either for seedling production and pot cultivation or for soil amelioration purposes (Dixon and Aldous, 2014). However, the increasing global demand for growth substrates has resulted in increasing prices of peat-based products and reduction of available natural resources which further increase production cost of horticultural products (Barrett et al., 2016; Bustamante et al., 2008). Moreover, peat based production is associated with environmental issues related with gas emissions and natural resources depletion (Bonaguro et al., 2017). The use of peat-based growth substrates is very common for seedling production and soilless cultivation of leafy vegetables and/or microgreens (Di Gioia et al., 2017), although various alternative substrates have been suggested as peat substitutes with very diverse physicochemical properties and contrasting results (Dixon and Aldous, 2014; Moore, 2015). *Cichorium spinosum* L. is a wild species with edible tender leaves which constitute a common ingredient of the Mediterranean diet and recently has gained research interest due to its bioactive compounds profile and its high nutritional value (Petropoulos et al., 2017b; Psaroudaki et al., 2012). During the last few years, increasing market demands have created a niche for commercial cultivation of the species and agricultural practices must be elucidated and compiled in best practice guidelines.

Several studies have confirmed the effect of growth substrates not only on yield parameters but also on quality features of the final products related with their nutritional value, chemical composition and bioactive compounds content (Saleh et al., 2019). So far, only municipal solid waste compost has been evaluated as a potential substrate for *C. spinosum* plants (Papafilippaki et al., 2015). In the same context, olive oil industry byproducts have been proposed as ingredients of growth substrates and according to (Chrysargyris et al., 2019) olive mill waste could be a promising substrate for seedling production of *Brassica* species in nurseries. Biochar is also a candidate substrate for soilless cultivation of leafy vegetables since research studies demonstrated that its use increased plant growth and mineral composition and decreased the undesirable growth of algae in nutrient solution (Awad et al., 2017). Other potential peat substitutes include composts from distillery wastes (Bustamante et al., 2008), mixtures of vegetable waste, manure and sawdust (Gavilanes-Terán et al., 2016), paper waste (Chrysargyris et al., 2018a), hazelnut husks (Dede et al., 2011), and sawdust (Marinou et al., 2013) among others.

Cotton industry byproducts have been also used as growth substrates for ornamental crops, where according to Cole et al. (2005) the use of substrate blends containing cotton ginning byproducts resulted in plant growth comparable to traditional substrates for azalea plants, while a higher water use efficiency was also recorded. Moreover, the same raw material showed promising results in the production of leafy vegetables such as lettuce, spinach and radish (Khah et al., 2012), and Alburquerque et al. (2006) further suggested the use of cotton waste as a bulking agent in composts intended for greenhouse production of pepper. Composts from cotton stalks and gin byproducts have been proposed for use as amendments to commercial nursery substrates to improve their physicochemical properties (Riley et al., 2016; Warren et al., 2009). Moreover, incorporation of cotton gin thrash composts in growth substrate resulted in compact growth of potted chrysanthemum, reducing the required dose of daminozide for the production of compact ornamental plants (Papafotiou and Vagena, 2012). The addition of crushed cotton gin compost as soil amendment under Mediterranean semi-arid conditions was also reported to have a beneficial effect on rice crop yield, plant nutrient uptake and soil biological properties (Tejada and Gonzalez, 2006). As it is difficult to resemble and fully substitute the properties of peat-based growing media with waste material, a strategy commonly used is to substitute only a fraction of the peat to prepare *ad hoc* mixes combining different materials (Abad et al., 2001). In this perspective, zeolite a very common aluminosilicate mineral has been proposed as a conditioner to ameliorate the properties of soilless media and enhance soilless crops nutrient use efficiency (Gül et al., 2005). Recently, zeolite has found several uses in agricultural production, including its use in slow release fertilizers and as pesticide carrier, for soil amelioration through heavy metals binding, as a water retention agent and so forth (Eroglu et al., 2017; Nakhli et al., 2017; Zuorro et al., 2016). For that reason, its use in substrate blends is very promising in the adoption of sustainable agriculture practices for horticultural crops in general, and especially in potted plants where growth substrates are most commonly used (Bonaguro et al., 2017; Chen et al., 2018).

The bulky nature of field crops byproducts and the high amounts of waste produced annually, necessitates the finding of alternative end-use solutions for mitigating environmental stress as well as for the adoption of sustainable production systems within the circular economy concept. Considering the importance of cotton and cardoon crops in the Mediterranean basin for the textile and energy production industry, the aim of the present study was to evaluate the effect of various cotton and cardoon byproducts-based substrates supplemented with zeolite on the growth and chemical composition of *C. spinosum* plants. This particular species was selected due to its high market value and its ability to adapt and grow under arduous conditions that could make economically feasible the use of alternative growth substrates. For this purpose, a pot experiment was carried out for two consecutive growing seasons and growth substrates of variable composition were tested, namely: a) soil, b) peat, and c) waste from agroindustry byproducts (cardoon seedcake, cotton ginning byproducts) with the addition of zeolite. The evaluation criteria included *C. spinosum* yield in terms of fresh biomass production, as well bioactive compounds content and antioxidant and antimicrobial properties of the leaves.

2. Materials and methods

2.1. Plant material and experimental conditions

The experiment took place at the experimental farm of the University of Thessaly, Greece. Plant material has been previously described in the study of Kolovou et al. (2017). Briefly, seeds of *Cichorium spinosum* (Asteraceae) were sown on September 27th, 2016 (1st sowing date) and on December 5th, 2016 (2nd sowing date) in seed trays containing peat. When plants reached the stage of 3–4 assimilatory leaves, young seedlings were transplanted in 2 L pots on December 4th, 2016 (1st sowing date) and on February 14th, 2017 (2nd sowing date). The following substrates were used in the 1st sowing date: a) soil (SUB1), b) soil: peat (Klassman-Deilmann TS 3) (1:1 v/v) (SUB2), c) soil + peat + cardoon seedcake (80%) and zeolite (20%) mix (1:1:1 v/v) (SUB3), d)

soil + peat + cotton ginning waste (80%) and zeolite (20%) mix (1:1:1 v/v) (SUB4), e) soil + peat + cardoon seedcake (60%) and zeolite (40%) mix (1:1:1 v/v) (SUB5), f) soil + peat + cotton ginning waste (60%) and zeolite (40%) mix (1:1:1 v/v) (SUB6). For the 2nd sowing date, the most promising substrates were used based on the yield results of the 1st sowing, namely SUB1, SUB2 and SUB6. Physicochemical properties of soil and raw material were determined according to the method described by Chrysargyris et al. (2019) (Table 1). Size of zeolite particles was within the range of 1.8–3.5 mm.

Each pot was filled up to a total volume of 2 L after homogenization of substrate. Plants from all treatments were fertigated throughout the experiment with nutrient solution containing the same amount of nitrogen (300 mg/L) using amounts of 50–300 mL per pot, depending on weather conditions. Water was provided in excess to assure a minimum drainage of 20%. Twenty pots, each one containing one plant were used for each treatment (120 pot for the 1st sowing date, 60 pots for the 2nd sowing date and 180 pots in total). In both experiments, treatments were arranged in a randomized complete block design with three replications.

Harvest of plants was carried out once for each growing period and when rosettes of leaves reached marketable size, namely February 5th, 2017 for the 1st sowing date and on April 26th, 2017 for the 2nd sowing date. After harvest, fresh and dry weight of leaves and number of leaves were recorded. For dry weight evaluation, samples of leaves were dried at 72 °C until constant weight. Samples of fresh leaves were put in freezing conditions, then lyophilised, ground with a mortar and pestle, put in air-sealed food bags and stored at deep freezing conditions (−80 °C) until further analysis.

2.2. Extracts preparation

Lyophilised powdered of different *Cichorium spinosum* treated plants were submitted to heat assisted extraction by maceration in an aqueous ethanolic solution (80%, v/v; 30 g/L) at 25 °C for 60 min. Afterwards, extract suspension was filtered using a Whatman nº4 filter and the above procedure was repeated using the same conditions to maximize the extraction yield. Afterwards, the solvent was evaporated at 40 °C, under reduced pressure, in a rotary evaporator (Büchi R-210, Flawil, Switzerland) and the residual aqueous extract freeze dried (FreeZone 4.5 model 7750031, Labconco, Kansas, USA).

2.3. Phenolic compounds characterization

The dry extracts were re-dissolved in aqueous ethanol (80%, v/v) at a concentration of 10 mg/mL and filtered using 0.2 μm disposable LC filter disk, 30 mm, nylon, before loading on the HPLC column. HPLC analysis was performed using liquid chromatography with diode-array detector (280, 330, and 370 nm wavelengths) coupled to an electrospray ionization mass spectrometry operating in negative mode (Dionex Ultimate 3000 UPLC and Linear Ion Trap LTQ XL, Thermo Scientific, San Jose, CA, USA). The chromatographic separation conditions were similar to the ones previously described by Bessada et al. (2016). The phenolic compounds were identified according to their chromatographic characteristics (retention times, UV-VIS and mass spectra) and by comparison to those obtained with standard compounds, as also with data available from already reported studies. Calibration curves of appropriate standards were obtained in the range 200–5 μg/mL, for the quantitative analysis. For compounds with no available commercial standards, quantification was carried out using calibration curves of the most similar available compound. The results were expressed in mg per g of extract (mg/g).

2.4. In vitro antioxidant assays

Each sample was dissolved in water and from the stock solution of the aqueous extracts (10 mg/mL), successive dilutions were made (5000 and 6.25 μg/mL). The antioxidant activity was measured through the thiobarbituric acid reactive substances (TBARS) assay and anti-haemolytic activity. The TBARS assay was performed following a methodology described by Barreira et al. (2013) and results were expressed in IC_{50} values, which represent sample concentration providing 50% of antioxidant activity. The anti-haemolytic activity of the extracts was evaluated by the oxidative haemolysis inhibition assay (OxHLIA), as previously described by Lockowandt et al. (2019). The results were presented as IC_{50} values, which represent extract concentration that delayed the haemolysis time for 60 min, with 50% of intact erythrocytes. Trolox was used as positive control.

2.5. Antimicrobial assays-Microbial and fungal strains

Three Gram (+) bacteria [*Bacillus cereus* (clinical isolate), *Staphylococcus aureus* (ATCC 6538), and *Listeria monocytogenes* (NCTC 7973)], and three Gram (−) bacteria [*Escherichia coli* (ATCC 35210), *Enterobacter cloacae* (human isolate) and *Salmonella enterica* subsp. *enterica* (ATCC 13311)], were used for testing antibacterial activity of leaves extracts, while seven fungi [*Aspergillus fumigatus* (ATCC 1022), *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger* (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9122) and *Penicillium aurantiogriseum* (food isolate)] were used to test antifungal activity. The bacteria and fungi were obtained from the Mycological laboratory, Department of Plant Physiology, Institute for biological research "Sinisa Stanković", University of Belgrade, Serbia.

2.5.1. Microbial and fungal inhibition assay

The antimicrobial activity was evaluated with a microdilution method (Tsukatani et al., 2012), following a procedure previously described by Soković and Van Griensven (2006). The concentrations that completely inhibited bacterial growth were defined as the lowest concentrations without visible growth, at the binocular microscope (MICs; minimal inhibitory concentration) and were

Table 1
Physicochemical properties of the tested growing media mix components.

Substrate components	Bulk density (g/cm[b])	WHC[a] (%)	pH	EC dS/m	OM[2] (%)	C (%)	N (%)	C/N	K (cmol/kg)
Cardoon seedcake	0.63	117.6	6.1	0.56	82.3	47.8	0.25	191.4	1.67
Cotton waste	0.13	180.0	6.8	5.42	82.9	48.1	0.19	253.4	0.87
Zeolite	0.82	61.3	7.0	n/a	n/a	n/a	n/a	n/a	n/a
Soil[b]	1.07	49.9	7.5	0.14	2.7	1.6	0.08	19.9	0.91
Peat	0.12	218.5	6.0	0.35	47.5	27.5	0.14	196.8	46.03

[a] WHC: water holding capacity; OM: organic matter.
[b] Soil texture was classified as Sandy-Clay-Loam.

determined by the colorimetric microbial viability assay based on reduction of INT (p-iodonitrotetrazolium violet) and by reinoculation of 10 μL of medium with inoculum and tested extracts in fresh clean medium. After 24 h for bacteria and 72 h for fungi, the lowest concentrations without visible microbial growth was defined as the MICs, the lowest concentration indicating 99.5% death of bacteria strain as the MBC, and the lowest concentration indicating 99.5% death of fungal strain as the MFC. Streptomycin (Sigma-Aldrich S6501) and Ampicillin (Sigma-Aldrich A9393) were used as positive controls for antibacterial activity, while Ketoconazole (Zorka-pharma, Serbia) and Bifonazole (Srbolek, Serbia) were used as positive controls for antifungal activity. 5% DMSO was used as a negative control.

2.6. Statistical analysis

Plant growth measurements were recorded on 20 plants per substrate treatment for both sowing dates ($n = 20$). For chemical analyses and for each growth substrate and growing season the harvested leaves from all the plants were divided into three batch samples of ($n = 3$) for further analysis. All chemical composition assays were carried out in triplicate. Data were analyzed with a two-way analysis of variance (ANOVA), and when significant differences were observed means comparison was carried out with the Tukey's HSD Test ($p = 0.05$). The statistical package IBM SPSS v.21 statistical software (IBM Corp., Armonk, NY, USA) was implemented for data analyses.

3. Results and discussion

3.1. Physicochemical properties of the growth substrate components

Examining the physicochemical properties of all the growth substrate components tested it was observed that only peat and cotton waste had bulk density values below 0.4 g/cm^3 which is the maximum threshold for an ideal growing medium (Table 1) (Di Gioia et al., 2017). As expected, soil had the highest bulk density (1.07 g/cm^3), followed by zeolite and cardoon seedcake. Lower bulk density values are usually associated with good porosity and lower transportation costs and on that account are preferable to a higher bulk density. Cotton waste had a bulk density similar to peat, suggesting that other physical properties may be similar. Peat had the highest WHC (218.5%), followed by cotton waste, cardoon seedcake, zeolite, and soil which compared to peat had 17.6%, 46.2%, 71.9%, and 77.2% less WHC, respectively. Peat and cardoon seedcake had similar pH values on the lower hand of the sub-acid range, which is considered optimal for the growth of most plants (5.5–6.6) (Islam et al., 1980). Cotton waste pH was on the higher hand of the sub-acid range and was on that account sub-optimal for plant growth. Zeolite had neutral pH, while the pH of the soil was sub-alkaline. Soil had the lowest EC (0.14 dS/m), followed by peat and cardoon seedcake that showed values within the optimal range for growing media. Instead, cotton waste showed a high EC (5.42 dS/m), which could limit the growth of crops sensitive or moderately sensitive to salinity stress; but may not limit the growth of *C. spinosum* which is considered relatively tolerant to salinity stress (Petropoulos et al., 2017b). Examining the chemical composition of the growth substrate component tested, soil had the lowest total C, OM, and total N content. While both cotton waste and cardoon seedcake had the highest values of total C, OM, and total N. The C/N ratio was similar in peat and cardoon seedcake and was higher in the case of cotton waste, suggesting that both cardoon seedcake and cotton waste are very stable materials. When looking at the concentration of K in each substrate blend component, cardoon seedcake and cotton waste showed low K concentrations, similar to that of the soil. While peat had considerably higher K concentration. A low nutrient concentration in soilless media is usually highly desirable, because nutrient supply may be controlled more easily, although the availability of some of the nutrients may allow to save money on fertilizers.

3.2. Effect of growth substrate on plant growth and yield

Plant growth and yield parameters are presented in Table 2. Substrate blends containing cotton ginning byproducts and zeolite (SUB4 and 6 in the 1st sowing date and SUB6 in the 2nd sowing date) exhibited the best results in terms of fresh weight and number of leaves, whereas dry weight was the highest for plants grown in soil (SUB1). The beneficial effects of cotton ginning byproducts and zeolite on *C. spinosum* plant growth could be attributed to the improved water holding capacity and better water availability of the substrate blends. While the combination of cardoon seedcake with zeolite in the substrate blends SUB3 and SUB5 provided lower fresh yield and number of leaves, compared to the combined application of cotton ginning byproducts and zeolite, it cannot be excluded that zeolite had a beneficial effect on *C. spinosum* plant growth. On lettuce grown in perlite the addition of zeolite improved plant growth and enhanced plant nutrient uptake (Gül et al., 2005). Moreover, the addition of cotton byproducts combined with zeolite in substrate blends regulated pH and EC values to proximate to optimum growing conditions for the tested species, while it increased the overall organic matter content comparing to peat and soil. Although Chatzigianni et al. (2017) reported that hydroponically grown *C. spinosum* plants did not respond to pH fluctuations due to different nitrogen sources in the nutrient solution, these contradicting results could be attributed to different growing media (perlite) and genetic material used in that study. Moreover, soil type may affect plant growth and fresh biomass yield of *C. spinosum*, since according to Papafilippaki et al. (2015) it seems that it prefers sandy soils due to its natural growth habits. In another report, Khah et al. (2012) suggested the beneficial effects from incorporating cotton ginning byproducts in peat-based substrates on the yield of leafy vegetables (lettuce and spinach) and radish in comparison to plants grown solely in peat. In contrast, Barcelos et al. (2016) highlighted the beneficial effect of peat-based substrates on spinach growth comparing to coir and blends of forest residues, peat and husks, while according to Di Gioia et al. (2017), the use of recycled cotton fibers in growth substrates for microgreens production resulted in fresh biomass yields similar to peat substrates. Regarding the combined effect of growth substrate and salinity, Klados and Tzortzakis (2014) reported that the selection of substrate as well as the severity of high salinity are pivotal for plant growth of *C. spinosum*. On that account, the use of cotton ginning byproducts and zeolite in growth substrates may partially substitute peat which could result in reduction of production cost while at the same time high yields are retained and environmental burden from bulky byproducts is reduced.

3.3. Effect of growth substrate on phenolic compounds profile

A total of thirteen compounds were identified in *C. spinosum* hydroethanolic extract (Table 3). They include four phenolic acids (hydroxycinnamic acid derivatives) and nine flavonoid glycoside derivatives, of which eight flavonols (mainly quercetin, kaempferol and isorhamnetin derivatives) and one flavone (apigenin derivative). All the identified compounds have been previously identified in *C. spinosum* by the authors (Petropoulos et al., 2018b; c; 2017a; b; c). Similarly, phenolic compounds profile in decoction extracts prepared from *C. spinosum* edible parts (healthy, clean leaves) were also reported by Brieudes et al. (2016) and Mikropoulou et al. (2018)

Table 2
Fresh and dry weight and number of leaves of *Cichorium spinosum* plants in relation to growth substrate and growing season (means ± SD).

Sowing date [a]	Substrates [b]	Fresh weight (g/plant)	Dry weight (%)	Number of leaves
1st	SUB1	3.7 ± 0.7e	15.3 ± 1.1a	18.3 ± 1.1e
	SUB2	14.3 ± 1.1b	11.4 ± 0.9c	27.9 ± 2.3 d
	SUB3	5.8 ± 0.6 d	12.7 ± 0.8b	15.3 ± 1.5f
	SUB4	15.8 ± 1.3a	10.1 ± 0.7 d	28.9 ± 2.1c
	SUB5	12±1c	10.3 ± 0.6 d	29.3 ± 2.1b
	SUB6	16.0 ± 0.9a	10.2 ± 0.9 d	32.9 ± 2.9a
2nd	SUB1	0.94 ± 0.02c*	18.3 ± 1.3a*	8.1 ± 0.7c*
	SUB2	2.96 ± 0.08b*	17.3 ± 1.1b*	13.9 ± 1.3b*
	SUB6	5.21 ± 0.12a*	14.9 ± 0.9c*	19.4 ± 1.4a*

Different Latin letters in the same column and the same sowing date indicate significant differences between the substrates. The asterisk (*) symbol indicates differences between the sowing dates for the same substrate ($p_{=}0.05$).

[a] 1st sowing date: September 4th, 2016; 2nd sowing date: December 5th, 2016.

[b] SUB1: soil; SUB2: soil + peat (1:1 v/v); SUB3: soil + peat + cardoon seedcake (80%) and zeolite (20%) mix (1:1:1 v/v); SUB4: soil + peat + cotton ginning waste (80%) zeolite (20%) mix (1:1:1 v/v); SUB5: soil + peat + cardoon seedcake (60%) zeolite (40%) mix (1:1:1 v/v); SUB6: soil + peat + cotton ginning waste (60%) zeolite (40%) mix (1:1:1 v/v).

Table 3
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data and tentative identification of phenolic compounds in *Cichorium spinosum* leaves' extract.

Peaks	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification
1	4.23	328	311	179(85), 149(54), 135(100)	Caftaric acid
2	6.37	328	353	191(100), 179(71), 135(43)	5-*O*-Caffeoylquinic acid
3	11.78	330	473	313(68), 293(83), 219(13), 179(93), 149(100), 135(42)	*cis* Chicoric acid
4	12.45	330	473	313(68), 293(83), 219(13), 179(93), 149(100), 135(42)	*trans* Chicoric acid
5	18.19	358	477	301 (100)	Quercetin-3-*O*-glucuronide
6	18.67	350	461	285 (100)	Kaempferol-*O*-glucuronide
7	20.27	356	505	463(10), 301(100)	Quercetin-7-*O*-(6″-*O*-acetyl)-glucoside
8	21.14	348	593	285 (100	Kaempferol-3-*O*-rutinoside
9	22.03	348	461	285 (100	Kaempferol-3-*O*-glucuronide
10	23.1	336	445	269 (100)	Apigenin-7-*O*-glucuronide
11	23.51	358	491	315 (100)	Isorhamnetin-3-*O*-glucuronide
12	24.71	338	489	285 (100)	Kaempferol-3-*O*-(6″-*O*-acetyl)-glucoside
13	25.95	358	519	315 (100)	Isorhamnetin-3-*O*-(6″-*O*-acetyl)-glucoside

who revealed the presence of caftaric acid, 5-*O*-caffeoylquinic acid, quercetin-3-*O*-glucuronide and kaempferol-*O*-glucuronide.

Plants grown in plain soil exhibited the highest content of total phenolic acids, flavonoids and phenolic compounds, regardless of the sowing date (SUB1; Table 4). However, not all the individual compounds were positively affected by soil substrate, namely caftaric and 5-*O*-Caffeoylquinic acids which showed the highest content when plants were grown in substrate blends. Moreover, similarly to Petropoulos et al. (2017c) the overall content of total and individual phenolic compounds was higher in the 2nd sowing date for the tested substrates, which indicates the importance of sowing date on phenolic profile of *C. spinosum* apart from the impact of growing substrate. Considering the results related to plant growth presented in Table 2, it seems that plants grown in plain soil were subjected to severe stress condition and their agronomic performance (yield) was considerably low. According to Klados and Tzortzakis (2014), salinity stress induces the biosynthesis of phenolic compounds in *C. spinosum*, whereas Petropoulos et al. (2017b) reported no significant effect. These contrasting results could be attributed to the different growth substrates and growing systems implemented in these studies, as well as to the severity of salinity stress which was higher in the study of Klados and Tzortzakis (2014). Higher values of total phenolic compounds content were also reported for parsley and dill plants grown in Germany soil in comparison to peat-based substrates by Saleh et al. (2019), while Chrysargyris et al. (2018a) demonstrated the effect of using paper in growth substrate blends on total phenols content of ornamental plants. In contrast, Dannehl et al. (2015) reported no significant differences in total phenolic compounds in fruit of tomato plants grown in peat moss, rock wool, sheep wool and hemp which indicates that there is also a species depended response to growth substrate.

3.4. Effect of growth substrate on antioxidant activity

Plants grown in soil had the highest antioxidant activity for both TBARS and OxHLIA assays showing the lowest IC_{50} values comparing to the other tested growth substrates, regardless of the sowing date (Table 5). In regard to OxHLIA assay, half of the erythrocytes were protected after 30, 60 and 120 min when 16 ± 2, 61 ± 4 and 173 ± 6 μg/mL of leaves extracts from plants of the 1st sowing date were used, respectively, whereas for the 2nd sowing date the amounts for the same time periods were 42 ± 2, 79 ± 2 and 153 ± 3, respectively. The same trend was observed for the TBARS assay, although IC_{50} values were higher than those reported for the inhibition of haemolysis of erythrocytes after 120 min for both sowing dates (199 ± 5 and 90 ± 1, for the 1st and 2nd sowing date, respectively). These results could be attributed to the higher content of total and individual phenolic compounds for the plants grown in plain soil comparing to the other substrate treatments. According to Dalar and Konczak (2014), the antioxidant activities of *Cichorium intybus* are correlated with total phenolic compounds content and individual compounds such as hydroxycinnamic acids and apigenin. Moreover, Brieudes et al. (2016) attributed antioxidant activities of *C. spinosum* and *C. intybus* decoctions to the presence of chicoric acid, which was also the major phenolic

Table 4
Quantification (mg/g) of phenolic compounds of *Cichorium spinosum* leaves' extract in relation to growth substrate and sowing date.

Compounds	1st sowing date [e]						2nd sowing date		
	SUB1 [f]	SUB2	SUB3	SUB4	SUB5	SUB6	SUB1	SUB2	SUB6
Caftaric acid[a]	0.58 ± 0.02c	0.673 ± 0.002b	1.01 ± 0.04a	0.496 ± 0.008 d	0.71 ± 0.01b	0.70 ± 0.09b	0.87 ± 0.04b*	0.97 ± 0.03a*	0.474 ± 0.003c*
5-*O*-Caffeoylquinic acid[b]	1.00 ± 0.01b	0.78 ± 0.02c	0.54 ± 0.01 d	0.39 ± 0.01f	0.495 ± 0.002e	1.10 ± 0.05a	3.48 ± 0.04a*	1.9 ± 0.2b*	1.87 ± 0.05b*
cis Chicoric acid[a]	1.52 ± 0.05a	0.65 ± 0.02b	0.32 ± 0.01e	0.46 ± 0.02 d	0.51 ± 0.01c	0.46 ± 0.01 d	2.82 ± 0.02a*	1.37 ± 0.03c*	2.27 ± 0.05b*
trans Chicoric acid[a]	1.06 ± 0.05a	0.77 ± 0.03b	0.45 ± 0.01f	0.655 ± 0.007 d	0.52 ± 0.02e	0.70 ± 0.05c	2.84 ± 0.03a*	1.32 ± 0.05c*	2.0 ± 0.2b*
Quercetin-3-*O*-glucuronide[c]	1.18 ± 0.02a	0.87 ± 0.01b	0.705 ± 0.001f	0.814 ± 0.005 d	0.781 ± 0.002e	0.851 ± 0.003c	1.89 ± 0.01a*	1.36 ± 0.03c*	1.79 ± 0.02b*
Kaempferol-*O*-glucuronide[c]	1.218 ± 0.001a	1.053 ± 0.003b	0.827 ± 0.00e1	0.866 ± 0.003 d	0.886 ± 0.003c	0.823 ± 0.006e	2.14 ± 0.03a*	1.666 ± 0.007c*	1.92 ± 0.01b*
Quercetin-7-*O*-(6″-*O*-acetyl)-glucoside[c]	0.595 ± 0.002a	0.526 ± 0.001b	0.499 ± 0.001e	0.514 ± 0.001c	0.513 ± 0.001c	0.506 ± 0.002 d	0.831 ± 0.003b*	0.705 ± 0.001c*	0.85 ± 0.01a*
Kaempferol-3-*O*-rutinoside[c]	0.524 ± 0.001a	0.485 ± 0.001 d	0.477 ± 0.002e	0.496 ± 0.001b	0.487 ± 0.001c	0.486 ± 0.001cd	0.63 ± 0.01a*	0.557 ± 0.001c*	0.590 ± 0.001b*
Kaempferol-3-*O*-glucuronide[c]	1.00 ± 0.01a	0.732 ± 0.003c	0.693 ± 0.003 d	0.728 ± 0.001c	0.747 ± 0.001b	0.743 ± 0.002b	1.42 ± 0.01a*	1.147 ± 0.001c*	1.354 ± 0.003b*
Apigenin-7-*O*-glucuronide[d]	0.79 ± 0.03a	0.588 ± 0.001c	0.59 ± 0.01c	0.642 ± 0.002b	0.64 ± 0.03b	0.558 ± 0.001 d	0.93 ± 0.03c*	0.995 ± 0.002b*	1.17 ± 0.03a*
Isorhamnetin-3-*O*-glucuronide[c]	0.65 ± 0.01a	0.608 ± 0.003c	0.60 ± 0.01 d	0.595 ± 0.001 d	0.59 ± 0.01 d	0.620 ± 0.001b	1.07 ± 0.02a*	0.78 ± 0.02c*	1.000 ± 0.003b*
Kaempferol-3-*O*-(6″-*O*-acetyl)-glucoside[c]	0.557 ± 0.002a	0.482 ± 0.002f	0.487 ± 0.002e	0.500 ± 0.001c	0.511 ± 0.001b	0.496 ± 0.001 d	0.67 ± 0.01b*	0.62 ± 0.01c*	0.71 ± 0.01a*
Isorhamnetin-3-O-(6″-O-acetyl)-glucoside[c]	0.484 ± 0.001a	nd	nd	0.463 ± 0.001 d	0.468 ± 0.001b	0.465 ± 0.001c	0.497 ± 0.001b*	0.516 ± 0.002a*	0.495 ± 0.001b*
Total phenolic acids	4.15 ± 0.07a	2.87 ± 0.01c	2.33 ± 0.03 d	2.00 ± 0.02f	2.24 ± 0.04e	2.95 ± 0.07b	10.00 ± 0.04a*	5.5 ± 0.1c*	6.6 ± 0.2b*
Total flavonoids	7.00 ± 0.01a	5.35 ± 0.01 d	4.87 ± 0.02e	5.62 ± 0.01b	5.62 ± 0.02b	5.549 ± 0.001c	10.08 ± 0.01a*	8.34 ± 0.02c*	9.89 ± 0.02b*
Total phenolic compounds	11.15 ± 0.08a	8.21 ± 0.01c	7.20 ± 0.01f	7.62 ± 0.02e	7.86 ± 0.02 d	8.50 ± 0.07b	20.08 ± 0.03a*	13.9 ± 0.1c*	16.5 ± 0.3b*

Nd - not detected, calibration curves used.
Different Latin letters in the same row and for the same sowing date indicate significant differences between the substrates. The asterisk (*) symbol indicates differences between the sowing dates for the same substrate (SUB 1–7; SUB 2–8; SUB 6–9) at $p = 0.05$.
[a] Caffeic acid ($y = 388345x + 406369$, $R^2 = 0.999$).
[b] Chlorogenic acid. ($y = 168823x - 161172$, $R^2 = 0.999$).
[c] Quercetin-3-*O*-glucoside ($y = 34843x - 160173$ $R^2 = 0.999$).
[d] Apigenine-7-*O*-glucoside ($y = 10683x - 45794$ $R^2 = 0.997$).
[e] 1st sowing date: September 4th, 2016; 2nd sowing date: December 5th, 2016.
[f] SUB1: soil; SUB2: soil + peat (1:1 v/v); SUB3: soil + peat + cardoon seedcake (80%) and zeolite (20%) mix (1:1:1 v/v); SUB4: soil + peat + cotton ginning waste (80%) zeolite (20%) mix (1:1:1 v/v); SUB5: soil + peat + cardoon seedcake (60%) zeolite (40%) mix (1:1:1 v/v); SUB6: soil + peat + cotton ginning waste (60%) zeolite (40%) mix (1:1:1 v/v).

Table 5
Antioxidant activity of *Cichorium spinosum* leaves' extract in relation to growth substrate and sowing date.

Sowing date [a]	Substrates [b]	TBARS (IC_{50}; μg/mL) [c]	OxHLIA (IC_{50}; μg/mL)		
			Δt = 30 min	Δt = 60 min	Δt = 120 min
1st	SUB1	199±5 d	16±2f	61±4e	173±6b
	SUB2	272±7c	20±2e	90±5c	n.a.
	SUB3	348 ± 16a	25±2 d	80±4 d	n.a.
	SUB4	321 ± 14b	201±8a	404 ± 12a	n.a.
	SUB5	332 ± 13a	120 ± 10b	n.a.	n.a.
	SUB6	336±3a	n.a	n.a.	n.a.
2nd	SUB1	90±1c*	42±2c*	79±2c*	153±3c*
	SUB2	167±2a*	52±3a*	110±5a*	233±2a*
	SUB6	139±5b*	46±2b*	85±2b*	183±4b*

Different Latin letters in the same column and the same sowing date indicate significant differences between the substrates. The asterisk (*) symbol indicates differences between the sowing dates for the same substrate (SUB 1–7; SUB 2–8; SUB 6–9) at $p = 0.05$.
[a] 1st sowing date: September 4th, 2016; 2nd sowing date: December 5th, 2016.
[b] SUB1: soil; SUB2: soil + peat (1:1 v/v); SUB3: soil + peat + cardoon seedcake (80%) and zeolite (20%) mix (1:1:1 v/v); SUB4: soil + peat + cotton ginning waste (80%) zeolite (20%) mix (1:1:1 v/v); SUB5: soil + peat + cardoon seedcake (60%) zeolite (40%) mix (1:1:1 v/v); SUB6: soil + peat + cotton ginning waste (60%) zeolite (40%) mix (1:1:1 v/v).
[c] IC_{50}: Extract concentration corresponding to 50% of antioxidant activity; n. a.: no activity.

compound in our study, while in similar study with wild edible species total phenolic compounds content was also associated with antioxidant properties of decoctions (Balabanos et al., 2018). This was also the case in the studies of Chrysargyris et al. (2018a,b; 2019) who reported the effect of growth substrate composition on antioxidant activities of ornamental plants and *Brassica* seedlings. Especially for TBARS assay, a significantly higher antioxidant activity was observed for plants grown in the second growing period regardless of the growing medium which is in accordance with results previously published by the authors (Petropoulos et al., 2017c).

Table 6
Antibacterial activity (mg/mL) of *Cichorium spinosum* leaves' extract in relation to growth substrate and sowing date.

Sowing Dates[a]	Substrates[b] ↓	MO[c] →	*Bacillus cereus*	*Staphylococcus aureus*	*Listeria monocytogenes*	*Escherichia coli*	*Enterobacter cloacae*	*Salmonella enterica* subsp. *enterica*
1st	SUB1	MIC	14.54	14.54	7.27	14.54	7.27	7.27
		MBC	14.54	14.54	14.54	14.54	14.54	14.54
	SUB2	MIC	7.27	7.27	7.27	14.54	7.27	7.27
		MBC	7.27	14.54	7.27	14.54	7.27	7.27
	SUB3	MIC	3.64	7.27	7.27	14.54	1.82	1.82
		MBC	3.64	14.54	14.54	14.54	1.82	1.82
	SUB4	MIC	7.27	14.54	3.64	14.54	3.64	3.64
		MBC	7.27	14.54	3.64	14.54	3.64	3.64
	SUB5	MIC	7.27	7.27	3.64	14.54	3.64	3.64
		MBC	7.27	14.54	3.64	14.54	3.64	7.27
	SUB6	MIC	7.27	14.54	14.54	14.54	14.54	14.54
		MBC	7.27	14.54	14.54	14.54	14.54	14.54
2nd	SUB1	MIC	7.27	14.54	14.54	14.54	14.54	14.54
		MBC	7.27	14.54	14.54	14.54	14.54	14.54
	SUB2	MIC	7.27	14.54	14.54	14.54	14.54	14.54
		MBC	7.27	14.54	14.54	14.54	14.54	14.54
	SUB6	MIC	14.54	14.54	14.54	14.54	14.54	14.54
		MBC	14.54	14.54	14.54	14.54	14.54	14.54
Streptomycin		MIC	0.0015	0.006	0.20	0.05	0.003	0.20
		MBC	0.003	0.012	0.30	0.10	0.006	0.30
Ampicillin		MIC	0.006	0.012	0.40	0.10	0.006	0.75
		MBC	0.025	0.025	0.50	0.20	0.012	1.20

[a] 1st sowing date: September 4th, 2016; 2nd sowing date: December 5th, 2016.
[b] SUB1: soil; SUB2: soil + peat (1:1 v/v); SUB3: soil + peat + cardoon seedcake (80%) and zeolite (20%) mix (1:1:1 v/v); SUB4: soil + peat + cotton ginning waste (80%) zeolite (20%) mix (1:1:1 v/v); SUB5: soil + peat + cardoon seedcake (60%) zeolite (40%) mix (1:1:1 v/v); SUB6: soil + peat + cotton ginning waste (60%) zeolite (40%) mix (1:1:1 v/v).
[c] MO: microorganisms; MIC: Minimal inhibitory concentration; MBC: Minimal bactericidal concentration; MFC: Minimal fungicidal concentration.

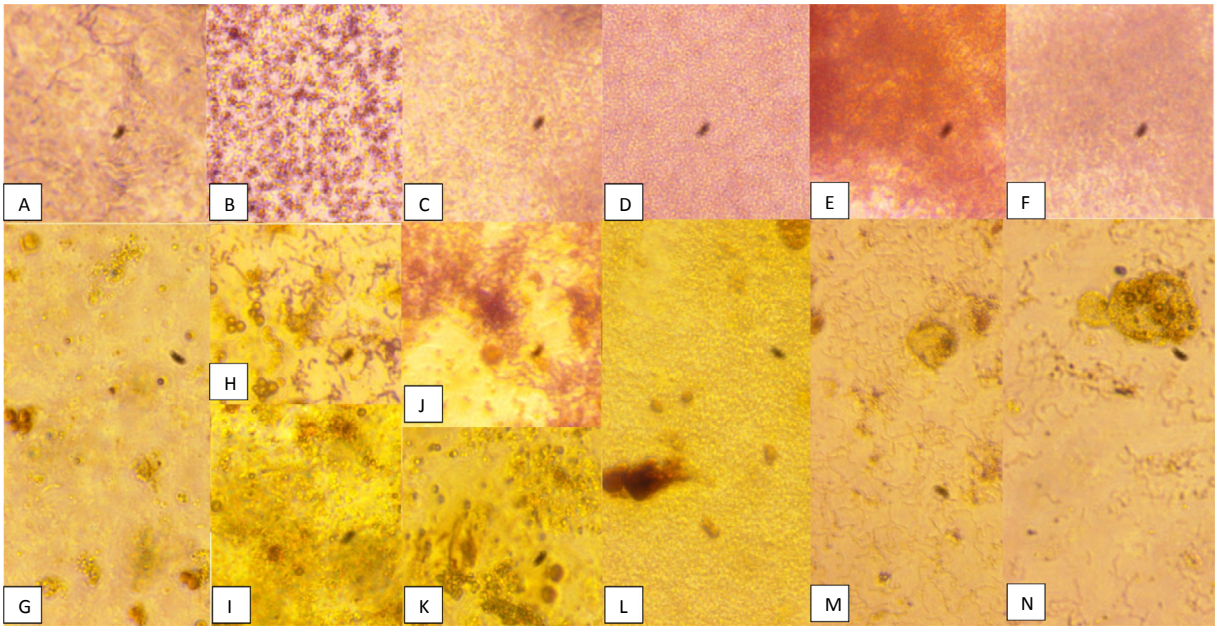

Fig. 1. Inverted light microscopy (magnification 40 ×) (Nikon eclipse TS2, Netherlands) of control bacteria (**A-F**) and bacteria treated (**G-N**, MIC and MBCs) with the most efficient SUB3 extract in microdilution method. **A** - *Bacillus cereus*; **B** - *Staphylococcus aureus*; **C** - *Listeria monocytogenes*; **D** - *Escherichia coli*; **E** - *Enterobacter cloacae*; **F** - *Salmonella typhimurium*; **G** - MIC and MBC of SUB3 on *B. cereus*, **H** - MIC and **I** - MBC on *S. aureus*; **J** - MIC and **K** - MBC on *L. monocytogenes*, **L** - MIC and MBC on *E. coli*, **M** - MIC and MBC on *E. cloacae*, **N** - MIC and MBC on *S. typhimurium*.

3.5. Effect of growth substrate on antimicrobial and antifungal activity

The effect of growing medium composition on the antibacterial properties of *C. spinosum* leaves' extracts is presented in Table 6. The most sensitive species to the effect of leaves extracts was *Enterobacter cloacae*, whereas the most resistant species was *Escherichia coli*. The activity of leaves' extracts from plants grown in the 1st sowing date and in substrate blends containing cardoon byproducts (SUB3) was the most prominent, especially against *E. cloacae* and *Salmonella* Tympimurium, while the weakest antibacterial activity was observed for plants grown in the 2nd sowing date and in growing medium containing cotton ginning byproducts (SUB6) (Fig. 1). In any case, *C. spinosum* extracts were less effective than positive controls, regardless of the growth substrate and sowing date. To the best of our knowledge, this is the first time that

Table 7
Antifungal activity (mg/mL) of *Cichorium spinosum* leaves' extract in relation to growth substrate and sowing date.

Sowing date[a]	Substrates[b] ↓	MO[c] →	*Aspergillus fumigatus*	*Aspergillus versicolor*	*Aspergillus niger*	*Penicillium funiculosum*	*Penicillium ochrochloron*	*Penicillium aurantiogriseum*
1st	SUB1	MIC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
		MFC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
	SUB2	MIC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
		MFC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
	SUB3	MIC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
		MFC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
	SUB4	MIC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
		MFC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
	SUB5	MIC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
		MFC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
	SUB6	MIC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
		MFC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
2nd	SUB7	MIC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
		MFC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
	SUB8	MIC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
		MFC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
	SUB9	MIC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
		MFC	>14.54	>14.54	>14.54	>14.54	>14.54	>14.54
Ketoconazole		MIC	0.2	0.2	0.2	0.2	1.0	0.2
		MFC	0.5	0.5	0.5	0.5	1.5	0.3
Bifonazole		MIC	0.15	0.1	0.15	0.20	0.20	0.1
		MFC	0.2	0.2	0.20	0.25	0.25	0.2

[a] 1st sowing date: September 4th, 2016; 2nd sowing date: December 5th, 2016.
[b] SUB1: soil; SUB2: soil + peat (1:1 v/v); SUB3: soil + peat + cardoon seedcake (80%) and zeolite (20%) mix (1:1:1 v/v); SUB4: soil + peat + cotton ginning waste (80%) zeolite (20%) mix (1:1:1 v/v); SUB5: soil + peat + cardoon seedcake (60%) zeolite (40%) mix (1:1:1 v/v); SUB6: soil + peat + cotton ginning waste (60%) zeolite (40%) mix (1:1:1 v/v).
[c] MO: microorganisms; MIC: Minimal inhibitory concentration; MBC: Minimal bactericidal concentration; MFC: Minimal fungicidal concentration.

antibacterial properties of *C. spinosum* leaves' extracts against various bacteria are reported, since so far the effect of decoctions against *S.* Tympimurium TA102 strain has been reported (Balabanos et al., 2018). Moreover, the results of the present indicate that such activities are not associated with phenolic compounds content and other bioactive compounds should be attributed with such properties.

The effect of growing medium composition on the antifungal properties of *C. spinosum* leaves' extracts is presented in Table 7. However, none of the evaluated extracts showed significant activity against the tested fungi for concentrations up to 14.54 mg/mL, indicating a week antifungal activity.

4. Conclusion

The use of cotton ginning byproducts and zeolite in growth substrates may partially substitute peat in *C. spinosum* commercial cultivation which could result in reduction of production cost while at the same time high yields would be retained and environmental burden from bulky agroindustry byproducts will be reduced. The higher content in phenolic compounds that was observed in plants grown in soil was probably the result of stress conditions due to high pH values and unfavorable nutrient soil conditions. In any case, the considerably lower yields comparing to substrate blends cannot support the commercial cultivation of the species, even for pharmaceutical purposes and/or the recovery of bioactive compounds. Antibacterial properties of leaves' extracts were also affected by growing media and sowing date with promising results against bacteria such as *Enterobacter cloacae* and *Salmonella enterica* subsp. *enterica*. Finally, it could be suggested that the use of cotton ginning byproducts and zeolite showed high potential in alleviating unfavorable soil conditions and further experiments are required with different types of soils and stressors (salinity and/or water deficit) in order to evaluate the use of such byproducts as soil amendments and growth substrates of horticultural crops.

Conflicts of interest

None to declare.

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