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Biochar *versus* hydrochar as growth media constituents for ornamental plant cultivation

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ABSTRACT: Biochar and hydrochar have been proposed as novel materials for providing soilless growth media. However, much more knowledge is required before reliable advice can be given on the use of these materials for this purpose. Depending on the material and the technology applied (pyrolysis or hydrothermal carbonization), phytotoxicity and greenhouse gas emissions have been found for certain chars. In this study, our aim was to assess the feasibility of three chars as substrate constituents. We compared two biochars, one from forest waste and the other from olive mill waste, and a hydrochar from forest waste. We studied how chars affected substrate characteristics, plant performance, water economy and respiratory CO_2 emission. Substrates containing biochar from forest waste showed the best characteristics, with good air/water relationships and adequate electrical conductivity. Those with biochar from olive mill waste were highly saline and, consequently, low quality. The substrates with hydrochar retained too much water and were poorly aerated, presenting high CO_2 concentrations due to high respiratory activity. Plants performed well only when grown in substrates containing a maximum of 25 % biochar from forest waste or hydrochar. After analyzing the char characteristics, we concluded that biochar from forest waste could be safely used as a substrate constituent and is environmentally friendly when applied due to its low salinity and low CO_2 emission. However, biochar from olive mill waste and hydrochar need to be improved before they can be used as substrate constituents.

Keywords: *Calendula officinalis*, *Petunia hybrida*, char, growth media characteristics, pyrolisis

Abbreviations: A_N (net CO_2 assimilation); BCH-FW (BioCHar from Forest Waste); BCH-OMW (BioCHar from Olive Mill Waste); CF (coir); CI (coarseness index); Db (bulk density); Dp (particle density); EC (electrical conductivity); Fv/Fm (maximum photochemical efficiency); g_s (stomatal conductance); HYD-FW (HYDrochar from Forest Waste); P_T (total pore space); Vair (air volume); Vwater (water volume); WHC (water holding capacity); Φ_{PSII} (effective quantum yield of photosystem II).

Introduction

The search for new alternatives to peat as a substrate constituent is a recurrent objective for horticulturalists. The use of biochar and hydrochar as substrate constituents is promising. Although biochar is now being studied as a substrate constituent (Fornes et al., 2017; Méndez et al., 2015; Nieto et al., 2016; Sáez et al., 2016; Steiner and Harttung, 2014; Vaughn et al., 2015), hydrochar has received less attention (Belda et al., 2016; Fornes et al., 2017).

Biochar and hydrochar are products obtained from organic matter by pyrolysis (dry process at 300 to 800 °C in anoxic environment) and by hydrothermal carbonization (wet process at 180 to 230 °C under high pressure), respectively. Chars with different characteristics can be obtained by varying the raw material and the manufacturing conditions (Fornes et al., 2015; Libra et al., 2011) which are relevant to potential agricultural application as well as to other uses. For instance, biochars from feedstock that is poor in nutrients are usually alkaline and non-saline whilst biochars from feedstock which is rich in nutrients are usually highly saline (Fornes et al., 2015). Hydrochars are usually acidic and contain large amounts of labile organic carbon which makes them phytotoxic and causes N immobilization in the soil (Bargmann et al., 2013; Fornes et al., 2017).

Containerized soilless plant production requires specific physical and physico-chemical characteristics of the substrates used (Bunt, 1988). This is especially true of the physical properties as these cannot be manipulated once the substrate has been placed in the container. Because of this, the application of standardized, internationally accepted methods to the characterization of new substrate materials is crucial. Notwithstanding, char-containing substrates have only recently started to be characterized according to standardized methods (Bedussi et al., 2015).

The aim of this study was partly to compare a biochar with a hydrochar, both having been produced from a similar raw material (forest waste); and partly to compare two biochars produced from two relatively different raw materials (forest waste and olive mill waste). Additionally the effect of substrates containing the three chars on plant performance was assessed.

Materials and Methods

Biochars, hydrochar, coir and plant material

Several properties of the three chars used in this study (BCH-FW [BioCHar from Forest Waste]; BCH-

OMW [BioCHar from Olive Mill Waste]; HYD-FW [HYDrochar from Forest Waste]) including the origin, physico-chemical and chemical characteristics have previously been published by Fornes et al. (2015). The materials were chosen in order to compare on the one hand two biochars with very different origins and characteristics and on the other, a biochar and a hydrochar both produced from a similar raw material (wood). All the materials were compared with coir (CF), which is one of the most common materials used in horticulture, after peat.

Two ornamental species, *Calendula officinalis* cv. Nana Gitana and *Petunia hybrida* cv. Costa Rosa Vivo, were used in this experiment. These species were chosen for their different response to salinity (Fornes et al., 2007) and, in the case of petunia, for its sensitivity to high pH (Smith et al., 2004).

Treatments, experimental design, and plant growth conditions

Treatments consisted of the following mixes of substrates; CF:BCH-FW, CF:BCH-OMW, and CF:HYD in the following proportions; 50:50, 75:25, 90:10, and 100:0 (control). The substrates were fertilized with 4 g L^{-1} substrate of a controlled release fertilizer. The substrate constituents and fertilizer were thoroughly mixed by hand. The composition of the fertilizer as indicated by the manufacturer was 15 % N (8 % ammonia N and 7 % nitrate N), K (K_2O) 12 %, P (P_2O_5) 9 %, S 6 %, Mg 1 %, Fe 4600 ppm, Mn 600 ppm %, Cu 500 ppm, Zn 500 ppm, B 200 ppm, Mo 200 ppm. For each plant species, four replicates of four plastic pots (12 cm diameter; 8.8 cm height; 8° slant; 600 mL volume) were filled with each of the substrates (16 pots per treatment). The pots were distributed following a random block design in a greenhouse, located in Valencia, Spain (29°39′0″ N, 0°20′30″ E, 14 MAMSL), equipped with both heating and cooling systems. Petunia and calendula seeds were sown on 14 Apr 2013 and the experiment finished on 16 June. At the end of the experiment, data on total leaf chlorophyll-related SPAD (Soil-Plant Analyses Development) units, parameters related to photosynthesis, and parameters related to growth and development were collected. Irrigation consisted of an automatized micro-sprinkler system and the volume of water supplied was adjusted to the necessities of the plants (as a factor of water evaporation and plant size): 150 mL (Apr) to 300 mL (May-June) d^{-1} for petunia and calendula, distributed in four short irrigation pulses a day (2 to 4 min). Minimum temperatures registered inside the glasshouse ranged from 15 °C in Apr to 20 °C in June whilst maximum temperatures ranged from 20-25 °C in Apr to 30-35 °C in June. Photosynthetically active radiation (PAR) reaching the plants was measured using the external quantum sensor incorporated in a portable photosynthesis system at noon (solar time) on sunny days at the beginning of each month of the experimental period. The measured PAR ranged from 400 µmol m^{-2} s^{-1} in Apr to 750 µmol m^{-2} s^{-1} in June. Treatments against whitefly and fungal diseases were carried out where necessary.

Physical, physico-chemical and chemical characterization of the substrates

Characterization of substrates was carried out following the European Standards (EN) for soil improvers and growing media.

For characterization of the physical properties, particle size was determined in 200 mL air-dried aliquots. Particles were separated according to size by means of an electromagnetic vibratory shaker for 10 min, using sieves of square mesh sizes of 0.125, 0.25, 0.50, 1, 2, 4, 8 and 16 mm. The material collected in each sieve was weighed and expressed as a percentage by weight of the whole sample (European Standards, 2007). A coarseness index (CI) was calculated expressing the accumulated percentage by weight of particles larger than 1 mm. Bulk density, water capacity and total water-holding capacity were determined using loosely packed cores and the methods in the European Standards (1999c). For this study, steel cylinders measuring 40 mm in height and 82.3 mm in internal diameter (210 mL) were used. Shrinkage was the percentage of bulk volume loss after drying the material contained in the cylinder at 105 °C. Total pore space is the percentage of the volume of material which can be filled with water. Air capacity is the difference in percentage by volume between the total pore space and the moisture content at a suction of 1 kPa (European Standards, 1999c).

For the characterization of the physico-chemical and chemical characteristics, the pH (European Standards, 1999a), electrical conductivity (EC) (European Standards, 1999b), and available (water soluble) mineral element concentration (European Standards, 2001) of the substrates were determined in a 1:5 (v:v) substrate:water suspension. To obtain this suspension, 60 mL of the substrates were mixed with 300 mL of distilled water and shaken for 1 h. The pH was measured using a pHmeter. EC and water soluble elements were determined in the suspension filtrate after filtering through filter paper. EC was determined with a conductimeter. Water-soluble C and N were determined with a Total Organic Carbon analyser. Water-soluble elements (P, K, Ca, Mg) were determined by Atomic Emission Spectrophotometry with Inductively Coupled Plasma. Total carbon (TC) (which was mainly organic carbon in all the substrates) and total nitrogen (TN) were determined by burning the material at 1020 °C in an elemental analyser. Total element content (P, K, Ca, Mg) was determined after HNO_3/H_2O_2 digestion by Atomic Emission Spectrophotometry with Inductively Coupled Plasma. Total and water soluble mineral contents were expressed as mg L^{-1} substrate.

All determinations were replicated three times

Determination of the water retention capacity of biochars, hydrochar, and coir used as growth media constituents

In order to determine the ability of the chars and the coir to delay water loss by evaporation when containerized, 0.6 L containers were filled with the dry materials (three containers per material), weighed, and saturated with water for 24 h. After this, they were allowed to drain for another 24 h, then weighed, and the water content calculated. Containers were maintained at room temperature on a laboratory bench for 30 days. Water loss rhythm was gravimetrically determined by weighing the containers at days 4, 8, 15, 22 and 30. The water content (% by weight) and water lost (% of water lost from start) were calculated.

Determination of microbial respiration and its effect on the composition of the atmosphere inside biochars, hydrochar and coir

Microbial respiration was determined as indicated by Fornes et al. (2015). 10 g samples of each substrate were incubated for 30 days at 25 °C and 60 % of water holding capacity, in 250 mL glass flasks (3 flasks per material) equipped with a septum plug. Respiration was measured daily by sampling the air contained in the flasks and determining the O_2 and CO_2 concentrations with a portable gas analyser. When necessary, the flasks were opened to allow for aeration. Average absorption of O_2 and emission of CO_2 were expressed as μmol O_2/CO_2 kg^{-1} d^{-1}.

In order to determine the composition of the atmosphere inside the materials when containerized, 0.6 L containers were filled with each material (three containers per material) and maintained at 60 % of the water holding capacity on a laboratory bench for 30 days. At days 10, 20 and 30, a hypodermic needle connected to a portable gas analyser was immersed in the substrates and the O_2 and CO_2 concentrations (%) recorded. The data presented are the average of the three sampling dates.

Effects of substrates on seed germination, plant growth, leaf chlorophyll and nutrient contents, and photosynthesis

In order to evaluate seed germination, four seeds were sown in each pot in all cases. Previously seeds had been disinfected by soaking them in a 2 % sodium hypochlorite solution for 5 min and rinsing them afterwards three times with sterile distilled water. Germination was recorded over a month and germination percentage calculated ([n° of emerged seedlings/n° of sown seeds]*100). Only the first seedling to emerge from the surface was kept for the experiment (one plant per pot).

As indicated above, at the end of the growing period, total leaf chlorophyll-related SPAD units, parameters related to photosynthesis, and parameters related to growth and development were recorded.

SPAD measurements were carried out with a Chlorophyll Meter SPAD on four leaves per plant, and the average calculated.

Photosynthetic activity and chlorophyll fluorescence were determined for one leaf per plant. CO_2 fixation rate (A_N, μmol CO_2 m^{-2} s^{-1}) and stomatal conductance to water vapor (g_s, mol H_2O m^{-2} s^{-1}) were measured in all plants at steady-state while maintaining the plants at 1000 μmol photons m^{-2} s^{-1} for 10-15 min and 400 ppm CO_2 with a portable photosynthesis system. Photochemical PSII efficiency (Φ_{PSII}) was computed as the quotient (F'_m – F_s)/ F'_m (Genty et al., 1989), the parameters of which were determined in the presence of an actinic illumination of 1000 μmol photons m^{-2} s^{-1}. The maximum quantum yield of PSII (F_v/F_m) was measured in leaves after 30 min in the darkness, using a portable pulse amplitude modulation fluorometer.

The percentage of flowering plants was recorded due to the interest of this parameter to ornamentals. A plant was recorded as flowering when it showed at least one open flower, independent of the number of developing buds or open flowers that it finally produced. Shoot dry weight was obtained by oven-drying at 65 °C for 72 h. Root size (visual rating) was evaluated by taking the root ball out of the pot and evaluating the extension of the root system on an arbitrary scale ranging from 1 to 5, in which 1 represents roots which do not reach the surface of the substrate, and 5 represents a root system forming a compact mesh that colonizes all the substrate (Fornes et al., 2007).

Oven-dried leaf tissue was finely ground for analysis. Leaf N, P, K, Ca and Mg contents were determined as previously indicated for the substrate analysis.

Statistical analysis

Analysis of variance was carried out to determine statistically significant differences between substrates (Tables 1 to 5; Figures 2 and 3). In order to analyse the percentage of flowering plants, an arcsine transformation was performed. Where differences were significant, the Tukey test at $p \leq 0.05$ was carried out to establish significant differences between means. Additionally, the O_2 consumed and CO_2 released by each of the substrates were compared with the *t*-Student test (Figure 2). Linear or polynomic regressions in Figure 1 were fitted by least squares and the determination coefficients (R^2) calculated.

Results

Physical and physico-chemical characteristics of the substrates

Table 1 shows the main physical and physico-chemical characteristics of the substrates. As the particle size was larger in BCH-FW and BCH-OMW (data not shown) than in coir, the CI increased in the substrates in proportion to the number of chars used in the mix. An opposite effect was observed for the HYD-FW containing substrates. Db also increased in the substrates as char content was increased, with the highest Db seen in the BCH-FW containing substrates. P_T was largest for CF and decreased in the substrates as the char proportion increased. Vwater was similar in all substrates containing BCH-FW or BCH-OMW, in all cases being lower than in CF. In contrast, HYD-FW at a high proportion (50 %) caused an increase in Vwater. Vair was greater in the substrates containing

Table 1 – Physical and physico-chemical properties of substrates based on two biochars (BCH-FW and BCH-OMW), one hydrochar (HYD-FW), and coir (control).

Material	Dose	[3]CI > 1 mm	Db	P_T	Vwater	Vair	WHC	Shrinkage	pH	EC
	%	% w/w	kg m^{-3}		% v/v		g water/100 g mix	% v/v		dS m^{-1}
BCH-FW[1]	50	61 b	265 a	83.0 g	52.9 cd	30.2 e	198 g	13.2 c	9.0 b	0.93 de
	25	56 c	157 cd	88.2 de	48.7 d	39.5 bc	312 f	18.0 b	8.2 c	0.80 e
	10	45 e	118 e	90.4 bc	49.2 d	41.2 ab	415 d	19.0 b	7.8 d	0.83 e
BCH-OMW	50	63 a	170 c	88.4 e	52.3 cd	36.1 d	308 f	12.0 c	9.7 a	5.68 a
	25	51 d	143 d	89.6 d	51.0 d	38.6 c	354 e	19.3 b	9.6 a	3.16 b
	10	42 f	100 f	92.1 ab	49.8 d	42.3 a	498 c	26.0 a	9.1 b	1.39 c
HYD-FW	50	30 h	210 b	86.8 f	65.3 a	21.5 f	306 f	26.4 a	7.4 e	1.04 d
	25	32 h	126 e	91.3 cd	56.0 bc	35.3 d	434 d	26.2 a	7.7 d	0.92 de
	10	34 gh	95 f	93.5 a	50.2 d	43.3 a	515 b	25.4 a	7.3 e	0.86 e
Control (CF)		40 fg	81 g	94.8 a	58.7 b	36.1 d	722 a	24.2 a	6.0 f	0.81 e
p^2		***	***	***	***	***	***	***	***	***

[1]BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir; [2]***Indicates significant at $p \leq 0.001$; Different letters in columns differ at $p \leq 0.05$ (Tukey test); [3]CI = Coarseness index; Db = Bulk density; P_T = Total porosity; Vwater = Water volume; Vair = Air volume; WHC = Water holding capacity; EC = Electrical conductivity.

Table 2 – Chemical composition (total [T] and water soluble [WS] C and nutrients; mg L^{-1} substrate) of substrates based on two biochars (BCH-FW and BCH-OMW), one hydrochar (HYD-FW), and coir (control).

Material	Dose	C		N		P		K		Ca		Mg	
		T	WS	T	WS	T	WS	T	WS	T	WS	T	WS
	%												
BCH-FW[1]	50	151,140 a	213 d	1840 b	13 c	256 e	5 d	2158 c	279 f	6992 b	11 de	616 bc	5 e
	25	95,810 bc	173 e	1130 d	8 cd	208 f	3 de	1323 d	184 g	4108 e	7 e	396 d	5 e
	10	62,612 e	148 fg	700 g	5 d	179 fg	1 e	822 f	126 h	2378 gh	5 e	264 f	5 e
BCH-OMW	50	101,755 b	154 f	1085 e	9 cd	1313 a	52 a	9039 a	4497 a	5270 c	16 d	924 a	19 b
	25	70,820 d	143 ge	747 g	6 d	732 b	26 b	4732 b	2276 b	3230 f	10 de	547 c	12 c
	10	52,497 f	136 e	547 h	4 de	387 d	11 c	2173 c	957 c	2020 h	6 e	323 e	8 de
HYD-FW	50	91,682 c	1032 a	2816 a	50 a	566 c	0.7 e	1443 d	549 d	7951 a	156 a	671 b	26 a
	25	66,081 de	582 b	1616 c	27 b	363 d	0.5 e	965 e	319 e	4587 d	80 b	424 d	16 b
	10	50,544 f	310 c	890 f	13 c	240 e	0.5 e	676 g	179 g	2551 g	34 c	274 f	10 cd
Control (CF)		40,986 g	134 e	421 i	3 e	162 g	0.4 e	494 h	89 i	1239 i	4 e	178 g	6 e
p^2		***	***	***	***	***	***	***	***	***	***	***	***

[1]BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir; [2]***Indicate significant at $p \leq 0.001$; Different letters in columns differ at $p \leq 0.05$ (Tukey test).

Table 3 – Selected plant growth parameters of *Calendula officinalis* (C) and *Petunia hybrida* (P) grown on different substrates based on two biochars (BCH-FW and BCH-OMW), one hydrochar (HYD-FW), and coir (control).

Material	Dose	Seed germination		Shoot dry weight		Root ball - VR score[1]		Flowering plants	
		C	P	C	P	C	P	C	P
	%	%		mg per plant				%	
BCH-FW[2]	50	70 cd	24 d	851 bcd	977 bc	2.3 c	1.6 c	83 ab	33 c
	25	80 b	40 c	1166 abc	1135 bc	2.9 abc	2.4 bc	85 ab	50 b
	10	91 a	55 b	1207 abc	1299 bc	3.3 abc	3.2 ab	83 ab	66 a
BCH-OMW	50	0 f	0 e	–	–	–	–	–	–
	25	0 f	0 e	–	–	–	–	–	–
	10	24 e	27 d	502 d	980 bc	2.4 c	2.5 bc	19 e	6 d
HYD-FW	50	64 d	59 b	873 c	711 c	3.3 bc	2.5 bc	38 d	6 d
	25	73 bc	65 ab	1001 bc	1095 bc	3.6 ab	3.1 ab	56 c	25 c
	10	80 b	70 a	1399 a	2070 a	4.3 a	4.0 a	94 a	31 c
Control (CF)		84 ab	67 ab	1197 ab	1465 ab	4.3 a	4.0 a	75 b	69 a
p^3		***	***	***	***	***	***	***	***

[1]Visual rating (VR) score from 1 (least developed) to 5 (most developed); [2]BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir; [3]***Indicates a significant difference at $p \leq 0.001$, respectively; Different letters in numerical columns differ at $p \leq 0.05$ (Tukey test).

Table 4 – Leaf mineral contents (% dry weight) of *Calendula officinalis* (C) and *Petunia hybrida* (P) grown on different substrates based on two biochars (BCH-FW and BCH-OMW), one hydrochar (HYD-FW), and coir (control).

Material	Dose	Nitrogen		Phosphorus		Potassium		Calcium		Magnesium	
		C	P	C	P	C	P	C	P	C	P
	%										
BCH-FW[1]	50	4.53 a	2.70 ab	0.26 d	0.20 c	8.88 a	4.70 bc	1.71 c	2.52 a	0.88 a	0.65 ab
	25	4.45 a	2.87 ab	0.26 d	0.27 bc	7.80 ab	4.59 bc	1.82 bc	2.58 a	0.95 a	0.72 a
	10	4.40 a	2.78 ab	0.33 c	0.20 c	6.52 c	4.97 bc	1.76 c	2.06 ab	0.89 a	0.61 ab
BCH-OMW	50	–	–	–	–	–	–	–	–	–	–
	25	–	–	–	–	–	–	–	–	–	–
	10	3.53 b	3.01 ab	0.54 a	0.44 a	7.21 bc	7.67 a	1.13 d	1.19 e	0.70 b	0.67 ab
HYD-FW	50	4.16 ab	3.23 a	0.42 b	0.45 a	4.70 d	5.36 b	2.45 a	1.72 b	0.77 ab	0.55 b
	25	3.82 ab	3.17 ab	0.43 b	0.42 ab	4.98 d	5.18 b	2.26 ab	1.67 bc	0.78 ab	0.54 b
	10	3.59 b	2.60 b	0.46 b	0.35 b	4.33 de	4.35 bc	1.87 bc	1.49 cd	0.76 ab	0.54 b
Control (CF)		3.94 ab	2.78 ab	0.38 bc	0.27 bc	3.66 e	3.95 c	1.71 c	1.32 de	0.94 a	0.68 ab
p[2]		**	*	***	***	***	***	**	***	*	*

[1]BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir; [2]*, **and***Indicates a significant difference at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively; Different letters in numerical columns differ at $p \leq 0.05$ (Tukey test).

Table 5 – Photosynthesis parameters of *Calendula officinalis* (C) and *Petunia hybrida* (P) grown on different substrates based on two biochars (BCH-FW and BCH-OMW), one hydrochar (HYD-FW), and coir (CF).

Material	Dose	Chlorophyll content (SPAD units)		A_N[1]		g_s		Fv/Fm		Φ_{PSII}	
		C	P	C	P	C	P	C	P	C	P
	%										
BCH-FW[2]	50	28.0	35.0 ab	8.0 b	13.5	0.26	0.25	0.849 a	0.831	0.20	0.19
	25	30.3	35.8 ab	8.5 ab	14.5	0.25	0.29	0.847 a	0.827	0.20	0.19
	10	27.7	36.6 a	9.2 a	13.7	0.23	0.31	0.847 a	0.826	0.20	0.18
BCH-OMW	50	–	–	–	–	–	–	–	–	–	–
	25	–	–	–	–	–	–	–	–	–	–
	10	28.1	32.8 bc	8.3 ab	15.5	0.24	0.37	0.839 ab	0.827	0.20	0.18
HYD-FW	50	28.5	29.9 c	7.8 b	15.9	0.28	0.33	0.835 b	0.827	0.19	0.18
	25	30.4	34.7 ab	6.4 b	16.0	0.25	0.31	0.848 a	0.827	0.19	0.18
	10	31.6	38.4 a	8.3 ab	13.7	0.28	0.23	0.840 ab	0.828	0.20	0.18
Control (CF)		31.9	39.6 a	9.8 a	14.9	0.22	0.30	0.849 a	0.823	0.20	0.18
p[3]		ns	**	**	ns	ns	ns	*	ns	ns	ns

[1]A_N = net CO_2 assimilation; g_s = stomatal conductance; Fv/Fm = maximum photochemical efficiency; Φ_{PSII} = effective quantum yield of photosystem II; [2]BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir; [3]ns, * and ** indicates not significant or significant difference at $p \leq 0.05$ $p \leq 0.01$, respectively; Different letters in columns differ at $p \leq 0.05$ (Tukey test).

a lower proportion of char (10 %) than in CF but it decreased as the proportion of char increased, approaching the values of CF. Compared to CF, shrinkage was not affected by the presence of HYD-FW in the mix and decreased with the presence of BCH-FW and BCH-OMW.

Substrates containing BCH-FW and most substrates containing BCH-OMW were notably alkaline. HYD-FW containing substrates were alkaline but close to neutrality and CF was acidic. Substrates containing BCH-FW and HYD-FW, and CF had a low EC. BCH-OMW containing substrates were outstandingly saline, with EC increasing with the proportion of char in them.

Water retention capacity of the biochars, the hydrochar and the coir

Figure 1 shows the curves that best fitted the loss of water by evaporation from the materials used as substrate constituents in our experiment. CF was the material which contained the most water after saturation and drainage followed by HYD-FW, BCH-OMW, and BCH-FW in that order. CF lost water slowly, only 11 % of the initial water content after 30 days. HYD-FW and BCH-OMW lost practically the same proportion of the initial water content (33 % in average). Finally, BCH-FW was the material that lost the most water proportionally (50 % of the initial content).

Chemical composition of the substrates

Table 2 shows the main nutrient content of the substrates. In general, all of the substrates were richer in nutrients, both total and water soluble, compared to the CF control, and the nutrient content increased with the proportion of char used in the substrate. BCH-FW added the most total C to the mix; however, HYD-FW containing substrates were the richest in water soluble C. BCH-OMW was the material which contributed the

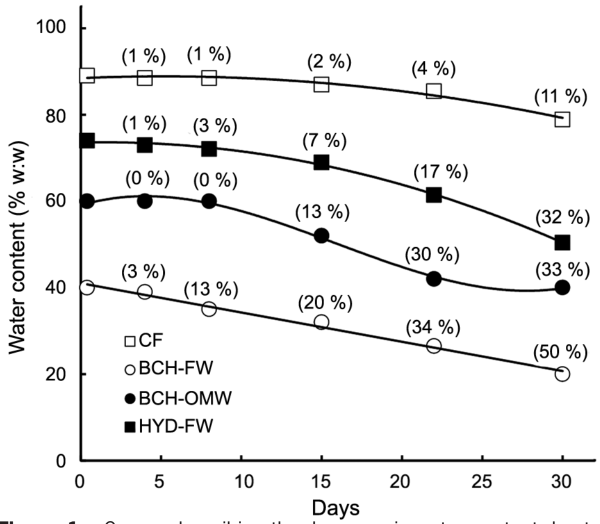

Figure 1 – Curves describing the decrease in water content due to evaporation in the water-saturated chars and the coir (CF) used as growth media constituents. BCH-FW (biochar from forest waste): $y = -0.678x + 41.05$, $R^2 = 0.989$ ($p \leq 0.001$); BCH-OMW (biochar from olive mill waste): $y = 0.003x^3 - 0.156x^2 + 1.098x + 59.06$, $R^2 = 0.994$ ($p \leq 0.001$); HYD-FW (hydrochar from forest waste): $y = -0.028x^2 + 0.081x + 73.57$, $R^2 = 0.998$ ($p \leq 0.001$); CF: $y = -0.016x^2 + 0.160x + 88.46$, $R^2 = 0.976$ ($p \leq 0.001$). Data in brackets indicate the percentage of water lost from the initial content in each sampling date.

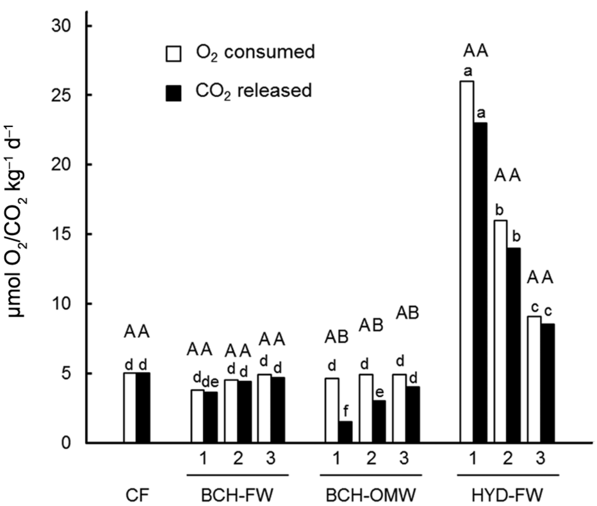

Figure 2 – Mean respiratory activity, measured as the uptake of O_2 and the release of CO_2, of the char (biochar from forest waste BCH-FW, biochar from olive mill waste BCH-OMW and hydrochar from forest waste HYD-FW) and coir (CF) containing growth media (1 = 50 % CF + 50 % char; 2 = 75 % CF + 25 % char; 3 = 90 % CF + 10 % char). Different lower case letters indicate significant differences at $p \leq 0.05$ (Tukey test) in O2 uptake or CO2 released between substrates. Different capital letters indicate a significant difference at $p \leq 0.05$ (t-Student test) between the O2 uptake and the CO2 emission from each substrate.

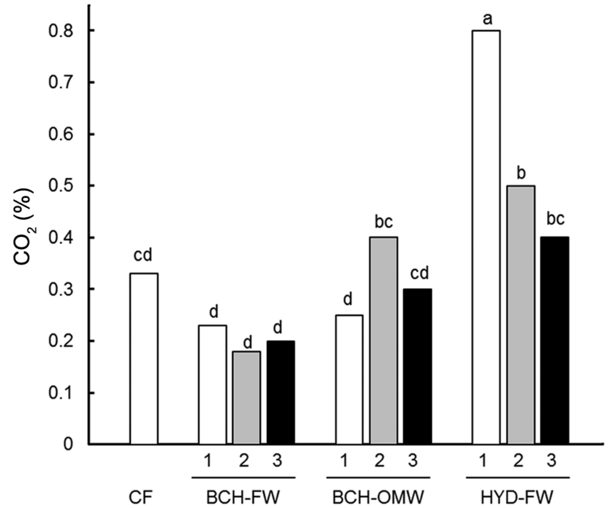

Figure 3 – CO_2 concentration in the atmosphere inside the char (biochar from forest waste BCH-FW, biochar from olive mill waste BCH-OMW and hydrochar from forest waste HYD-FW) and coir (CF) containing growth media (1 = 50 % CF + 50 % char; 2 = 75 % CF + 25 % char; 3 = 90 % CF + 10 % char). Different letters indicate significant differences at $p \leq 0.05$ (Tukey test).

most total and soluble P and K, and the most total Mg. HYD-FW contributed the most total and soluble N and Ca, and the most soluble Mg.

Microbial respiration and composition of the atmosphere inside substrates

Figure 2 shows the mean daily respiratory activity recorded in the substrates over 30 days of incubation. O_2 uptake in the substrates containing BCH-FW and BCH-OMW was equal to that of the CF control. HYD-FW containing substrates, however, presented greater respiratory activity than the others. Moreover, in these substrates O_2 uptake increased with the proportion of HYD-FW in the mix. As regards the respiratory process, all substrates, with the exception of those containing BCH-OMW, presented no differences between the amount of O_2 consumed and the amount of CO_2 released. In the case of the BCH-OMW containing substrates the release of CO_2 was significantly lower than the O_2 consumed.

This differential respiratory activity affected the gas composition of the atmosphere inside the substrates (Figure 3). In all substrates, the internal atmosphere had the same concentration of O_2 (about 20 %; data not shown). Nevertheless, the concentration of CO_2 differed between substrates being significantly higher in the HYD-FW containing mixes, and particularly in those with the largest proportion (50 %) of this char.

Seed germination, plant growth, leaf chlorophyll and nutrient content, and photosynthesis

Table 3 shows several parameters related to seed germination and plant growth. Remarkably no calendula or petunia seeds germinated in the substrates containing BCH-OMW at 25 % and 50 %. BCH-OMW at 10 % markedly reduced the germination percentage, shoot and root growth, and the percentage of flowering plants in these species compared to the control.

In the case of BCH-FW substrates, germination was reduced at the 50 % dose for calendula and at both the 25 % and 50 % doses for petunia. Compared with the control, shoot growth of both species was not affected by the substrates, whilst root growth was significantly diminished with the 50 % dose for calendula and the 25 % and 50 % doses for petunia. In the case of petunia, the number of flowering plants was also significantly reduced in substrates with 25 % and 50 % doses of BCH-FW.

For the HYD-FW containing substrates, germination and plant growth of calendula and petunia were significantly reduced compared with the control only at a dose of 50 %. The percentage of flowered plants was reduced at all doses in both species with the exception of petunia at the 10 % dose, at which an increase in flowered plants was observed.

Table 4 shows the nutrient leaf content. BCH-OMW at a low dose caused an increase in P and K in both species. This substrate negatively affected the content of Ca and Mg in calendula but had no such effects on petunia.

HYD-FW at medium (25 %) and high (50 %) doses caused an increase in K and Ca in both species, and in P only in petunia.

BCH-FW tended to cause a decrease in P and an increase in K in calendula, whereas Ca increased in petunia.

Table 5 shows the chlorophyll content (SPAD units) and certain important photosynthetic parameters measured in the plants. Chlorophyll content was not affected in calendula whilst in petunia it decreased with BCH-OMW 10 % and HYD-FW 50 %. Photosynthesis was not affected in petunia. Calendula suffered a decrease in net photosynthesis (A_N) when grown in substrates with 25 % and 50 % of HYD-FW, grown in substrates with 50 % of BCH-FW, although, with the exception of Fv/Fm in HYD-FW 50 %, no other parameter was affected.

Discussion

The physico-chemical and chemical characteristics of the chars used in this study had been previously analysed by Fornes et al. (2015). In this study, it was suggested that BCH-OMW was phytotoxic due to its high pH and, in particular, its high salinity. BCH-FW showed no phytotoxicity and HYD-FW was also suspected to be phytotoxic due to its high content in organic toxic-related labile C (Bargmann et al., 2013). These suspicions have recently been confirmed by Fornes and Belda (2017) using seed germination and seedling hydroponic culture bioassays.

Due to the characteristics of the raw materials mentioned above, some of the assayed substrates fell outside the proposed adequate ranges (AR) for pH (5.5 to 6.3) and EC (≤ 3.5 dS m^{-1} in saturation extract; ≤ 0.5 dS m^{-1} in 1:5 (v:v) extract) for substrates (Abad et al., 2001; Bunt, 1988). This was indeed the case for the BCH-OMW-50 %, which contained a high proportion of soluble K, probably in the form of a hydroxide or a carbonate (Bedussi et al., 2015). BCH-OMW-25 % was still too alkaline and saline. In fact, these substrates were harmful to plant development for calendula and petunia, although other species like tomato have been shown to be tolerant to BCH-OMW (Fornes et al., 2017). Even at 10 % BCH-OMW, plants showed an unbalanced nutrient uptake and low chlorophyll content. However, photosynthesis was not negatively affected in this substrate.

BCH-FW containing substrates were also too alkaline, yet the above-ground growth of both species did not significantly differ from the CF control, though root growth and seed germination decreased. In this sense, petunia proved slightly more sensitive than calendula to the presence of BCH-FW in the growth media. Moreover, as alkalinity causes poor P solubility and availability for plants, P content was low in plant tissues. Alkalinity has also been related to the impairment of chlorophyll synthesis and thylakoid function through low iron uptake (Marschner, 2012). Although the negative effect of alkaline substrates on iron uptake and on chlorophyll concentration in petunia had already been reported (Smith et al., 2004), our results showed no effect on these parameters in petunia and calendula with the exception of a slight decrease in net photosynthesis caused by the highest dose of BCH-FW in the latter. This effect was not accompanied by any symptoms of injury in the photosynthetic apparatus (chlorophyll content, Fv/Fm and Φ_{PSII}). In previous experiments, we also found no effect of BCH-FW water extracts on photosynthesis in either tomato or pea (Fornes et al., 2017). Hence, we concluded that the described effect on calendula was a circumstantial situation with no physiological relevance. As calendula and petunia have similar growth requirements (Aspden et al., 1989), the reasons for their differential behaviour probably rest on a different sensitivity to some chemical present in the biochar, but this fact remains unknown. Other authors also found differential responses, from stimulation to inhibition, of different species to biochars, Belda et al. (2016) comparing myrtle and mastic; Sáez et al. (2016) comparing milk thistle and sunflower; Solaiman et al. (2012) comparing wheat, mung bean and subterranean clover.

At any rate, excessively high pH of an organic material can be easily improved by acidifying the material with elemental sulphur (García de la Fuente et al., 2007), and diluted sulphuric (Costello and Sullivan, 2014) or phosphoric acids (Mazuela et al., 2005). In addition, EC can be easily reduced by leaching the materials with good quality water (Fornes et al., 2010). In the case of the chars used in this study, Fornes and Belda (2017) demonstrated that alkalinity and salt excess were easily reduced by treating chars with diluted nitric acid, thus removing the phytotoxic factors.

Considering EC and pH values together, the HYD-FW containing substrates were the most adequate for plant growth. Notwithstanding, mixes containing medium to high proportions of this char markedly reduced seed germination, plant growth and negatively affected important processes such as nutrition and photosynthesis of both species. Similar effects were described by Bargmann et al. (2013) and Belda et al. (2016) for seed germination, Bargmann et al. (2014), Belda et al. (2016) and Fornes et al. (2017) for plant growth, and Fornes et al. (2017) for photosynthesis.

Determining and adjusting the physical properties of chars and the substrates derived from them is more relevant because these cannot be manipulated once the substrate has been placed in the container. For the main physical properties AR have been established (Bunt, 1988). All the assayed substrates can be considered light as their Db lay within the AR (< 400 kg m^{-3}) and all complied with the recommended minimum porosity of 85 %, with the exception of BCH-FW-50 % which, nonetheless, was close to this limit. The water contents and particularly the air contents of growth media are of paramount importance to containerized plant production (Fernandes and Corá, 2004). In our case, HYD-FW is a material which strongly limits aeration of the substrate (8 % Vair in the pure material; data not shown). Because of this the mixes with medium to high proportions of HYD-FW (25-50 %) had the lowest air content and the highest water content, close to the AR limits (20-30 % for air and 55-70 % for water). Other substrates such as those containing BCH-FW or BCH-OMW were also outside the AR for Vair and Vwater. However, in these cases this was due to excessive aeration and low water content, which is a lesser problem than excess water content, as it can be solved by increasing irrigation frequency. WHC, which is equivalent to field capacity in soils, depends on P_T and Vwater. CF had markedly more P_T than the other substrates and a high Vwater value, which led to the highest WHC in the experiment. This means that saturated CF offers more water to plants than the other substrates, which offer progressively less water to plants as the CF proportion decreases. In addition, the strength with which water is retained by matric forces in the solid phase of a substrate will affect the readiness of plants to absorb water. This is also related to substrate readiness to evaporate water. Water loss by evaporation was lowest in CF, followed by HYD-FW and BCH-OMW, with the greatest water loss seen in BCH-FW. This means that, although greater in WHC, CF-rich substrates will create stronger barriers to water uptake by plants than HYD-FW, BCH-OMW and, particularly, BCH-FW. Shrinkage is a factor which must be taken into account where irrigation is not continuous. Between two irrigation cycles substrates can dry and shrink. Once shrunk, rewetting the substrates will not bring them back to their initial volume (Abad et al., 2001). Shrinkage can injure the roots and hinder irrigation due to fast and useless drainage. With respect to shrinkage, BCH-FW containing substrates were the most adequate, followed by those containing BCH-OMW and, finally, those containing HYD-FW.

Microbial biomass and activity can also affect substrate characteristics if the substrate is not well stabilized (i.e. high C/N ratio). Organic matter decomposition can lead to changes in chemical composition and to structural degradation of solid particles, which changes the physical properties of the substrate. This can be counteracted by the addition of biochar to the substrate (Tian et al., 2012). Additionally, an abundant and varied microbial population often endows the substrates with the capacity to suppress phytopathogens (Hoitink et al., 1996) leading to healthier plant growth. This effect has in fact been proven for biochar (Harel et al., 2012). Among the substrates assayed in our study, those containing high amounts of HYD-FW showed higher respiratory activity than the rest. This higher respiratory activity led to the consumption of large amounts of O_2 and the release of large amounts of CO_2, in a near-equimolecular ratio. This means that, although aeration was relatively poor in these substrates, no anaerobic respiration (fermentation) was taking place. Due to this, an accentuated increase of CO_2 concentration inside the substrate was recorded. We argue that the poor structure of HYD-FW, its poor aeration, its high Vwater and water retention capacity, and its high respiratory activity (which leads to an abnormally high CO_2 concentration in the substrate), strongly contribute to the growth failure observed in both species when large amounts of this char were used. This, combined with the presence of phytotoxic compounds in hydrochars (Bargmann et al., 2013; Fornes et al., 2017) and the recognized effect of this type of char of immobilizing N (Bargmann et al., 2014), were the most probable cause of the deleterious effect of HYD-FW substrates on plant growth. With respect to the presence of phytotoxic compounds, Bargmann et al. (2013) demonstrated that phytotoxicity was markedly reduced after washing or storing hydrochars. With respect to N immobilization, Fornes et al. (2015) showed that pure HYD-FW does not immobilize N but it does so when mixed with coir or sand (Belda et al., 2016).

From the results presented we can conclude the following: 1) Biochar presents better characteristics than hydrochar as a constituent of soilless growth media, as was demonstrated by comparing both biochar and hydrochar prepared from a similar feedstock (forest waste), and 2) The raw materials used for producing biochars deeply affect the characteristics of the final products, and will eventually determine whether these products are adequate for use as constituents of soilless growth media, as was the case for forest waste and olive mill waste, (adequate and not adequate, respectively).

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