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TITLE

Assessment of biochar and hydrochar as minor to major constituents of growing media for containerized tomato production

RUNNING TITLE

Assessment of biochar and hydrochar in growing media for tomato production

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ABSTRACT

BACKGROUND: Chars are emerging materials as constituents of growth media. However, chars of different origin differ in their characteristics and more studies are needed to ratify them for such role. The characteristics of coir mixed with 0, 10, 25, 50, 75, and 100 (% v:v) of two biochars, from forest waste (BCH-FW) and from olive mill waste (BCH-OMW), and one hydrochar, from forest waste (HYD-FW), and their effects on growth, yield and fruit quality of two tomato cultivars (Gransol RZ and Cuarenteno) were assessed.

RESULTS: Chars negatively affected plant growth and yield but not fruit quality. The effect was related to the char dose and was larger in HYD-FW and BCH-FW than in BCH-OMW, despite the high salinity of the latter, and more acute in Cuarenteno than in Gransol RZ. The results were discussed on the basis of: the large particle size of BCH-FW, which could have caused low nutrient solution retention and, hence, reduced plant nutrient uptake, and the high water holding capacity, poor aeration and large CO_2 emission of HYD-FW, which could lead to root anoxia.

CONCLUSION: BCH-OMW can be used at high proportion in media for tomato cultivation. The use of BCH-FW at high proportion might be taken under consideration after adjusting particle size yet this needs additional assays. HYD-FW is inadequate for soilless containerized tomato cultivation.

KEYWORDS

biochar; growing media characteristics; hydrochar; tomato production; *Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a highly produced and consumed horticultural crop worldwide. Apart from its nutritional value, flavour and aroma, tomato is considered a functional food of interest due to the presence of significant amounts of antioxidant compounds such as vitamin C, phenols, carotenoids, etc.[1] Different cultivars (genotypes) differ not only in these compound contents but also in growth, yield potential and tolerance to stress (salinity, drought, toxics, etc), which are related to the growth media.[2]

Growing tomato in soilless conditions is a good option where soils are of poor quality or unavailable. The main constituent of soilless growing media has been peat for the last fifty years.[3] However, environmental concerns about peat extraction are forcing media suppliers to look for alternative materials. In this sense, the use of biochar and hydrochar as substrate constituents for horticulture is novel and promising. Biochar and, mainly hydrochar, have been scarcely studied as substrate constituents and always as minor constituents of the growth mix.[4-8]

Biochar and hydrochar are charred organic matter (ideally obtained from organic wastes) produced by dry pyrolysis and wet hydrothermal carbonization, respectively. Physical and chemical characteristics of biochars and hydrochars are quite different and may vary greatly depending on the raw material and manufacturing conditions.[9,10]Biochars from nutrient-poor feedstock such as wood normally happen to be alkaline, non-saline and recalcitrant to decomposition[11] whilst biochars from nutrient-rich feedstock such as olive mill waste are highly saline.[10] Hydrochars tend to be acidic and contain large amounts of labile organic carbon, hence, causing N immobilization in soil.[12] Moreover, hydrochar has been considered phytotoxic due to the presence of organic toxics such as phenolic compounds and organic acids in relation to its high content in labile organic carbon.[13] All these features indicate that not all biochars and hydrochars ought to be considered for horticultural use. Besides, the effect of biochar as soil amendment[14,15] or as soilless growing medium constituent[8] on tomato has been scarcely studied and that of hydrochar has not been assayed.

The aim of this study was to ascertain the effect of two biochars produced from forest waste and olive mill waste and a hydrochar produced from forest waste[10] on growth, and fruit yield and quality of two tomato cultivars (Gransol Rijk Zwaan [RZ] and Cuarenteno) differing in their productivity and fruit characteristics. A second objective was to establish the limiting reliable proportion of char in the growing media which did not harm the crop. To reach this objective, chars were assayed from low (10% v:v) to high (100 %) proportions in the growth media.

MATERIALS AND METHODS

Biochars, hydrochar, coir, and plant material

Three chars, a biochar from forest waste (BCH-FW) [CARBOEXPOR S.L. (Cañizal, Spain)], a biochar from olive mill waste (BCH-OMW) [CARBONES ALCARAZ S.L. (Aldaia, Spain)], and a hydrochar from forest waste (HYD-FW) [INGELIA S.L. (Naquera, Spain)], and a coir (CF), which was selected to compare one of the most common materials used in horticulture with the chars, were used in this study. The origin, physicochemical and chemical characteristics, and some other specific properties of the three chars and the coir were previously published.[10]

Two tomato varieties with clearly different performance were selected for this work. The commercial hybrid Gransol RZ [Rijk Zwaan (Almería, Spain)] is characterized by slightly flattened, large-sized fruits. It is a hybrid typical of intensive production with high use of inputs. The obsolete material, Cuarenteno [COMAV-UPV

(Valencia, Spain)] was selected as representative of the traditional tomato varieties of Spain. Cuarenteno is characterized by intermediate-sized flattened-ribbed fruits and an early production.[16]

Treatments, experimental design, and plant growth conditions

Two experiments were conducted to test chars at low proportion (year 2013) and at high proportion (year 2014) as components of the growing media for tomato cultivation. In experiment I, treatments consisted of the following mixes (substrates): CF:BCH-FW, CF:BCH-OMW, and CF:HYD in the proportions 75:25, 90:10, and 100:0 (control of pure coir). In experiment II, the proportions of CF:chars assayed were 0:100 (pure chars), 25:75, 50:50, and 100:0 (control of pure coir). For each tomato cultivar, four replicates of three pots (10 L volume) were filled with each of the substrates (12 pots per treatment). The pots were distributed following a random block design in a climatic greenhouse equipped with heating and cooling systems. With this system, the minimum temperatures registered inside the greenhouse ranged from 15ºC in February to 25ºC in June whilst maximum temperatures ranged from 20-25ºC in February to 30-35ºC in June. In February of 2013 and 2014, three weeks old seedlings of each of the cultivars were transferred to the pots (one seedling per pot). The plants were cultivated for five months. During the growth period plants were daily drip fertigated with a solution of the following composition and characteristics: 9.63 mM NO_3^-; 3.75 mM SO_4^{2-}; 2.25 mM Cl^-; 1.71 mM $H_2PO_4^-$; 0.72 mM HCO_3^-; 7.20 mM K^+; 4.25 mM Ca^{2+}; 2.20 mM Mg^{2+}; 1.26 mM Na^+; 0.28 mM NH_4^+; pH = 5.8; Electrical conductivity (EC) = 2.0 dS m^{-1}. The amount of water and fertilizer was increased progressively (from 1 to 1.5 L pot^{-1} day^{-1}) as the plant demand increased and the temperature rose through the spring-summer season. Two treatments (middle March and middle May) with Confidor 20 LS® (imidacloprid), BAYER, against whitefly, and three treatments (beginning of March, middle April, and middle May) with Pelt® (thiophanate-methyl), BAYER, against fungal diseases, were carried out.

Physical and physicochemical characterization of the substrates

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

Physical properties. Particle size was determined on 200 mL air-dried aliquots. Particle sizes were separated 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 percentage by weight of the whole sample.[17] Coarseness index (CI) was calculated as the accumulated percentage in weight of particles larger than 1 mm. Bulk density (D_B), water capacity (V_{water}) and total water-holding capacity (WHC) were determined using the official methods in the European Standards for Soil Improvers and Growing Media EN 13041.[18] 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 105ºC. Total pore space (P_T) is the percentage of the volume of the material that can be filled with water. Air capacity (V_{air}) is the difference −in percentage by volume− between the total pore space and the moisture content at a suction of 1 kPa.[18]

Physicochemical. pH[19] and electrical conductivity (EC)[20] of the substrates were determined in a 1:5 (v:v) substrate:water suspension. The pH was measured using a

Crison mod. 2000 pHmeter (Barcelona). EC was determined with a Crison mod. 522 conductivity meter (Barcelona).

All determinations were replicated three times.

Determination of the moisture and the salinity in the substrates throughout the growth period

Periodically, i.e. every month from the end of February to the end of June (5 measurements), the moisture (%) and the salinity (EC; dS m^{-1}) were measured using a Wet-2 Sensor (AT Delta-T devices, Cambridge) probe. As the variability among different months was low, the mean of the five measurements is presented as a unique value for each substrate.

Determination of the O_2 and CO_2 concentration in the internal atmosphere of the substrates throughout the growth period

The composition of the atmosphere inside the containerized substrates will be affected by multiple factors: physical properties of the substrate (porosity, air volume and water retention), microbial respiration, root density, irrigation regime, etc. To measure this composition, a hypodermic needle (15 cm long) connected to a CheckPoint portable gas analyzer (Dansensor®) was immersed into the substrates and the O_2 and the CO_2 concentrations (%) were recorded. The measurements were repeated at the end of March and at the end of May. The results presented are the average of the two sampling dates.

Effects of the substrates on plant growth and on leaf chlorophyll and nutrient content

For both experiments, SPAD measurements were carried out in May with a Chlorophyll Meter SPAD-502 (Konica, Minolta, Tokyo) in four leaves of each plant, and the average calculated.

At the end of both experiments (end of June) some parameters related to growth were recorded. The shoot and the root were separated by cutting the stem right above the substrate surface. Root size was immediately evaluated by taking the root ball out of the pot and evaluating the extension of the root system with a qualitative scale ranging from 1 to 5, in which value 1 represents roots which do not reach the surface of the substrate, whilst value 5 represents a root system forming a compact mesh that colonizes all the substrate.[21] As the aerial biomass was too large to be oven-dried, shoots were left to dry in the closed glasshouse for two weeks and weighted. In the summer conditions temperature in the closed glasshouse easily reaches 55ºC during the day, which was considered enough to dry the shoots.

Dry leaf tissue was finely ground for analysis of N, P, K, Ca, and Mg. N was determined by burning the material at 1020 ºC in an elemental analyzer (Flash EA 1112 Series-LECO Truspec). P, K, Ca, and Mg were determined by Atomic Emission Spectrophotometry with inductively coupled plasma (ICP-AES; ICAP 6500 DUO/IRIS INTREPID II XDL).

Effects of the substrates on yield and on fruit quality related parameters

Fruits were collected from plants at the red-ripe stage. Fruits were progressively picked as they ripened for two months (May and June) and the cumulative number and weight of fruits per plant was recorded. Two fruits were selected from each plant at the red-ripe stage from the second or third truss. The fruits were then homogenized obtaining a single homogenate per plant, which was kept frozen until analysis.

The contents of the sugars fructose, glucose and sucrose and of the organic acids citric, malic and glutamic were determined using capillary electrophoresis with an Agilent 7100 Series CE equipment (Agilent Technologies, Waldbronn, Germany) as described by Cebolla-Cornejo et al.[22] Briefly, fused silica capillaries (Polymicro technologies, Phoenix, AZ, USA) with 50 µm internal diameter, 363 µm external diameter, 67 cm total length and 60 cm effective length were conditioned by sequential rinses with NaOH 1 N, deionized water and the running buffer (20 mM 2,6-piridin dicarboxylic acid and 0.1% w:v hexadimethrine bromide, pH = 12.1). Analytical conditions included hydrodynamic injection (20 s, 0.5 psi) and −25 kV fixed voltage separation at 20◦C. The capillaries were flushed with 58 mM SDS and running buffer between runs. Sucrose equivalents (SEq) and the ratios SEq/citric acid and SEq/glutamic acid were also calculated, as described by Cebolla-Cornejo et al. (2011).[23]

Statistical analysis

One-way analysis of variance was carried out to determine statistically significant differences caused by the substrates (Table 1; Tables 3 to 7). When differences were significant, the Tukey test at $P \leq 0.05$ was conducted to establish significant differences between means. Additionally, the pH and EC of the substrates at the beginning and at the end of the growth period were compared by the *t*-Student test (Table 2). All statistical analyses were performed using the Statgraphics Plus for Windows 5.1 statistical package (Statistical Graphics Corp., 2000).

RESULTS

Physical and physicochemical characteristics of the substrates

Table 1 shows the main physical properties of the substrates. CI was largest in BCH-FW, followed by BCH-OMW, HYD-FW and CF, in which it was less than half of CI in BCH-FW. This parameter decreased in the substrates as the proportion of coir increased in the mix. D_B increased in the substrates with the increase of char content, the highest being for the BCH-FW treatment. P_T was largest for CF and lowest for BCH-FW (16.6% lower than for CF), with intermediate values for BCH-OMW and HYD-FW. This parameter decreased in the substrates as the char proportion increased. Vwater was about half in pure BCH-FW than in CF and increased in substrates as the proportion of CF increased. Pure BCH-OMW had slightly lower Vwater than CF but its Vwater also increased with the increase of CF in the mix. Conversely, pure HYD-FW had a significantly greater Vwater value than CF and this parameter decreased with increasing CF in the mix. Vair showed the opposite trend to Vwater. Vair was similar or greater than in CF in the substrates containing BCH-OMW and greater than CF in those containing BCH-FW. The low value for Vair in the substrates containing high proportion of HYD-FW was noteworthy (four times lower in pure HYD-FW than in CF). WHC was lowest for BCH-FW, followed by BCH-OMW, HYD-FW, and CF which held nine times more water than BCH-FW. WHC increased in the mixes as the proportion of CF increased. Compared to CF, shrinkage was only slightly affected by the presence of HYD-FW in the mix and decreased in the mixes with BCH-FW and BCH-OMW being negligible in pure biochars.

Substrates containing BCH-FW and, mainly, those containing BCH-OMW were alkaline due to the high pH of the pure biochars (Table 2). HYD-FW containing substrates were slightly alkaline and CF was slightly acidic. In BCH-FW and BCH-OMW substrates, pH decreased as the proportion of CF increased. However, this effect was less pronounced in HYD-FW. At the end of the growth period pH decreased in all heavily alkaline substrates due to the acidic pH of the fertigation solution, but it remained alkaline in all substrates containing BCH-OMW and in those containing 50% or more BCH-FW. This effect was negligible in HYD-FW containing substrates and no significant pH changes were found in these mixes throughout the experiment. Substrates containing BCH-FW and HYD-FW had low EC, which was similar to or even lower than that of CF. BCH-OMW containing substrates were saline, with EC concomitantly increasing with the proportion of BCH-OMW. At the end of the growth period the EC of the substrates approached the EC of the fertigation solution, i.e. it increased in substrates containing BCH-FW and HYD-FW and decreased in those containing BCH-OMW. The latter case was remarkable because EC decreased 6.6 dS m^{-1} from the beginning to the end of the experiment in pure BCH-OMW. Despite this trend, at the end of the experiment, EC was still high in substrates containing high proportion of BCH-OMW.

Moisture and salinity in the substrates throughout the growth period

Fig. 1 shows the mean salinity and moisture of the containerized substrates throughout the experiment. Therefore, the data of these two parameters have been affected by other factors which are associated with the culture (temperature, composition and volume of the fertigation solution, volume of leachate, plant growth [water uptake and transpiration rates, nutrient uptake], etc) rather than exclusively the nature of the constituent materials. As expected BCH-OMW mixes were more saline than the others and salinity increased with the increase of char proportion in the mix. Associated with salinity was the moisture content in the BCH-OMW substrates, which was higher than for other substrates. However, salinity cannot be the only factor affecting moisture because the mix containing 75% BCH-OMW with 25% CF had greater moisture content than pure BCH-OMW, which was the most saline. Moisture of pure HYD-FW was also larger than that of CF whilst the moisture of the BCH-FW substrates, mainly that of the pure one, was lower.

O_2 and CO_2 concentration in the internal atmosphere of the substrates throughout the growth period

Fig. 2 shows the concentration of O_2 and CO_2 in the air space inside the substrates. O_2 concentration was 20% in all substrates containing BCH-FW and BCH-OMW and in CF. In HYD-FW containing substrates O_2 concentration was slightly lower, the lower the more char was there in the mix. With respect to CO_2, its concentration was very low in all substrates with the exception of those containing HYD-FW in which CO_2 concentration increased with the increasing char proportion in the mix reaching near 3% in the pure HYD-FW.

Plant growth and chlorophyll and mineral nutrient contents in the leaf

The fact that the different doses of chars were assayed in different years led us to refer the results to their respective CF controls for comparison purposes. In experiment I the plants of both cultivars grew more than in experiment II (Table 3). Shoot growth of Gransol RZ decreased with the increased presence of BCH-FW (doses above 10%) and HYD-FW (all doses) in the substrate. Root growth was negatively affected at medium to high proportions of these two chars (doses above 25%). BCH-OMW affected the growth of both shoot and root negatively only at doses larger than 25%. For Cuarenteno, low doses of chars did not affect shoot growth whilst root growth was reduced by the doses of 10% and 25% in the BCH-OMW and HYD-FW treatments, respectively. For this cultivar, doses larger than 25% of BCH-FW and HYD-FW reduced shoot and root growth as the proportion of char in the substrate increased. With respect to BCH-OMW these high doses did not affect shoot growth although root growth was reduced.

SPAD results, which are related to leaf chlorophyll content, are presented in table 4. BCH-FW did not cause a clear effect on this parameter since only intermediate doses (25% and 50%) decreased the SPAD units in Gransol RZ but not in Cuarenteno, in which SPAD units even increased at the 100% dose. BCH-OMW caused a reduction in chlorophyll in Gransol RZ only at the dose of 100% and had no effect in Cuarenteno. HYD-FW caused a general reduction in chlorophyll in Gransol RZ although the effect was not significant for some of the doses (50% and 100%) whilst it affected Cuarenteno negatively at the largest doses of 75% and 100%.

There were differences in leaf mineral composition between the two cultivars (Table 4). Whilst Gransol RZ had larger concentration of P, K and Ca than Cuarenteno (0.9 vs. 0.5, 4.2 vs. 3.2 and 4.5 vs. 4.1 on average, respectively), Cuarenteno had larger N concentration than Gransol RZ (3.9 vs. 3.4 on average). Mg content was the same for both cultivars (1.1 on average). Low doses (experiment I) of the three chars in the substrates did not cause any effect on leaf nutrient content. Nevertheless, at high doses (experiment II) some significant effects were found. The most noteworthy was that BCH-FW at 75% and 100% caused a decrease in N in Cuarenteno but not in Gransol RZ, and in P, K and Ca in Gransol RZ but not in Cuarenteno; BCH-OMW caused a decrease in P in Gransol RZ in all the doses and an increase in Mg in doses 50% and 75%, whilst in Cuarenteno it caused an increase in K and a decrease in N in all the doses and a decrease in Ca and Mg in the highest dose; HYD-FW caused a decrease in P, Ca and Mg in Gransol RZ in the highest dose and in K in doses 50% and 75%, whilst the negative effect in Cuarenteno was small and only for N and Ca in some of the doses but showing no trend.

Yield and fruit characteristics

Table 5 shows the yield as affected by the different substrates assayed. Comparing yield results to the control, Gransol RZ was more productive than Cuarenteno. The former yielded 4.8 kg plant^{-1} and the latter 3.6 kg plant^{-1} on average. However, Cuarenteno bore more fruits than Gransol RZ although of smaller size. With respect to the char effect, at low dose only HYD-FW 10% caused yield reduction in Gransol RZ but not in Cuarenteno. At larger doses, BCH-FW caused the strongest reduction in yield (both in fruit weight and number of fruits per plant) in both cultivars, especially at the 100% dose. The negative effect of HYD-FW was less remarkable. In Gransol RZ all doses caused the same reduction expressed as kg plant^{-1}. However, doses 75% and 100% had less number of fruits though of larger size than dose 50%. In Cuarenteno the 75% and 100% doses also reduced yield. With respect to BCH-OMW, only the 100% dose caused a decrease both in kg per plant and fruit number in Gransol RZ. In Cuarenteno there was a decrease in kg per plant in the 50% dose due to a decrease in fruit weight.

Tables 6 and 7 shows some fruit quality related parameters of Gransol RZ and Cuarenteno at low and high char doses, respectively. The cultivars showed different sugar and acid profiles. Gransol RZ showed less malic and glutamic acid contents and more citric acid content than Cuarenteno. Regarding sugars, Gransol accumulated more glucose and fructose and presented higher sucrose equivalents (SE) than Cuarenteno in experiment I, but not in experiment II. Ratio SE:glutamic acid was larger in Gransol RZ than in Cuarenteno whilst ratio SE:citric acid was similar in both cultivars.

With regard to the effect of chars, no significant differences were observed at low doses. At high doses, BCH-FW and BCH-OMW at 100% caused an increase in citric acid, but this effect was found only in Gransol RZ.

DISCUSSION

The effect of biochar and, mainly, hydrochar as substrate constituents for containerized soilless plant production has been scarcely studied. Generally, biochar has been applied as a minor component in the growth mix (<50%; v.v).[4,5,8,14] Only in a few cases for biochar,[7,24] and never, to our knowledge, for hydrochar, high doses (50% to 100%; v:v) have been assayed.

Suitability of BCH-FW for tomato production

Our results indicated that BCH-FW can be used as substrate constituent for containerized tomato production up to a 25% dose, since doses of 50% or higher decreased plant growth and yield. Contrary to Graber et al.[4] who found a stimulating effect of low doses of biochar on tomato yield, we did not find this effect. As indicated above fruit quality was hardly affected by this char in our study. Similarly, Petruccelli et al.[8] did not detect any effect of poplar or wheat-straw biochars on fruit palatability although the latter caused an increase in the antioxidant properties of the fruits. Conversely, Akhtar et al.[14] reported that amending a sandy loam soil with 5% of biochar increased titratable acidity without affecting soluble solids in short irrigation conditions.

EC was not the pernicious factor at the high doses as this material had a low content in soluble salinizing ions[10] and, hence, a low EC value (Table 2; Figure 1). pH was alkaline in BCH-FW and far from the recommended range (5.5 to 6.3)[25] for soilless growing media (Table 2). The alkalinity and the nutrient adsorbing capacity of some biochars might affect nutrient availability, mainly that of P.[26] In fact, plants of Gransol RZ grown in substrates containing the highest doses (75% and 100%) of the char, which were the only that were still alkaline at the end of the experiment, had reduced leaf content of some nutrients such as P, K, and Ca, although these were still within adequate range for tomato.[27] These results did not agree with those presented by Dunlop et al.[24] who did not observe any decrease in nutrient content in tomato leaf in spite of the strong alkalinity of the biochar-containing substrates used in their experiments. A second, but also relevant, possible cause for the deficient nutrient supply of these substrates might be related to the physical properties of BCH-FW. The particle size of the batch used in our experiments was large. This conferred low total porosity, WHC, and water content, and excessive aeration to the substrates (Table 1; Figure 1) compared to the adequate ranges proposed by Bunt[25]: >85% for P_T, 20-30% for Vair, and 55-70% for Vwater. Low moisture retention by a 5 to 30 mm particle size biochar was also found by Steiner and Harttung.[7] The consequence of this was excessive drainage and low water and nutrient availability. Nevertheless, we believe that the inadequacy of these specific properties might be easily amended by grinding and sieving the char to the desired particle size. This, together with the fact that fruit quality was not affected by the char, led us to consider BCH-FW a potentially good material for horticultural purposes although some additional studies in this direction are still needed.

Suitability of BCH-OMW for tomato production

In our conditions, BCH-OMW also affected plant growth negatively when used at high doses. Different responses were found in Gransol RZ and Cuarenteno since the shoot weight of the former was negatively affected by the 50% to 100% doses, whilst the latter was only negatively affected by the highest dose. It was remarkable that yield was reduced in Gransol RZ only at the highest dose (100%), due to a decrease in fruit number. In Cuarenteno the only reduction in yield was found at an intermedium dose (50%) and was due to a reduction in fruit weight but not in fruit number. Gransol RZ is an F1 hybrid developed for high input agriculture, whilst Cuarenteno is a traditional rustic variety adapted to low input agriculture. Although traditional varieties are lower yielding, their rusticity makes them more tolerant to stressful conditions than hybrids and this can explain that Cuarenteno maintained a high fruit set which led to a fruit growth reduction.

Although some significant effects (positive and negative) were found on the foliar nutrient content in both cultivars, no deficiency was detected. The causes for the negative effects of BCH-OMW were different from those of BCH-FW. Although BCH-OMW had high aeration it held sufficient water due to its smaller particle size (Table 1). Beyond the high alkalinity of BCH-OMW-based substrates the main concern about them was their high salinity (Table 2) caused by the large content of soluble K in the char.[10] With respect to this, Schulz and Glaser[28] stated that biochar amendment initially increased pH in soil but it decreased it afterwards probably due to the leaching of base cations. In our case, the hydroponic fertigation used during the culture led to the reduction of EC to 50% of the initial value. This decrease in EC was probably progressive, being fast at the beginning and slow towards the end of the experiment, as we have observed in column leaching assays for this material (unpublished results) and for saline composts.[29] This was supported by the fact that EC hardly differed inside the container from the first measurement at the end of February to the last at the end of June. Nonetheless, the initial extremely high salinity, which might have lasted for the two or three first weeks of cultivation, might have caused a delay in plant growth, mainly in Gransol RZ, which seemed more sensitive to salinity than Cuarenteno, yet the reduction in substrate salt content through the culture enabled plants to produce a good yield. After rinsing an extremely saline biochar from tomato crop waste, Dunlop et al.,[24] did not find any negative effect in the growth and yield of tomato plants grown in it compared to those grown in a pine sawdust substrate. Besides, through ionic hydration, salinity contributed to the high moisture maintained in the BCH-OMW substrates (Figure 1) during the culture period. Hence, we believe that the previous rinsing of BCH-OMW will allow applying it at high proportion in the growth media.

The effect of BCH-OMW on fruit quality, as in the case of BCH-FW, was very limited. Petruccelli et al.[8] also studied a biochar produced from olive residues at the doses of 10%-20%. As in our case, these authors found little effects on sugar accumulation.

Suitability of HYD-FW for tomato production

HYD-FW presented the best combined (pH and EC) conditions for plant growth of the three chars assayed (Table 2). However, it was the most detrimental material for plant growth and yield, even at low doses. Again, this effect was more outstanding in Gransol RZ than in Cuarenteno. The reasons ought to be sought at the poor physical conditions that HYD-FW confers to the substrates. Low aeration (Table 1) together with high microbial respiration in this material[10] led to the accumulation of CO_2 and the reduction of O_2 in the container (Figure 2), which probably produced periodic conditions of anoxia. Moreover, a direct phytotoxic effect caused by the presence of toxic organic compounds in hydrochars[13] must also be considered. However, as the harvested fruit number, which depends on flowering and fruit set processes, and the fruit quality were not affected, this char did not alter important physiological and biochemical mechanisms in the tomato plant. It might have been the low plant growth, both of stem and root, which led to low nutrient supply to growing fruits, which finally reached small size.

CONCLUSIONS

Although highly saline, BCH-OMW can be used at high proportion in the growth media. This is due to the reduction of salinity with irrigation and to the tolerance of tomato to salinity. BCH-FW, which has large particle size, cannot be used at high proportion because of its low nutrient solution retention capacity. However, the use of a higher proportion of BCH-FW might be explored after adjusting the particle size in order to improve the retention of the nutrient solution and the nutrient supply to plants. HYD-FW was inadequate for soilless containerized tomato cultivation due to its large water retention, poor aeration and large CO_2 emission which probably led to root anoxia.

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Figure 1. Electrical conductivity (EC) (A) and moisture (B) inside the char-containing growing media (BCH-FW=Biochar from forest waste; BCH-OMW=Biochar from olive mill waste; HYD-FW=Hydrochar from forest waste; CF=coir). Data are the average of 12 pots and five sampling dates (monthly from February to June). Vertical bars represent error standard (n=60).

Figure 2. O_2 (A) and CO_2 (B) concentration in the atmosphere inside the char-containing growing media (BCH-FW=Biochar from forest waste; BCH-OMW=Biochar from olive mill waste; HYD-FW=Hydrochar from forest waste; CF=coir). Data are the average of 12 pots and two sampling dates (end of March and end of May). Vertical bars represent error standard (n=24).

Figure 1

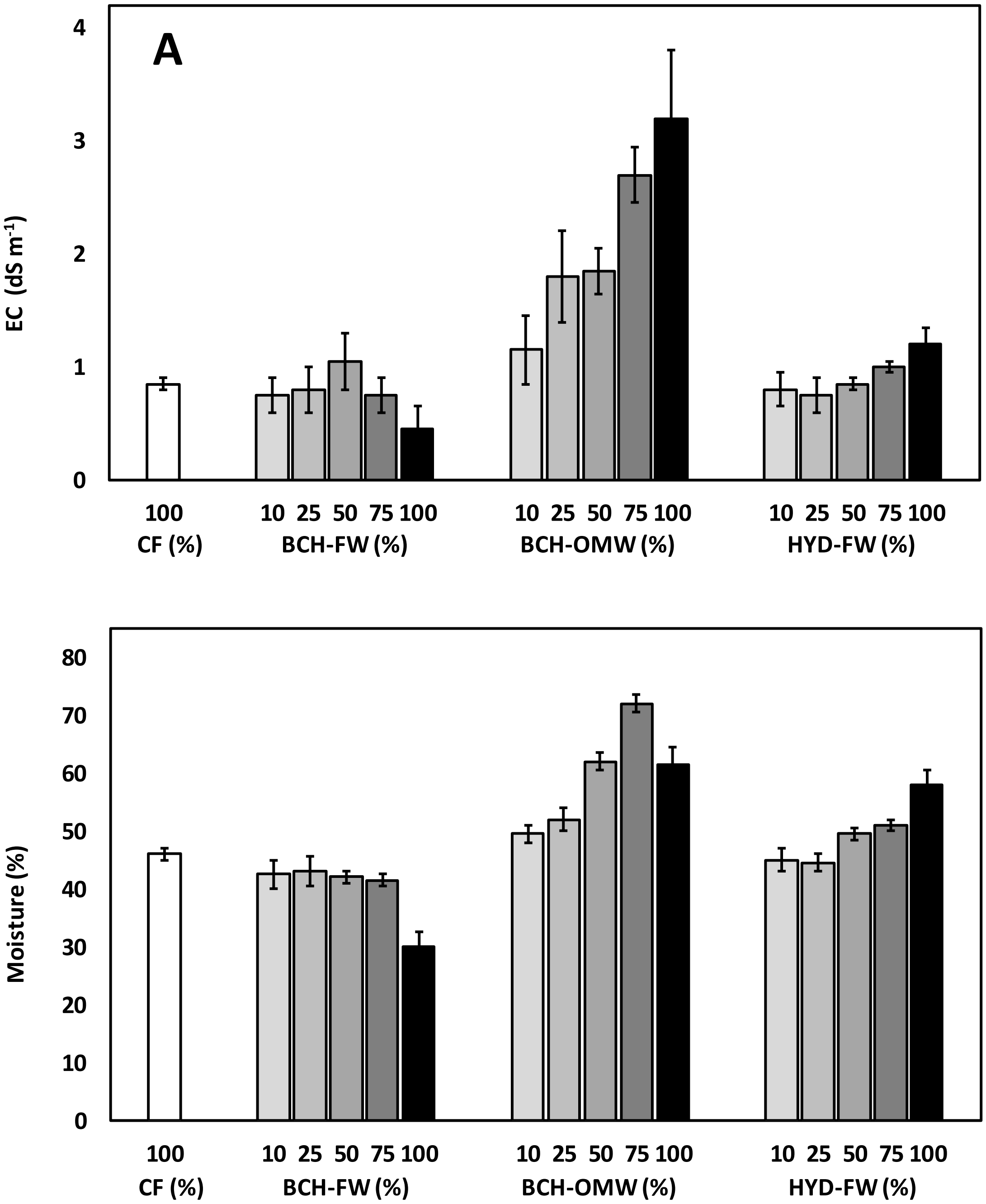
A
EC (dS m-1)
0
1
2
3
4
Moisture (%)
0
10
20
30
40
50
60
70
80
100
CF (%)
10 25 50 75 100
BCH-FW (%)
10 25 50 75 100
BCH-OMW (%)
10 25 50 75 100
HYD-FW (%)

Figure 2

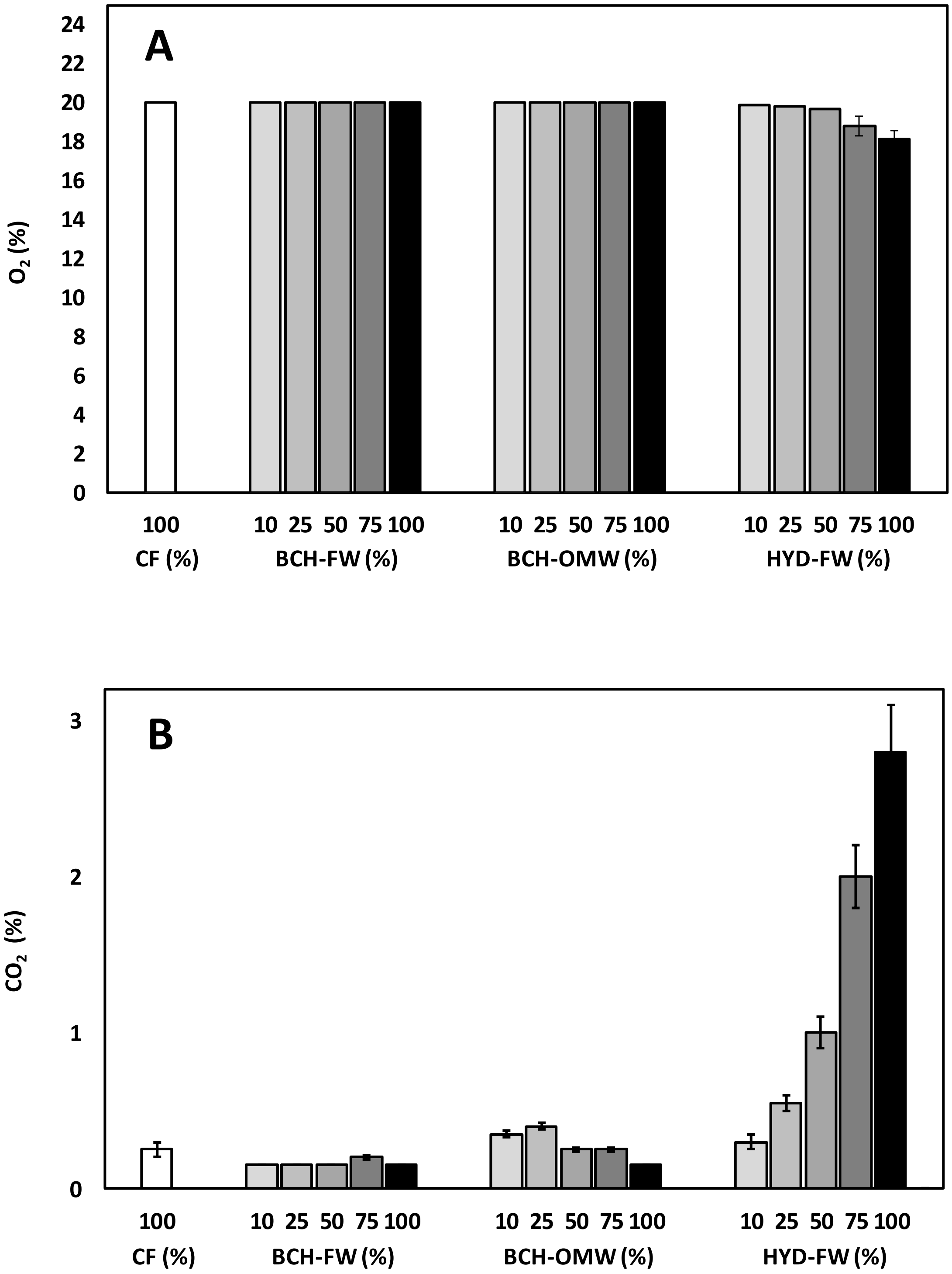
A
O$_2$ (%)
0
2
4
6
8
10
12
14
16
18
20
22
24
100
CF (%)
10 25 50 75 100
BCH-FW (%)
10 25 50 75 100
BCH-OMW (%)
10 25 50 75 100
HYD-FW (%)
B
CO$_2$ (%)
0
1
2
3
100
CF (%)
10 25 50 75 100
BCH-FW (%)
10 25 50 75 100
BCH-OMW (%)
10 25 50 75 100
HYD-FW (%)

Table 1. Physical properties of substrates based on two biochars, one hydrochar, and coir (control).

Growing media	Dose (%)	CI >1 mm[z] (% w/w)	D_B (kg m^{-3})	P_T (% v/v)	Vwater (% v/v)	Vair (% v/v)	WHC (g water kg mix^{-1})	Shrinkage (% v/v)
BCH-FW[y]	100	96a	360a	78.2i	27.0g	51.2a	820k	0f
	75	89b	290c	82.3g	32.2f	50.1a	1410j	9.0
	50	81c	230e	85.9f	44.0de	41.9bc	1900hi	12.5d
	25	59e	160fg	90.3cd	49.0d	41.3bc	3090e	18.4c
	10	48fg	120hi	92.4b	49.9d	42.5bc	4120c	18.6c
BCH-OMW	100	75d	260d	85.1f	42.6e	42.3bc	1730ij	0f
	75	62e	230e	87.2e	49.7d	37.5d	2680ef	8.9e
	50	60e	173f	89.4d	52.6d	36.8d	3050e	12.3d
	25	53f	146g	90.9c	51.4d	39.6cd	3510d	19.6c
	10	44g	101ij	93.7a	50.0d	43.7bc	4950b	26.7a
HYD-FW	100	55f	324b	80.3h	71.1a	9.2f	2190gh	21.0bc
	75	49fg	270cd	85.1f	67.3ab	17.8e	2580fg	23.2ab
	50	45gh	213e	87.0e	65.7b	21.3e	3080e	26.5a
	25	40hi	129h	91.8b	56.3c	35.5d	4370c	26.5a
	10	36i	97ij	93.9a	50.7d	43.2bc	5200b	25.9a
Control (CF)		40hi	81j	94.8a	58.8c	36.0d	7220a	24.0a

[z] CI = coarseness index; D_B = bulk density; P_T = total porosity; Vwater = water volume; Vair = air volume; WHC = water holding capacity

[y] BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir

Different letters in numerical columns differ at $P \leq 0.05$ (Tukey test)

Table 2. pH and electrical conductivity (EC; dSm^{-1}) of substrates based on two biochars, one hydrochar, and coir (control) at the beginning (initial) and at the end (final) of the culture period.

		pH		EC	
Growing media	Dose (%)	Initial	Final	Initial	Final
BCH-FW[z]	100	9.1a	8.5b	0.44a	0.68a
	75	9.0a	8.0b	0.53b	1.75a
	50	8.6a	7.3b	0.60b	2.06a
	25	8.2a	6.9b	0.80b	2.35a
	10	7.9a	6.4b	0.83b	2.54a
BCH-OMW	100	10.2a	9.9b	11.46a	4.94b
	75	10.1a	9.8b	8.87a	3.55b
	50	9.9a	9.6b	6.70a	3.05b
	25	9.6a	9.3b	3.13a	2.83b
	10	9.1a	8.8b	1.39b	2.52a
HYD-FW	100	7.6a	7.5a	0.90b	1.99a
	75	7.5a	7.4a	0.83b	2.30a
	50	7.5a	7.4a	0.70b	2.53a
	25	7.7a	7.6a	0.92b	2.54a
	10	7.3a	7.2a	0.86b	2.56a
Control (CF)		6.5a	6.1b	0.81b	2.55a

[z] BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir

Different letters in initial vs. final values indicate statistical significance at $P \leq 0.05$ (t-Student test)

Table 3. Effect of substrates based on two biochars, one hydrochar, and coir (control) on plant growth of *Solanum lycopersicum* cv. Gransol RZ and Cuarenteno. Experiment I with low doses (10; 25 % v:v) of chars. Experiment II with high doses (50; 75; 100 % v:v) of chars.

Material	Dose (%)	Shoot dry weight (g)	Root ball (Visual score; 1-5)	Shoot dry weight (g)	Root ball (Visual score; 1-5)
		Experiment I			
		Gransol RZ		Cuarenteno	
BCH-FW[z]	25	330b	4.0b	257ab	4.0a
	10	353ab	5.0a	275ab	4.5a
BCH-OMW	25	370ab	5.0a	271abc	4.0a
	10	417a	4.0b	301a	3.0b
HYD-FW	25	331b	4.0b	245bc	2.0c
	10	323b	3.5c	229c	4.0a
Control (CF)	0	401a	4.0b	266abc	4.0a
		Experiment II			
		Gransol RZ		Cuarenteno	
BCH-FW[z]	100	139e	2.5e	171e	2.0d
	75	237bc	4.0b	184de	2.0d
	50	235bc	3.5c	198cd	3.5b
BCH-OMW	100	223c	3.0d	211bc	2.5c
	75	205d	3.0d	218ab	1.5e
	50	248b	2.5e	230a	2.5c
HYD-FW	100	191d	2.5e	153f	1.5e
	75	229c	3.5c	170e	1.5e
	50	237bc	3.5c	197cd	2.5c
Control (CF)	0	313a	4.5a	226ab	4.5a

[z] BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir

Different letters in numerical columns differ at $P \leq 0.05$ (Tukey test)

Table 4. Effect of substrates based on two biochars, one hydrochar, and coir (control) on cholophyll and mineral elements content in leaves of *Solanum lycopersicum* cv. Gransol RZ and Cuarenteno. Experiment I with low doses (10; 25 % v:v) of chars. Experiment II with high doses (50; 75; 100 % v:v) of chars.

Material	Dose (%)	Chlorophyll (SPAD units)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Chlorophyll (SPAD units)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
		Experiment I											
		Gransol RZ						Cuarenteno					
BCH-FW^{z}	25	42.5d	3.2a	0.8a	4.4a	4.1a	1.1a	46.5a	3.7a	0.4a	3.1a	3.9a	1.0a
	10	46.8ab	3.3a	1.0a	4.1a	4.4a	1.1a	44.3a	3.6a	0.6a	3.2a	4.3a	1.0a
BCH-OMW	25	44.3abcd	3.3a	0.9a	4.2a	4.1a	1.1a	44.5a	3.6a	0.5a	3.4a	4.1a	1.2a
	10	45.8abc	3.3a	1.0a	4.0a	4.3a	1.2a	46.5a	3.6a	0.5a	3.1a	3.9a	1.1a
HYD-FW	25	42.9cd	3.2a	0.8a	4.0a	4.3a	1.0a	46.9a	3.7a	0.4a	3.2a	4.1a	1.1a
	10	43.9bcd	3.4a	1.0a	4.2a	4.3a	1.0a	44.6a	3.6a	0.5a	3.4a	4.2a	1.1a
Control (CF)	0	47.3a	3.1a	0.7a	4.4a	3.8a	1.2a	47.7a	3.6a	0.6a	3.1a	4.4a	1.1a
		Experiment II											
		Gransol RZ						Cuarenteno					
BCH-FW	100	40.6ab	3.5a	0.4c	3.1b	4.2cd	1.1c	54.2a	4.0b	0.4a	2.4e	4.7a	1.1bc
	75	38.4abc	3.6a	0.8b	3.0b	4.2cd	1.0c	47.1bc	4.1b	0.5a	2.8d	4.4ab	1.2ab
	50	35.9cd	3.4a	1.2a	3.6a	5.5a	1.3abc	46.7bc	4.4a	0.6a	3.1cd	4.8a	1.4a
BCH-OMW	100	36.1cd	3.7a	0.8b	3.3ab	4.6b	1.1c	45.9bcd	4.1b	0.4a	4.0a	2.8d	0.7d
	75	41.8a	3.4a	0.8b	3.4a	4.5bc	1.5a	48.8b	3.6c	0.4a	3.9a	4.3b	1.2ab
	50	39.8abc	3.4a	0.9b	3.4a	4.7b	1.4a	48.5b	4.0b	0.5a	3.3bc	4.7a	1.2ab
HYD-FW	100	38.6abc	3.8a	0.8b	3.2ab	3.7d	0.7d	44.1cd	3.9b	0.6a	2.9d	4.3b	1.1bc
	75	33.5d	3.5a	1.0ab	2.8b	5.4a	1.0c	43.0d	4.5a	0.6a	2.6d	3.6c	0.9cd
	50	37.5bc	3.7a	0.9ab	2.9b	4.8b	1.0c	45.9bcd	3.9b	0.6a	2.8d	3.6c	0.9cd
Control (CF)	0	41.2ab	3.8a	1.2a	3.5a	5.0ab	1.2bc	48.0b	4.5a	0.6a	3.1cd	4.3b	1.1bc

^{z}BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir

Different letters in numerical columns differ at $P \leq 0.05$ (Tukey test)

Table 5. Effect of substrates based on two biochars, one hydrochar, and coir (control) on yield of *Solanum lycopersicum* cv. Gransol RZ and Cuarenteno. Experiment I with low doses (10; 25 % v:v) of chars. Experiment II with high doses (50; 75; 100 % v:v) of chars.

Material	Dose (%)	kg plant^{-1}	Nº fruits plant^{-1}	Fruit weight (g)	kg plant^{-1}	Nº fruits plant^{-1}	Fruit weight (g)
		Experiment I					
		Gransol RZ			Cuarenteno		
BCH-FW[z]	25	3.6a	18ab	208a	2.7a	23a	123a
	10	3.5a	19ab	193a	2.4a	21a	117a
BCH-OMW	25	3.6a	18ab	204a	2.8a	23a	119a
	10	3.6a	20a	181a	2.5a	22a	118a
HYD-FW	25	3.2ab	18ab	187a	2.5a	21a	119a
	10	2.9b	17b	178a	2.5a	21a	122a
Control (CF)	0	3.7a	20a	183a	2.6a	20a	131a
		Experiment II					
		Gransol RZ			Cuarenteno		
BCH-FW	100	3.1e	16e	199a	3.3f	36c	98de
	75	4.2d	23cd	180a	3.8ef	38bc	102bcde
	50	4.6cd	24bcd	191a	4.1cdef	39abc	107bcde
BCH-OMW	100	4.4cd	22d	198a	4.9abcd	42abc	117ab
	75	5.1abc	24bcd	215a	5.2ab	46a	113abcd
	50	5.4ab	26abc	208a	4.0def	41abc	96e
HYD-FW	100	4.7bcd	22d	216a	3.8ef	36c	106bcde
	75	4.6bcd	24bcd	198a	3.7ef	36c	103bcde
	50	4.7bcd	27ab	177a	4.9abc	43ab	114abc
Control (CF)	0	5.8a	29a	200a	5.0abc	46a	109bcde

[z] BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir

Different letters in numerical columns differ at $P \leq 0.05$ (Tukey test)

Table 6. Effect of substrates based on two biochars, one hydrochar, and coir (control) on fruit quality related parameters of *Solanum lycopersicum* cv. Gransol RZ and Cuarenteno. Experiment I with low doses (10; 25 % v:v).

		Acids and sugars					Taste derived-variables		
Material	Dose (%)	Malic acid (g kg^{-1})	Citric (C) acid (g kg^{-1})	Glutamic (G) acid (g kg^{-1})	Fructose (g kg^{-1})	Glucose (g kg^{-1})	Sucrose equivalents (SE) (g kg^{-1})	SE/C Ratio	SE/G Ratio
		Gransol RZ							
BCH-FW[z]	25	1.59a	5.77a	2.06a	18.08a	17.83a	44.47a	7.7a	21.6a
	10	1.54a	5.30a	2.01a	17.80a	17.64a	43.85a	8.3a	21.8a
BCH-OMW	25	1.37a	5.00a	1.78a	15.19a	15.09a	37.44a	7.5a	21.0a
	10	1.66a	5.60a	1.58a	16.60a	16.76a	41.11a	7.3a	26.0a
HYD-FW	25	1.28a	4.85a	1.61a	15.65a	15.40a	38.47a	7.9a	23.9a
	10	1.63a	5.28a	1.93a	17.42a	17.25a	42.90a	8.1a	22.2a
Control (CF)	0	1.29a	4.94a	1.80a	16.98a	17.54a	42.35a	8.6a	23.5a
		Cuarenteno							
BCH-FW	25	2.00a	4.44a	2.71a	14.97a	14.47a	36.60a	8.2a	13.5a
	10	2.07a	4.63a	2.59a	16.68a	15.33a	40.20a	8.7a	15.5a
BCH-OMW	25	2.03a	4.10a	2.46a	15.35a	13.66a	36.67a	8.9a	14.9a
	10	1.66a	3.72a	2.28a	12.94a	11.35a	30.78a	8.3a	13.5a
HYD-FW	25	2.02a	4.33a	2.42a	16.76a	15.60a	40.53a	9.4a	16.8a
	10	1.60a	4.10a	2.33a	13.33a	12.38a	32.22a	7.9a	14.0a
Control (CF)	0	1.74a	4.22a	2.56a	14.13a	13.00a	34.06a	8.1a	13.3a

[z] BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir

Different letters in numerical columns differ at $P \leq 0.05$ (Tukey test)

Table 7. Effect of substrates based on two biochars, one hydrochar, and coir (control) on fruit quality related parameters of *Solanum lycopersicum* cv. Gransol RZ and Cuarenteno. Experiment II with high doses (50; 75; 100 % v:v)

		Acids and sugars					Taste derived-variables		
Material	Dose (%)	Malic acid (g kg^{-1})	Citric (C) acid (g kg^{-1})	Glutamic (G) acid (g kg^{-1})	Fructose (g kg^{-1})	Glucose (g kg^{-1})	Sucrose equivalents (SE) (g kg^{-1})	SE/C Ratio	SE/G Ratio
						Gransol RZ			
BCH-FW^{z}	100	1.69b	5.25a	1.16a	16.77a	17.16a	41.71a	7.9a	36.0a
	75	2.05ab	4.77ab	1.35a	16.50a	16.58a	40.93a	8.6a	30.3a
	50	2.23ab	4.47ab	1.37a	16.82a	16.17a	41.06a	9.2a	30.0a
BCH-OMW	100	2.66a	5.45a	1.40a	19.42a	20.17a	48.52a	8.9a	34.7a
	75	2.22ab	4.75ab	1.40a	17.43a	16.58a	42.42a	8.9a	30.3a
	50	2.33ab	4.98ab	1.44a	16.52a	16.96a	41.12a	8.3a	28.6a
HYD-FW	100	2.12ab	4.00b	0.99a	15.74a	15.27a	38.53a	9.6a	39.3a
	75	2.32ab	4.50ab	1.17a	16.58a	16.09a	40.59a	9.0a	34.7a
	50	2.31ab	4.82ab	1.24a	18.14a	17.36a	44.22a	9.2a	35.7a
Control (CF)	0	2.19ab	4.15b	1.34a	17.09a	16.87a	42.05a	10.1a	31.4a
						Cuarenteno			
BCH-FW	100	2.10a	4.14a	2.09a	17.95a	18.18a	44.51a	10.8a	21.3a
	75	2.41a	4.30a	1.83a	18.35a	17.22a	44.48a	10.3a	24.3a
	50	2.13a	4.44a	1.83a	19.42a	18.07a	46.98a	10.6a	25.7a
BCH-OMW	100	2.34a	3.69a	1.85a	17.94a	15.50a	42.49a	11.5a	23.0a
	75	2.59a	4.39a	1.65a	19.50a	17.75a	46.87a	10.7a	28.4a
	50	2.32a	4.62a	2.14a	20.49a	19.66a	50.00a	10.8a	23.4a
HYD-FW	100	3.60a	3.28a	2.41a	17.10a	18.40a	44.20a	13.5a	18.3a
	75	2.28a	3.72a	1.78a	17.71a	16.34a	42.73a	11.5a	24.0a
	50	2.38a	3.62a	1.88a	17.91a	16.44a	42.93a	11.9a	22.8a
Control (CF)	0	2.52a	4.08a	1.91a	19.26a	18.36a	46.90a	11.5a	24.6a

^{z}BCH-FW = biochar from forest waste; BCH-OMW = biochar from olive mill waste; HYD-FW = hydrochar from forest waste; CF = coir

Different letters in numerical columns differ at $P \leq 0.05$ (Tukey test)