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# Biochar or Biochar-Compost Amendment to a Peat-Based Substrate Improves Growth of Syngonium podophyllum

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**Abstract:** Increasing demand for sustainable and low-cost alternatives to peat is a challenge in the production of container-grown plants. Biochar (BC) and compost, as eco-friendly materials, could be used to completely or partially substitute for peat. However, information regarding plant responses to the substitution is limited. This study evaluated effects of the amendment of a BC or a BC-compost mixture (BioComp) to a peat-based substrate at 20% by volume on the growth of *Syngonium podophyllum*. BC was pyrolyzed from wheat straw at 350 °C. Compost was made from farm green waste. BC or BioComp amendment elevated the pH and electrical conductivity of formulated substrates and improved plant growth. Concentrations of nitrogen, phosphorus, potassium, and chlorophyll in leaves and the net photosynthetic rate of plants grown in BC or BioComp amended substrates were significantly higher than those grown in the control substrate. Total soluble protein and total phenolic contents were greater in plants grown in BC- or BioComp-amended substrates as well, but no significant difference occurred in reactive oxygen-related enzymatic activities, reducing power or proline contents across substrates. Our results show that BC or BioComp can be used to replace 20% of peat by volume, and such replacement enhanced *S. podophyllum* growth.

Keywords: Biochar; compost; growing media; house plants; leaf gas exchange; peat; proline; substrate

# 1. Introduction

The production of container-grown plants has increased significantly [1,2]. For example, about 90% of greenhouse, nursery, and floriculture crops in the United States (U.S.) are produced in containers [2,3]. A distinct feature of container production is that plants are grown in confined volume filled with growing medium or substrate [4]. A major component of substrate is peat [5] due to its desirable physiochemical characteristics [6]. In Europe, the volume of organic substrate used for the nursery sector amounts to 34.6 million m<sup>3</sup> per annum, of which 27 million m<sup>3</sup> is composed of peat [7]. The use of peat as growing media, however, has caused ecological concerns [8] because peat is a non-renewable resource, and peatland is a sink of carbon dioxide [9,10]. As a result, peat has become costlier for commercial use [5,11], and there is an increasing need for alternative organic materials to replace or partially replace peat for the production of container plants [5,12,13].

One promising alternative to peat is biochar (BC) [5,13,14]. It is a charcoal-like solid with a high content in recalcitrant carbon created during pyrolysis of organic feedstock, such as crop residues, manure, and wood in an oxygen-limited environment at a temperature ranging from 300 to 900 °C [15]. BC is considered sustainable and environmentally friendly as it is carbon negative and derived from agricultural or forest residues [1,5,16]. BC has the potential to partially replace peat in growing

substrates. For instance, Méndez et al. [17] demonstrated positive effects of a peat and BC mixture at a 1:1 ratio by volume on lettuce yield compared to a similar amount of BC incorporated into a coir-based growing medium. Dispenza et al. [18] reported that incorporation of conifer BC (60% or 80% by volume) in a brown peat-based substrate increased the dry weight and canopy height of potted *Euphorbia* × *lomi* plants due to improved physiochemical characteristics of the substrate. Margenot et al. [19] found that softwood BC in a soilless substrate can completely replace peat at 70% (v/v) without pH adjustment for producing containerized marigold. Similarly, Guo et al. [20] demonstrated that pinewood BC produced at 450 °C can be used in a peat-based substrate for up to 80% (v/v) without negative effects on the growth and development of containerized Easter lily (*Lilium longiflorum*). The effects of BC on plant growth are promising but also variable depending on feedstock, pyrolysis temperature, residence time, and plant species [21].

Compost is another alternative to peat for formulation of substrates, which has been extensively studied [9,22,23]. Recent studies show that mixtures of BC with compost appear to have synergistic effects on container plant growth [1]. Indeed, the combination of compost and BC improves nutrient availability to plants and decrease incidence [24,25]. Zulfiqar et al. [1] reported that 10% BC mixed with 10% compost significantly increased the growth of *Dracaena deremensis* relative to standard peat-perlite-based growing media due to improved chemical properties of the substrate. Alvarez et al. [26] also reported enhanced shoot dry weight in geranium (*Pelargonium peltatum*) and petunia (*Petunia hybrida*) as a result of some combinations of BC with vermicompost.

Accumulating evidence suggests that substrates amended with BC or BC-compost mixture improve plant growth, but limited information is available on plant physiological responses to BC or BC-compost amendments [27]. In the present study, a peat-based substrate was amended with BC or BC-compost. Morphological, physiological, and biochemical responses of an important ornamental foliage plant, *Syngonium podophyllum*, to the formulated substrates were evaluated. Our results showed that the amendment of substrates with eco-friendly materials improved plant physiological parameters and ultimately enhanced plant growth.

# 2. Materials and Methods

## 2.1. Chemical and Physical Properties of Growth Substrates

Peat, perlite, BC, and green-waste-based compost were selected for formulating substrates. Imported peat and perlite were purchased from a reputable local nursery in Faisalabad, Pakistan. BC was pyrolyzed from crushed wheat straw at 350 °C for 3 h residence time using a muffle furnace (Gallonhop, England). The wheat straw was free of contaminants, such as metal, rubber, plastic, and stones. The pyrolyzed BC had a particle size of  $\leq 2$  mm. Compost was developed from on-farm green wastes which included grass clippings (30% by weight), garden litter (40% by weight), and dairy-farm wastes (containing cow manure and hay; (30% by weight)). All these components were well mixed in the proportion weight and sprayed with urea and water. Composting was done in a polyvinyl concrete trench ( $3 \times 4 \times 1$  m;  $W \times L \times D$ ), where the moisture content was regularly checked using a Hydrosense moisture meter (Campbell Scientific, Inc., Logan, UT, USA) to maintain a level of 50–60%. The compost was turned in 4 days intervals to maintain porosity. The composting procedure take two and half months. After composting, samples were taken for analysis. Nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca) were analyzed as described by Zulfiqar et al. [1]. Electrical conductivity (EC), pH, and cation-exchange capacity (CEC) were tested in triplicate using the methods mentioned blow.

Three growing media were formulated based on volume: (1) Control: 70% peat + 30% perlite, (2) BC: 50% peat + 30% perlite + 20% BC, and (3) BioComp: 50% peat + 30% perlite + 10% BC + 10% green-waste compost. The ratios of BC or BC-compost to peat and perlite were based on our previous work on the production of another foliage plant *Dracaena* [1]. The components were mixed in a rotary cement mixer to ensure homogeneity. The pH and EC of the formulated substrates were determined using a sample:water ratio of 1:10 (weight: volume) (AB 15 pH meter, Thermo Fisher Scientific,

Waltham, MA, USA; Digital EC meter, Lovibond Senso Direct 150, Dortmund, Germany) [28,29]. Substrate CEC was measured with NH<sub>4</sub>OAc/HOAc at pH 7.0 [30]. All measurements were performed in triplicate.

Physical properties of the three substrates, each with three replicates, were analyzed using the method described by Méndez et al. [17] and Nieto et al. [31]. A container of known volume with a tightly sealed drainage hole at the bottom was filled with a substrate. The substrate was slowly, but completely, saturated by gradually pouring water onto the surface and letting it percolate into the medium. The container of saturated medium was held over a watertight pan and the seal removed from the drain hole to allow water to freely drain for 10 min. The volume of drained water was measured to determine the volume of air space in the medium. The saturated and drained substrate was weighed, kept in an aluminum pan, and allowed to dry for 24 h at 105 °C. The weight of the dried substrate was measured, and the water-holding capacity was calculated as the amount of water that dried from the substrate. The total porosity was determined as the sum of the air space and the water-holding capacity.

## 2.2. Plant Material and Experimental Design

The experiment was conducted from mid-May to mid-September 2017 in a partially shaded glasshouse at the Floriculture Experimental Site, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. Tissue cultured liners of *Syngonium podophyllum* at the 2 to 3 true-leaf stage were locally purchased (Best Garden Nursery, Faisalabad, Pakistan). Each liner was carefully transplanted into a 4 L plastic pot (20 cm dia. top, 15.5 cm dia. base, 15.6 cm high) filled with the formulated substrates. The experiment was arranged in a completely randomized design with six replications. Potted plants were set out in 35% shading and arranged 30 cm apart. Plants were fertilized once per week using the Peters Professional 20-20-20 General Purpose fertilizer (Scotts, Maryville, OH, USA) with a N concentration of 200 mg L<sup>-1</sup>. Plants were also irrigated with water as needed so that they never showed drought symptoms. Irrigation water pH ranged from 6.2 to 6.6, while EC ranged from 0.42 to 0.60 dS m<sup>-1</sup>. The average monthly minimum temperature ranged from 16 to 18 °C, and the average monthly maximum temperature varied from 26 to 36 °C.

## 2.3. Morphological Evaluation

Plant growth parameters were recorded when plants reached a marketable size (about 30 cm height). Canopy height and number of leaves (>5 cm length) per plant were recorded prior to harvest. Plants were also graded for overall appearance and marketability based on scores (1–4): 1 = non-marketable (necrotic tissues with retarded growth), 2 = medium quality (small size with decolorized leaves); 3 = good quality/salable (medium size and no decolorization), 4 = high quality (large size without decolorized leaves). The plants were removed from the pots, and roots were washed with tap water to remove the substrate. The plant was separated into leaf and roots and weighed. Leaves and roots were dried at 70 °C for 72 h and weighted to a constant weight for assessing plant dry matter.

## 2.4. Physiological Parameters

Three days before harvesting, leaf gas exchange of three fully-expanded leaves on each plant was measured. A portable infrared  $CO_2/H_2O$  gas exchange system (LI-COR 6400, LI-COR, Lincoln, Nebraska, USA) set at 400 µmol m<sup>-2</sup> s<sup>-1</sup> CO<sub>2</sub> and 300 µmol m<sup>-2</sup> s<sup>-1</sup> flow rate was used to perform onsite measurements from 9:00 am to 11:00 am including net photosynthetic rate ( $P_N$ ), intercellular CO<sub>2</sub> concentration (*Ci*), transpiration rate (*E*), and stomatal conductance (*Gs*). For leaf chlorophyll analysis, fresh leaves were cut from the plant, sealed in plastic bags, and transported to the laboratory on ice. Chlorophyll (chl) a and b and carotenoid concentrations were determined following the method of Arnon [32] using 500 mg fresh leaf extracted overnight with 80% acetone and centrifuged at 10,000× *g* for 5 min. Total chlorophyll was measured as the sum of chl a and chl b. Leaf N concentration was

determined by the Kjeldhal method according to Jackson [33]. Leaf P and K concentration were determined according to Chapman and Pratt [34].

#### 2.5. Biochemical Parameters

Assay of total phenolic content (TPC). Fresh leaf (0.5 g) was extracted in 10 mL of 80% acetone and centrifuged at 10,000× g for 15 min. Leaf TPC was quantified in sample extracts at 765 nm following the method of Folin and Ciocalteu [35]. Briefly, 1 mL of sample was mixed with 4 mL of 20% (w/v) sodium carbonate and 5 mL of 10% Folin–Ciocalteu reagent (MP Biomedicals, LLc, lllkirch, France) followed by 1 h incubation. The absorbance was measured at 765 nm (Optizen POP, Mecasys Co., Ltd., Daejeon South Korea). TPC was expressed as gallic acid equivalents (GAEs) in reference to a calibration curve constructed using methanolic gallic acid solutions ranging from 0.01 to 0.10 mg mL<sup>-1</sup>. Finally, GAEs were calculated using the following formula:  $T = C \times V/M$ , where T: TPC, mg GAE/g plant extract; C: the concentration of gallic acid equivalents determined from the calibration curve, mg/mL; V: the volume of extract, mL; and M: the weight (g) of extract.

Antioxidant potential assay. Fresh leaf material (0.5 g) was extracted in 10 mL of 80% acetone and centrifuged at  $10,000 \times g$  for 15 min. The antioxidant potential of leaf extracts was measured on the basis of their scavenging activity against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to Yen and Chen [36] with slight modifications where 3 mL of leaf extract at different concentrations was added to 1 mL of 0.004% DPPH in methanol. The mixture was vigorously shaken and kept in the dark for 30 min. The absorbance was measured spectrophotometrically at 517 nm relative to a blank sample of methanol solution. The tests were carried out in triplicate. The percent of inhibition of DPPH radicals were calculated by following equation: DPPH Inhibition (%) = (absorbance of blank – absorbance of sample/absorbance of blank)  $\times 100$ .

Assay of reducing power (RP). The RP of leaf extracts was measured by direct electron donation in the reduction of Fe<sup>3+</sup> (CN)<sub>6</sub> to Fe<sup>2+</sup> (CN)<sub>6</sub> based on the method described by Yadav et al. [37]. Briefly, 1 mL of extract was mixed with 2.5 mL of 1% potassium Fe<sup>3+</sup> (CN)<sub>6</sub> and 2.5 mL 0.2 M phosphate buffer (pH 6.6), and the mixture was incubated at 50 °C for 20 min. Then, trichloroacetic acid (2.5 mL 1% w/v) was added, and the reaction mixture was centrifuged (Heraeus Fresco 17 Centrifuge, 133 Thermo Fisher Scientific Inc., Waltham, MA, USA) at 3000 × g for 10 min. The upper layer (2.5 mL) of supernatant was mixed with 0.5 mL 0.1% ferric chloride and 2.5 mL of deionized water. The absorbance was measured at 700 nm.

*Enzyme assays*. Each leaf sample (0.5 g) was homogenized in 4 mL of 0.05 M sodium phosphate buffer (pH 7.8) containing 2% (w/v) polyvinylpyrrolidone and 1.0 mM Ethylenediamine tetraacetic acid. The homogenate was centrifuged at 10,000× g for 17 min at 4 °C. The supernatant was used directly for the enzyme assays described below using a spectrophotometer (Optizen POP, Mecasys Co., Ltd., Daejeon South Korea).

*Catalase (CAT).* The CAT activity was assayed based on the change in absorbance at 240 nm in 0.1 mL of reaction mixture comprising 0.9 mL of 5.9 mM  $H_2O_2$ , 2 mL of 50 mM phosphate buffer with a pH at 7.0 and 0.1 mL of enzyme extract. The absorbance was read every 30 sec for 5 min to measure  $H_2O_2$  decomposition by CAT [38]. The enzyme activity was expressed as  $\mu$ mol min<sup>-1</sup> mg<sup>-1</sup> protein.

*Peroxidase (POD).* The POD activity was estimated following the method of Chance and Maehly [38]. Reaction mixtures contained 100  $\mu$ L of enzyme, 400  $\mu$ L of 20 mM guaiacol, 500  $\mu$ L of 40 mM H<sub>2</sub>O<sub>2</sub>, and 2 mL of 50 mM sodium phosphate buffer with a pH at 5.0. The absorbance of the mixture was read at 470 nm every 20 sec. The change in absorbance with time was used to calculate POD activity in  $\mu$ mol min<sup>-1</sup> mg<sup>-1</sup> protein.

Superoxide dismutase (SOD). SOD activity was estimated by Giannopolitis and Ries [39]. One mL reaction mixture comprising 50  $\mu$ L of nitroblue tetrazolium, 50  $\mu$ L riboflavin, 100  $\mu$ L L-methionine, 50  $\mu$ L of leaf extract, 100  $\mu$ L of triton-X, 250  $\mu$ L of buffer and 400  $\mu$ L of H<sub>2</sub>O were added in the test tube. The absorbance of the reaction mixture was read at 560 nm. The SOD was calculated in  $\mu$ mol min<sup>-1</sup> mg<sup>-1</sup> protein.

*Total soluble proteins (TSP).* Extraction of TSP was carried out following the method described by Sambrook and Russell [40] and determined according to the protocol described by Bradford [41]. Absorbance of a reaction mixture containing 200  $\mu$ L of leaf extract, 20  $\mu$ L of Coomassie blue dye, and 780  $\mu$ L of deionized water was read at 595 nm. The TSP was calculated in mg mL<sup>-1</sup>.

*Leaf free-proline content.* A ninhydrin-based method was used to measure leaf free-proline content [42]. Briefly, 0.5 g fresh leaf tissue was extracted in 10 mL of 3% sulfo-salicylic acid. Afterwards, 2 mL of the filtered solution was added to 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. Samples were incubated at 80 °C for 60 min and shifted to an ice bath to terminate the reaction. Then 4 mL of toluene was added to the solution and vigorously mixed by vortex for 30 sec. The chromophore-containing toluene was separated from the aqueous phase and the absorbance of the chromophore was read at 520 nm.

#### 2.6. Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using Minitab<sup>®</sup> 17 software package (LEAD Technologies Inc., Charlotte, NC, USA). If significance occurred among treatments, means were separated by Tuckey's honestly significant difference (HSD) test at p < 0.05 or 0.001 level.

# 3. Results and Discussion

## 3.1. Substrate Chemical Properties

Characterization of substrate components prior growing medium formulation and analysis of formulated substrates before their use for plant production are critically important as such effort generates essential information regarding the suitability of the formulated substrates for the growth of particular plants [43,44].

In this study, the BC had higher pH and carbon (C) contents than compost, whereas compost had much higher N, P, K, and Ca contents than BC (Table 1). The C:N ratio of compost was 24, suggesting that it was appropriate for commercial use since a compost with a C:N ratio of 25 or less is considered to be matured [22]. Due to the incorporation of BC, formulated substrates BC and BioComp had higher pH values than the control substrate (Table 1). This result was consistent with reports of other peat-based growing media where biochar amendment generally increased substrate pH [23,45,46]. Moreover, the elevated pH was within or close to the appropriate range of 5.3–6.5 recommended for container ornamental plants [47,48]. Specifically, Chen et al. [49] reported that the suitable pH for production of foliage plants such as Syngonium ranged from 5.5 to 6.5. The EC represents soluble salts in growing media [50]. The control substrate had an extremely low EC, only 0.03 dS m<sup>-1</sup>, compared to 0.47 dS m<sup>-1</sup> in BC and 1.04 in BioComp, indicating the control substrate had little available nutrients compared to the BC and BioComp substrates. The increased EC in BioComp was likely attributed to the higher content of N, P, K, and Ca ions in the compost. Although the recommended EC range for growing media is between 1.5 and 2.0 dS  $m^{-1}$  [51], lower EC may not be a great concern as substrates are generally fertilized after plants are transplanted. The CEC influences plant nutrient availability [14]. BioComp substrate had significantly higher CEC than both BC and control substrates (Table 1). The values are similar to those of a sewage-sludge biochar-based peat substrate used for Latuca sativa production [46].

Component	pН	EC (dS m <sup>-1</sup> )	CEC (cmol kg <sup>-1</sup> )	C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	P (g kg <sup>-1</sup> )	K (g kg <sup>-1</sup> )	Ca (g kg <sup>-1</sup> )
Peat	5.6	0.04	101.4	212	0.4	0.04	0.5	11.2
Biochar	8.4	2.2	115.0	635	2.1	1.05	3.95	0.91
Compost	7.7	3.8	-	303	12.5	4.7	11.4	3.3
Growing media								
Control	5.64 a	0.03 a	101.5 a					
BC	6.12 b	0.47 b	101.1 a					
BioComp	6.60 c	1.04 c	106.4 b					

Table 1. Chemical properties of biochar, compost, and growing media at the beginning of the experiment.

Notes: EC: Electric conductivity; CEC: cation exchange capacity; Control: peat:perlite (70:30 v/v); BC: peat/perlite/ biochar (50/30/20 v/v); BioComp: peat/perlite/biochar/compost (50/30/10/10 v/v); Values in column followed by different letters are significantly different at p < 0.05 based on the HSD-Tuckey test. Table shows the mean value (n = 3) for growing media analysis. "-" indicates that not determined.

#### 3.2. Substrate Physical Properties

Physical properties of growing media include bulk density, air space, water-holding capacity, and total porosity, which measure the distribution of air, water, and solid phases in relation to water status of the media [52]. With the addition of BC or BC-compost mixture, bulk density, air space, water-holding capacity, and total porosity significantly increased (Table 2). Although there are no universal standards for physical properties of growing media, Yeager et al. [53] reported that a substrate with an air space from 10% to 30%, a total porosity of 50–85%, and a water-holding capacity of 45–65% was appropriate for container plant production. Abad et al. [54] suggested that the ideal bulk density should be lower than 0.4 g cm<sup>-3</sup>. Similar ranges were also recommended by Chen et al. [44] for substrates used for foliage plant production. As shown in Table 2, the physical properties of the three substrates were within the recommended ranges for producing *Syngonium*.

Table 2. Physical properties of formulated three growing media.

Growing Media	Bulk Density (g cm <sup>-3</sup> )	Air Space (vol %)	Water-Holding Capacity (vol %)	Total Porosity (vol %)
Control	0.16 a	9.06 a	61.6 a	78.6 a
BC	0.22 b	13.76 b	64.0 b	82.6 b
BioComp	0.25 c	16.36 c	64.6 b	83.6 b

Notes: Mean values in column (n = 3) followed by different letters are significantly different (p < 0.05) using the Tuckey test.

#### 3.3. Plant Growth

*Syngonium* is an important ornamental foliage plant and priced at its growth form, leaf color, growth vigor, overall size, and appearance [44]. Plants grown in BC and BioComp substrates had increased canopy height, leaf number, leaf fresh-weight, and root fresh and dry weights compared to those grown in the control substrate (Table 3). Canopy height, leaf number, leaf fresh and dry masses, and root fresh and dry masses of plants grown in the BioComp substrate were significantly greater than those grown in the BC substrate. Even though plants grown in BC and BioComp were significantly larger, plant grading and the overall quality were not significantly different among the treatments.

Growing Media	Canopy Height (cm)	Number of Leaves	Leaf FM (g)	Leaf DM (g)	Root FM (g)	Root DM (g)	Plant Grade
Control	21.2 a	19.7 a	14.3 a	1.3 a	6.4 a	1.7 a	3.6 a
BC	24.4 b	24.3 b	16.4 b	2.0 a	9.0 b	2.4 b	3.6 a
BioComp	29.2 c	30.1 c	26.2 c	2.4 b	14.4 c	3.2 c	4.1 a

Table 3. Growth characteristics of Syngonium podophyllum cultivated in three growing media.

Notes: Mean values (n = 6) in column followed by different letters are significantly different (p < 0.05) using the Tuckey test. FM = fresh mass, DM = dry mass.

The higher biomass production in plants grown in BC or BioComp substrates was related to physiological responses of plants, including the enhanced uptake of mineral nutrients, elevated chlorophyll contents, and increased net photosynthetic rates. Leaf P and K contents of plants grown in BC were significantly higher than those grown in the control substrate, while N was significantly higher only in BioComp (Table 4). The higher tissue N, P, and K contents in plants were likely attributed to the higher concentrations of these elements in the substrate components mentioned above. In our previous experiment, substrates formulated with similar BC or BC-compost had N and P at least two times higher and K 34% higher than the control substrate formulated with peat and perlite [1]. As a result, plants grown in substrates amended with BC or BC-compost had increased leaf nutrient contents, whereas the relatively low tissue N in plants grown in BC substrate might be due in part to the binding ability of BC to N [55]. Tian et al. [56] reported that the available N decreased after the addition of 50% biochar in peat.

Table 4. Leaf N, P, and K contents (%) in Syngonium podophyllum produced in three growing media.

Growing Media	Ν	Р	K
Control	2.70 a	0.14 a	2.58 a
BC	2.42 a	0.32 b	2.87 b
BioComp	3.93 b	0.38 c	3.11 c

Notes: Mean values in column (n = 3) followed by different letters are significantly different (p < 0.05) using the Tuckey test.

Increased nutrient uptake was concomitant with increased chlorophyll contents in leaves of Syngonium grown in BC and BioComp substrates (Table 5). Chlorophyll a and total chlorophyll were highest in plants grown in BioComp substrate, followed by those grown in BC and control substrates. But chlorophyll b content was higher only in plants grown in BC substrate. Carotenoid contents did not respond to the amendments. Net photosynthetic rates of plants grown in the three substrates had the same trend as chlorophyll a: the highest in BioComp and the lowest in control substrates (Table 6). These results illustrate the importance of chlorophyll a, as it is the primary photosynthetic pigment. There was no significant difference in either stomatal conductance or transpiration rate among plants grown in the three substrates, which is similar to the findings of Guo et al. [57]. Intercellular CO<sub>2</sub> concentrations of plants grown in BioComp, BC, and control substrates were 144, 154, and 175  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, respectively, exhibiting an opposite trend of chlorophyll a and net photosynthetic rate. These results suggest that the difference in net photosynthetic rates is not due to the effect of stomatal conductance but is probably attributed to the activity of ribulose-l,5-bisphosphate carboxylase (RuBPC); i.e., the amendments may affect RuBPC activities of plants. Further research is needed to confirm this proposition. Syngonium is an important ornamental foliage plant in the family Araceae [48]; however, its photosynthetic characteristics appear to be different from its relative Spathiphyllum [58] in which photosynthesis was affected by both stomatal and biochemical factors.

Growing Media	Chlorophyll a (mg g <sup>-1</sup> FW)	Chlorophyll b (mg g <sup>-1</sup> FW)	Total Chlorophyll (mg g <sup>-1</sup> FW)	Carotenoids Content (mg $g^{-1}$ FW)
Control	4.3 a	4.5 a	8.8 a	8.3 a
BC	5.5 b	5.1 b	10.6 b	8.2 a
BioComp	6.34 c	4.9 a	11.2 c	8.5 a

Table 5. Chlorophyll and carotenoids contents of Syngonium podophyllum grown in different growing media.

Notes: Mean values in column (n = 6) followed by different letters are significantly different (p < 0.05) using the Tuckey test. FW = fresh weight.

Table 6. Physiological	characteristics of Syngonium	podophyllum cultivated in	different growing media.
	<i>J B</i>	1 1 3	0 0

Growing Media	<i>Pn</i> (µmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	Gs (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	<i>Ci</i> (µmol m <sup>-2</sup> s <sup>-1</sup> )	<i>E</i> (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )
Control	5.2 a	0.42 a	175 a	4.23 a
BC	6.3 a	0.44 a	154 b	4.40 a
BioComp	8.1 b	0.46 a	144 c	4.69 a

Note: Mean values in column (n = 6) followed by different letters are significantly different (p < 0.05) using the Tuckey test. Pn = net photosynthetic rate, Gs = stomatal conductance, Ci = intercellular CO<sub>2</sub> concentration, and E = transpiration rate.

#### 3.4. Biochemical Parameters

In addition to the evaluation of morphological and physiological characteristics, we also examined some biochemical parameters that are closely related to plant responses to stressful conditions. The total soluble protein (TSP) of plants grown in BC and BioComp substrates increased by 26% and 32%, respectively, compared to the control plants (Table 7). Total phenol contents (TPC) in plants produced in BC and BioComp substrates were 9% and 22% higher, respectively, in comparison to the control (Table 7). Such an increase in TPC is important, as TPC is linked with the development of scavenging systems for reactive oxygen species (ROS) in plants and thus can protect the plant from tissue oxidation by scavenging free radicals that might cause lipid peroxidation [59].

Table 7. Impact of different growing media on biochemical characteristics of Syngonium podophyllum.

Growing Media	TSP (mg mL <sup>-1</sup> )	TPC (mg GAE g <sup>-1</sup> )	DPPH (%)	SOD (µmol min <sup>-1</sup> mg <sup>-1</sup> protein)	POD (µmol min <sup>-1</sup> mg <sup>-1</sup> protein)	CAT (µmol min <sup>-1</sup> mg <sup>-1</sup> protein)	RP (%)	Leaf-Free Proline (µmol g <sup>-1</sup> FW)
Control	3.1 a	129.4 a	0.2 a	53.5 a	1.7 a	18.8 a	0.5 a	3.14 a
BC	4.2 b	143.0 a	0.2 a	54.0 a	1.5 a	16.8 a	0.5 a	3.20 a
BioComp	4.6 c	165.1 b	0.2 a	57.0 a	1.8 a	17.3 a	0.4 a	3.60 a

Notes: Mean values in column (n = 6) followed by different letters are significantly different (p < 0.05) sing the Tuckey test. RP = reducing power, FW = fresh weight.

It has been well documented that antioxidant capacity, measured as DPPH scavenging capacity, is linked with free radical scavenging and is, therefore, regarded as a marker for antioxidant potency. Our study revealed that DPPH scavenging in plants grown in the three substrates did not differ significantly (Table 7). Activities of SOD, POD, and CAT also failed to significantly respond to either BC or BioComp (Table 7). These enzymes represent the front line of plant protection against ROS under both biotic and abiotic conditions. Stressful conditions often lead to over accumulation of ROS, which trigger oxidative stress. The scavenging of ROS is then directly linked to the activity of antioxidant enzymes, such as SOD, POD, and CAT. The activities of these enzymes generally increase with the increase in stress severity [9,60,61]. Furthermore, reducing power was also not altered significantly in response to either BC or BioComp amendment (Table 7). Leaf-free proline content did not differ either, regardless of the growing media. The increased TPC, but the lack of increase in SOD,

POD, and CAT activities, as well as reducing power (RP) and proline content, may indicate that plants grown in BC or BioComp did not encounter significant stress.

# 4. Conclusions

In conclusion, the present study investigated physical and chemical properties of three peat-based substrates amended with or without BC or BioComp and their use for the production of an important ornamental foliage plant, *Syngonium*, and also evaluated morphological, physiological, and biochemical characteristics of the plant grown in the substrates. The amended substrates improved physical and chemical properties over the peat-based substrate. Plants grown in BC or BioComp substrates increased net photosynthetic rates and had a significantly higher biomass production. Furthermore, plants grown in the BC or BioComp substrates had significantly higher TSP and TPC but no significant increase in ROS-related detoxification activities, reducing power or proline concentration compared to those grown in the control substrate. As ornamental plant production is a heavy user of wetland peat, results from the present study indicate that BC and compost are valuable alternatives to peat and can be used to partially replace peat in container plant production, thus reducing peat mining and contributing to peatland preservation and conservation.

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# References

- 1. Zulfiqar, F.; Younis, A.; Abideen, Z.; Allaire, S.E.; Shao, Q. Evaluation of container substrates containing compost and biochar for ornamental plant *Dracaena deremensis*. *Pak. J. Agric. Sci.* **2019**, *56*, 613–621.
- 2. Judd, L.A.; Jackson, B.E.; Fonteno, W.C. Advancements in root growth measurement technologies and observation capabilities for container-grown plants. *Plants* **2015**, *4*, 369–392. [CrossRef] [PubMed]
- 3. USDA (U.S. Department of Agriculture). *Census of Agriculture*; 2007 Summary and State Data; USDA: Washington, DC, USA, 2009. Available online: http://www.agcensus.usda.gov/Publications/2007/Full\_Report/usv1.pdf (accessed on 6 August 2019).
- 4. Chen, J.; Wei, X. Controlled-released fertilizers as a means to reduce nitrogen leaching and runoff in container-grown plant production. In *Nitrogen in Agriculture-Updates*; Khan, A., Fahad, S., Eds.; InTechOpen: Rijeka, Croatia, 2018; pp. 33–52.
- Zulfiqar, F.; Allaire, S.E.; Akram, N.A.; Méndez, A.; Younis, A.; Peerzada, A.M.; Shaukat, N.; Wright, S.R. Challenges in organic component selection and biochar as an opportunity in potting substrates: A review. *J. Plant Nutr.* 2019, 42, 1386–1401. [CrossRef]
- 6. Li, Q.; Chen, J.; Caldwell, R.D.; Deng, M. Cowpeat as a substitute for peat in container substrates for foliage plant propagation. *HortTechnology* **2009**, *19*, 340–345. [CrossRef]
- Di Lonardo, S.; Baronti, S.; Vaccari, F.P.; Albanese, L.; Battista, P.; Miglietta, F.; Bacci, L. Biochar-based nursery substrates: The effect of peat substitution on reduced salinity. *Urban For. Urban Green.* 2017, 23, 27–34. [CrossRef]
- 8. Carlile, B.; Coules, A. Towards sustainability in growing media. Acta Hortic. 2013, 1013, 341–349. [CrossRef]
- Chrysargyris, A.; Antoniou, O.; Tzionis, A.; Prasad, M.; Tzortzakis, N. Alternative soilless media using olive-mill and paper waste for growing ornamental plants. *Environ. Sci. Pollut.* 2018, 25, 35915–35927. [CrossRef]
- Alvarez, J.M.; Pasian, C.; Lal, R.; Lopez-Nunez, R.; Fernandez, M. A biotic strategy to sequester carbon in the ornamental containerized bedding plant production. A review. J. Agric. Res. 2018, 16, e03R01. (In Spanish) [CrossRef]
- 11. Huang, L.; Niu, G.; Feagley, S.E.; Gu, M. Evaluation of a hardwood biochar and two composts mixes as replacements for a peat-based commercial substrate. *Ind. Crop. Prod.* **2019**, *129*, 549–560. [CrossRef]

- Chen, J.; McConnell, D.B.; Robinson, C.A.; Caldwell, R.D.; Huang, Y. Production and interior performances of tropical ornamental foliage plants grown in container substrates amended with composts. *Compost Sci. Util.* 2002, 10, 217–225. [CrossRef]
- 13. Huang, L.; Gu, M. Effects of biochar on container substrate properties and growth of plants—A review. *Horticulturae* **2019**, *5*, 14. [CrossRef]
- 14. Gruda, N.S. Increasing Sustainability of Growing Media Constituents and Stand-Alone Substrates in Soilless Culture Systems. *Agronomy* **2019**, *9*, 298. [CrossRef]
- 15. Yu, H.; Zou, W.; Chen, J.; Chen, H.; Yu, Z.; Huang, J.; Tang, H.; Wei, X.; Gao, B. Biochar amendment improves crop production in problem soils: A review. *J. Environ. Manag.* **2019**, *232*, 8–21. [CrossRef] [PubMed]
- 16. Woolf, D.; Amonette, J.E.; Street-Perrott, F.A.; Lehmann, J.; Joseph, S. Sustainable biochar to mitigate global climate change. *Nat. Commun.* **2010**, *1*, 56. [CrossRef] [PubMed]
- 17. Méndez, A.; Paz-Ferreiro, J.; Gil, E.; Gascó, G. The effect of paper sludge and biochar addition on brown peat and coir based growing media properties. *Sci. Hortic.* **2015**, *193*, 225–230. [CrossRef]
- 18. Dispenza, V.; De Pasquale, C.; Fascella, G.; Mammano, M.; Alonzo, G. Use of biochar as peat substitute for growing substrates of *Euphorbia× lomi* potted plants. *J. Agric. Res.* **2016**, *14*, 1–11. (In Spanish) [CrossRef]
- 19. Margenot, A.J.; Griffin, D.E.; Alves, B.S.; Rippner, D.A.; Li, C.; Parikh, S.J. Substitution of peat moss with softwood biochar for soil-free marigold growth. *Ind. Crop. Prod.* **2018**, *112*, 160–169. [CrossRef]
- 20. Guo, Y.; Niu, G.; Starman, T.; Gu, M. Growth and development of easter lily in response to container substrate with biochar. *J. Hortic. Sci. Biotechnol.* **2019**, *94*, 80–86. [CrossRef]
- 21. Li, S.; Harris, S.; Anandhi, A.; Chen, G. Predicting biochar properties and functions based on feedstock and pyrolysis temperature: A review and data syntheses. *J. Clean. Prod.* **2019**, *215*, 890–902. [CrossRef]
- 22. Chen, J.; McConnell, D.B.; Robinson, C.A.; Caldwell, R.D.; Huang, Y. Rooting foliage plant cuttings in compost-formulated substrates. *HortTechnology* **2003**, *13*, 110–114. [CrossRef]
- 23. Zhang, L.; Sun, X.Y.; Tian, Y.; Gong, X.Q. Composted green waste as a substitute for peat in growth media: Effects on growth and nutrition of *Calathea insignis*. *PLoS ONE* **2013**, *8*, 1–18. [CrossRef] [PubMed]
- 24. Barker, A.V.; Bryson, G.M. Comparisons of composts with low or high nutrient status for growth of plants in containers. *Commun. Soil Sci. Plant Anal.* 2006, 37, 1303–1319. [CrossRef]
- Kammann, C.I.; Schmidt, H.P.; Messerschmidt, N.; Linsel, S.; Steffens, D.; Müller, C.; Koyro, H.W.; Conte, P.; Joseph, S. Plant growth improvement mediated by nitrate capture in co-composted biochar. *Sci. Rep.* 2015, 5, 11080. [CrossRef] [PubMed]
- 26. Alvarez, J.; Pasian, C.; Lal, R.; López, R.; Fernández, M. Vermicompost and biochar as substitutes of growing media in ornamental-plant production. *J. Appl. Hortic.* **2017**, *19*, 205–214.
- 27. Álvarez, J.M.; Pasian, C.; Lal, R.; López, R.; Díaz, M.J.; Fernández, M. Morpho-physiological plant quality when biochar and vermicompost are used as growing media replacement in urban horticulture. *Urban. For. Urban Green.* **2018**, *34*, 175–180. [CrossRef]
- Rhoades, J.D. Salinity, electrical conductivity and total dissolved solids. In *Methods of Soil Analysis. Part 3. Chemical Methods*; Bigham, J.M., Ed.; Soil Science Society of America: Madison, WI, USA, 1996; pp. 417–435.
- 29. Thomas, G.W. Soil pH and soil acidity. In *Methods of Soil Analysis. Part 3. Chemical Methods*; Bigham, J.M., Ed.; Soil Science Society of America: Madison, WI, USA, 1996; pp. 475–489.
- Sumner, M.E.; Miller, W.P. Cation exchange capacity and exchange coefficients. In *Methods of Soil Analysis. Part 3. Chemical Methods*; Bigham, J.M., Ed.; Soil Science Society of America: Madison, WI, USA, 1996; pp. 1201–1229.
- 31. Nieto, A.; Gascó, G.; Paz-Ferreiro, J.; Fernández, J.M.; Plaza, C.; Méndez, A. The effect of pruning waste and biochar addition on brown peat based growing media properties. *Sci. Hortic.* **2016**, *199*, 142–148. [CrossRef]
- 32. Arnon, D.I. Copper enzyme in isolated chloroplast: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*. **1949**, 24, 1–15. [CrossRef]
- 33. Jackson, M.L. Soil Chemical Analysis; Constable and Co. Ltd.: London, UK, 1962.
- 34. Chapman, H.D.; Pratt, P.F. *Methods of Analysis for Soils, Plants and Water*; University of California, Division of Agriculture Science Riverside: Riverside, CA, USA, 1961.
- 35. Folin, O.; Ciocalteu, V. On tyrosine and tryptophane determinations in proteins. J. Biol. Chem. 1927, 73, 627–650.

- 36. Yen, G.C.; Chen, H.Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27–32. [CrossRef]
- 37. Yadav, M.; Yadav, A.; Yadav, J.P. In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from Eugenia jambolana Lam. *Asian Pac. J. Trop. Med.* **2014**, *7*, S256–SS261. [CrossRef]
- 38. Chance, B.; Maehly, A.C. Assay of catalases and peroxidases. Methods Enzymol. 1955, 2, 764–775.
- Giannopolitis, C.N.; Ries, S.K. Superoxide dismutase: I. Occurrence in higher plants. *Plant Physiol.* 1977, 59, 309–314. [PubMed]
- 40. Sambrook, J.; Russell, D.W. In vitro mutagenesis using doublestranded DNA templates, selection of mutants with DpnI. *Mol. Cloning* **2001**, *2*, 13–19.
- 41. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
- 42. Bates, L.S.; Waldren, R.P.; Teare, L.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
- Chavez, W.; Di Benedetto, A.; Civeira, G.; Lavado, R. Alternative soilless media for growing *Petunia x hybrida* and *Impatiens wallerana*: Physical behavior, effect of fertilization and nitrate losses. *Bioresour. Technol.* 2008, 99, 8082–8087. [CrossRef] [PubMed]
- 44. Chen, J.; McConnell, D.B.; Norman, D.J.; Henny, R.J. The foliage plant industry. Hortic. Rev. 2005, 31, 45–110.
- 45. Luo, J.; Fan, RQ.; Wang, T.; Gao, Y.; Liu, L.Z.; Yan, S.H.; Zhang, Z.H. Evaluation of spent pig litter compost as a peat substitute in soilless growth media. *Biol Agric. Hortic.* **2015**, *31*, 219–229. [CrossRef]
- 46. Méndez, A.; Cárdenas-Aguiar, E.; Paz-Ferreiro, J.; Plaza, C.; Gascó, G. The effect of sewage sludge biochar on peat-based growing media. *Biol. Agric. Hortic.* **2017**, *33*, 40–51. [CrossRef]
- 47. Bunt, A.C. *Media and Mixes for Container Grown Plants: A Manual on the Preparation and Use of Growing Pot Plants,* 2nd ed.; Unwin Hyman Ltd.: London, UK, 1988.
- 48. Abad, M.; Noguera, P.; Burés, S. National inventory of organic wastes for use as growing media for ornamental potted plant production: Case study in Spain. *Bioresour. Technol.* **2001**, *77*, 197–200. [CrossRef]
- 49. Chen, J.; McConnell, D.B.; Henny, R.J. The world foliage plant industry. Chron. Hortic. 2005, 45, 9–15.
- 50. Gascó, G.; Lobo, M.C.; Guerrero, F. Land application of sewage sludge: A soil columns study. *Water SA* **2005**, *31*, 309–318. [CrossRef]
- Abad, M.; Noguera, P.; Puchades, R.; Maquieira, A.; Noguera, V. Physico-chemical and chemical properties of some coconut coir dusts for use as a peat substitute for containerized ornamental plants. *Bioresour. Technol.* 2002, *82*, 241–245. [CrossRef]
- 52. Michel, J.C. Physical properties of growing media: State of the art and future challenge. *Acta Hortic.* 2007, *819*, 65–71. [CrossRef]
- 53. Yeager, T.; Guilliam, C.; Bilderback, T.E.; Fare, D.; Niemiera, E.; Tilt, K. *Best Management Practices Guide for Producing Container-Grown Plants*; Southern Nursery Association: Atlanta, GA, USA, 1997.
- 54. Abad, M.; Fornes, F.; Carrion, C.; Noguera, P.; Maguieira, A.; Puchades, R. Physical properties of various coconut coir dusts compared to peat. *HortScience* 2005, *40*, 2138–2144. [CrossRef]
- 55. Altland, J.; Locke, J. Biochar affects macronutrient leaching from a soilless substrate. *HortScience* **2012**, 47, 1136–1140. [CrossRef]
- 56. Tian, Y.; Sun, X.; Li, S.; Wang, H.; Wang, L.; Cao, J.; Zhang, L. Biochar made from green waste as peat substitute in growth media for *Calathea rotundifola* cv. *Fasciata. Sci. Hortic.* **2012**, *143*, 15–18. [CrossRef]
- 57. Guo, Y.; Niu, G.; Starman, T.; Volder, A.; Gu, M. Poinsettia growth and development response to container root substrate with biochar. *Horticulturae* **2018**, *4*, 1. [CrossRef]
- 58. Chen, J.; Wang, Q. Variation in photosynthetic characteristics and leaf area contributes to *Spathiphyllum* cultivar difference in biomass production. *Photosynthetica* **2003**, *41*, 443–447.
- Scherer, R.; Lemos, M.F.; Lemos, M.F.; Martinelli, G.C.; Martins, J.D.L.; da Silva, A.G. Antioxidant and antibacterial activities and composition of Brazilian spearmint (*Mentha spicata* L.). *Ind. Crop. Prod.* 2013, 50, 408–413. [CrossRef]

- 60. Foyer, C.H.; Noctor, G. Ascorbate and glutathione: The heart of the redox hub. *Plant Physiol.* **2011**, *155*, 2–18. [CrossRef] [PubMed]
- Chrysargyris, A.; Xylia, P.; Botsaris, G.; Tzortzakis, N. Antioxidant and antibacterial activities, mineral and essential oil composition of spearmint (*Mentha spicata* L.) affected by the potassium levels. *Ind. Crop. Prod.* 2017, *103*, 202–212. [CrossRef]



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