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Wood-Based Biochar Ratio Used for Partial Peat Replacement in Growing Media for *Antirrhinum majus* Pot Production

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Abstract: Biochar has been promoted mostly as a soil supplement that improves plant growth/yield and to a lesser extent as a growing medium component. The alarmed situation for peat substitution in growing medium renders biochar as a promising substitute for current research. In this study, biochar derived by wood-based materials was evaluated at different ratios (0, 5, 10, 15, and 20% *v*/*v*) for peat partial substitution for *Antirrhinum majus* pot production. Biochar had increased potassium content and pH, which affected the growing media properties (total pores space and water filled capacity) and decreased nitrogen and phosphorus content in the media. Adding ≥15% biochar increased plant height and decreased flowering, but no effect was observed on plant biomass produced. The presence of biochar increased the total phenols and flavanols content and antioxidant capacity, with greater effects at the higher biochar rates used. This resulted in lipid peroxidation and an increase in hydrogen peroxide content, causing oxidative stress. Potassium and magnesium accumulated more but nitrogen and phosphorus were accumulated less in snapdragon leaves. Biochar at 10% can be considered as a successful candidate to partially substitute peat, and efforts to improve growing media characteristics are required for *A. majus* pot production.

Keywords: biochar; peat; growth; snapdragon; mineral accumulation

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1. Introduction

Horticultural, ornamental and herbs seedlings, and potted plants are typically produced under nursery conditions, with soilless growing media, typically composed by inorganic and organic materials in various ratios to succeed the most appropriate physico-chemical characteristics fitted to each plant species [1,2]. Peat is as a non-renewable natural resource and has increased extraction and transportation cost as well as various associated environmental constraints, meaning that there is a need for the identification of alternative materials for growing media preparation [2,3]. Peat was the most important component of growing media, and even now much of the growing media is based on peat-based mixtures enriched with minerals, although in recent years other materials such as wood fiber, composted greenwaste, and bark are being added to dilute the peat [4,5]. The peat volume within Europe can be calculated to approximately 800 billion metric tons [5], while the amount of peat being used until recently is around 11 million metric tons yearly [6]. However, peat bogs are important habitats, carbon sinks, fragile ecosystem, and harvesting peat is environmentally unfriendly as it leads to CO_2 emissions [7,8]. Multiple countries in Europe have implemented national or regional initiatives to phase out peat usage in horticulture by 2030 [2].

In recent years interest in the use of biochar (a solid, carbonaceous, charcoal like material) has increased as evidenced by publications in this area [9]. Biochar has a high content of recalcitrant carbon and is accepted as a sustainable and ecological material, storing organic C, reducing CO_2 and N_2O emissions, and improving soil inorganic N

retention [10] as well as having decreased runoff pollutants [11]. Biochar has high porosity, low density, and high affinity for cations change [5,6], which render its use as a component in various growing media preparations. However, the biochar properties depend on feedstock and processing (e.g., temperature, residence time, etc.) [10,12]. As the temperature rises, biochars become more fragrant and carbonized [10]. This increases biochar pH, electrical conductivity, ash content, surface area, and total pore space while lowering water extractable organic content [13,14]. The particle size of biochar might has adverse effects on soil biota and could become a pollutant carrier [15] and affect the physicochemical characteristics of the biochar material [16]. Replacing peat moss with biochar as a container substrate largely reduces peatland restoration costs because biochar production does not degrade the peatland ecosystem [9].

The addition of biochar to peat has given variable results with expanding research interest to assess the advances of adding biochar into a growing media of several horticultural, ornamental, and medicinal crops with partially or entire peat substitution [1,17–21]. For example, there have been positive responses, negative responses, and no response depending not only on the biochar type and ratio in the growing media but also depending on the crop species and plant growth stage and even on the type of peat used [17,18,22]. The biochar particle size can affect the nutrient availability to plants, as finer fractions of biochar increased electrical conductivity and nutrient availability, especially potassium in the growing media [16]. Generally the negative effect of biochar is more pronounced to young seedlings rather than to the plants that are transplanted at a mature phase, whereas biochar phytotoxicity to young plants might be related to the organic compounds, salinity, and/or heavy metals [23]. The rate of dilution with positive or no detrimental effect is in the region <20% by volume especially for young seedlings as a result of increased nutrients availability, decreased nutrient and water losses as well as ameliorate peat acidity [17,19,24]. Notably, other studies suggested the complete replacement of peat by biochar derived from softwood for marigold (*Tagetes erecta* L.) growth [1]. The response of biochar is different depending on the crop species as well as the developmental stage, i.e., seedlings or potted plants. For example, in 3-year-old *Aloe arborescens* Mill. plants (mature plants), the complete replacement of peat by biochar in potting plants accelerated secondary metabolites [20].

Studies with the growing media and effects on plants development are minimally reported when biochar is used, while information on the plant morphological, physiological, and biochemical effects is often not reported. Since the biochar material has a high pH and contains nutrients, e.g., potassium, this is often not considered in the nutrient management of the plants grown in biochar-based growing media. Consequently, the present study investigated the effect of a well characterized biochar at various rates on plant growth, plant physiology, nutrient accumulation, and antioxidant capacity of snapdragon (*Antirrhinum majus* L.) during its growth under nursery conditions and before transplanting to the final place (i.e., ornamental pots, soil, etc.). Moreover, the changes in physicochemical parameters of the different substrates before plant transplanting and at the end of the cropping period were evaluated.

2. Materials and Methods

2.1. Biochar, Peat, and Plant Material

The biochar material (named as Terra) was selected from Germany. The raw material used was mostly forest wood-based materials, i.e., beech, spruce, and pine. Biochar was generated by using the Schotteredorf process at 700 °C with a retention time of 15–30 min. Due to business sensitivity, additional data about the biochar production process are not available. The chemical composition of biochar was determined as reported previously [25] and likewise for pH [26], Electrical Conductivity (EC) in a water extract at a 1:5 (*v*:*v*) ratio [27], calcium chloride/DTPA (CAT) extractable (1:5 *v*:*v*) potassium (K) and phosphorus (P), ammonium (NH_4-N) levels, nitrate (NO_3-N) levels, as well as total extractable N (NH_4^+-N+NO_3^--N) [26]. In brief, Biochar had a pH of 9.57; EC of 0.611 mS/cm; P of 2 mg/L, and K of 1085 mg/L; a negligible amount of nitrates and ammonium, N. Moreover, biochar had

64.1%, 35.4%, and less than 1.0% particle sizes of <1 mm, 1–5 mm, and >5 mm, respectively, considered as a fine material.

A commercial peat (standard professional grade H4–H5 on von Post scale, Gebr. Brill Substrate GmbH & Co. KG, Georgsdorf, Germany) was used as the reference/control and as basic material to which the biochar was added.

Seedlings of *A. majus* (L.), commonly known as snapdragon, were purchased from a commercial nursery at the growing stage of 1-true leaf.

2.2. Experimental Set up and Preparation of Growing Media

The study implemented at the greenhouse infrastructures of the Cyprus University of Technology, Cyprus. The multi-spam greenhouse was equipped with an automated climate control system.

Biochar was mixed into the fertilized peat in different ratios of 0%, 5%, 10%, 15% and 20% (*v*/*v*), while peat was served as a control treatment. Biochar levels were based on previous reports and/or preliminary trials [17,19]. Minerals contribution derived by the different biochar levels were subtracted by the fertilized peat. Peat was fertilized by using commercial fertilizers (Novatec, simple superphosphate, and potassium sulfate) to reach adequate levels of N, P, and K of 95, 30, and 105 mg/L, respectively. Moreover, peat was limed by using dolomitic lime at 4 g/L, and a relevant amount of trace elements was added. There were almost insignificant amounts of N, some P, and an excess of K in most cases. No K was added into the mixture in case of K excess. In each mixture, 10% (*v*/*v*) of perlite was added to improve aeration and drainage. Prior to seedlings' transplanting, all the mixtures were sampled and analyzed for their physicochemical features.

The five different growing mixtures were used to fill up plastic pots (0.5 L) before the seedling's transplanting. Each pot had a single seedling, while each mixture was tested with nine replicate pots. Seedlings were irrigated every other day with the same volume of water for all treatments, which adjusted accordingly throughout the growing period, while no fertilizers, or pesticides were used. The pots were placed on plastic trays to retain the drained solution after irrigation. The experimental design was completely randomized. During the cultivation period, temperature, humidity, and light conditions were recorded and averaged 19.5 °C, 60.1%, and 1317.3 Lux, respectively.

2.3. Growing Media Properties

The physicochemical properties of different growing media were evaluated. The growing media total porosity (TP), air-filled porosity (AFP), available water-holding capacity (AWHC), and bulk density (BD) by volume were assessed according to the European Standard EN 13,041 [28], as described previously [29]. One portion of growing media was mixed with five portions (1:5 *v*/*v*) of distilled water, and the extracts were used to measure the pH and the EC. Organic matter content and carbon (both expressed in percentage) were calculated after the media had been burnt at 550 °C in an ash furnace [17]. After the ash samples had been acid digested (2 N HCl), the produced extracts, were subjected to analysis of macronutrients, using a flame photometer (Lasany Model 1832, Lasany International, Panchkula, India), for K and sodium (Na) determination, while P was measured by spectrophotometry (Multiskan GO, Thermo Fisher Scientific, Waltham, MA, USA). The contents of magnesium (Mg) and calcium (Ca) were determined by an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK). Nitrogen was measured by the Kjeldahl method (BUCHI, Digest Automat K-439 and Distillation Kjelflex K-360, Flawil, Switzerland) as described previously [29]. Data were expressed in g/kg of dry weight. The carbon to nitrogen (C/N) ratio was also measured, indicating the organic matter decomposition. Moreover, growing media properties were tested by a postharvest analysis of substrates, collecting root free substrates.

2.4. Plant Growth and Mineral Content

Snapdragon, as a potting plant, was grown for four weeks under nursery conditions. At the end of the cultivation period, plant growth-related parameters were assessed in six plants/treatment. Plant height and the number of flowers produced of each plant were measured. Leaf chlorophyll fluorescence (chlorophyll fluorometer, opti-sciences OS-30p, UK) was measured on two fully developed, light-exposed leaves per seedling, from the same plants used for growth measurements. Leaves were incubated in the dark for 20 min prior to Fv/Fm measurements. Leaf chlorophyll content was assayed in six replicates/treatment either by SPAD meter or photometrically. Chlorophylls were extracted with dimethyl sulfoxide (DMSO) and Chlorophyll a (Chl a), Chlorophyll b (Chl b), and total Chlorophyll (total Chl) content was determined [25]. The ratios of Chl a/Chl b as well as carotenoids/total Chls were computed. Plants were sampled above substrate surface, the upper plant part was weighed (Fw; g), dried at 65 °C, then dry weight (Dw; g) was measured, and dry matter (DM; %) content was computed.

Mineral content in the upper plant biomass (including leaves and stems) was determined on four replicates/treatment (two pooled plants/replicate). Plant tissue was dried to constant weight (at 65 °C for 3 d) and sub samples (~0.6 g) were ashed (at 500 °C for 5 h) and acid (2 N HCl) digested [30]. Nitrogen content was determined with the Kjeldahl (BUCHI, Digest automat K-439 and Distillation Kjeldahl K-360) digestion method. Phosphorus content was determined with a spectrophotometer (Multiskan GO, Thermo Fischer Scientific, Oy, Vanda, Finland), and K, Mg, Ca, and Na content was determined by an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK) for plant tissue analysis. Plant mineral content was expressed in g/kg of dry weight.

2.5. Total Phenolics, Total Flavonoids, Total Flavanols, and Antioxidant Activity

For every treatment, six fresh leaf biological samples (derived from different plants) were processed with methanolic extraction [31]. Extracts were analyzed for the antioxidant activity by three methods (ferric-reducing antioxidant power- FRAP; 2,2-diphenyl-1-picrylhydrazyl -DPPH; and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid -ABTS). The extracts were also used to determine the total amount of phenolic, flavonoid, and flavanol content.

Total phenols were quantified by using the methanolic extracts with the Folin–Ciocalteu method (at 755 nm; using a microplate spectrophotometer Thermo Scientific, Multiskan GO, Waltham, MA, USA), as described previously [32]. Results were expressed in gallic acid equivalents (mg GAE/g of fresh weight-Fw). The content of total flavonoids was determined based on the aluminum chloride colorimetric method [33] as modified in Chrysargyris et al. [30]. The absorbance was measured at 510 nm. Results were expressed in rutin equivalents (mg rutin/g Fw). Total flavanol content was determined by measuring the absorbance of the reaction mixture at 500 nm according to Tabart et al. [34]. Results were expressed as catechin equivalents (mg catechin/g Fw).

Free radical scavenging activity was determined as described previously [35]. Briefly, DPPH radical scavenging activity of the plant methanolic extracts was measured at 517 nm, FRAP activity was measured at 593 nm as described in Chrysargyris et al. [35]. The ABTS assay was implemented according to the methodology described by Woidjylo et al. [36]. Results were expressed as Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalents (mg trolox/g Fw).

2.6. Lipid Peroxidation and Hydrogen Peroxide Content

The content of hydrogen peroxide (H_2O_2) was determined by employing the method described by Loreto and Velikova [37] from six biological samples (derived from different plants) for each treatment. The absorbance of samples and standards (5 to 1000 μM of H_2O_2) were measured at 390 nm and results were expressed as μmol H_2O_2/g fresh weight.

Lipid peroxidation was assessed according to De Azevedo Neto et al. [38] and measured in terms of malondialdehyde (MDA) content. The absorbance was determined at

532 nm and corrected for non-specific absorbance at 600 nm. MDA amount was determined using the extinction coefficient of 155 mM/cm. Results were expressed as nmol of MDA/g fresh weight.

2.7. Statistical Analysis

Measurements were performed in four to six biological replications/treatment. Statistical analysis was carried out using the SPSS statistical software (SPSS v.22; IBM, Armonk, New York, NY, USA). Data means were also compared with one-way analysis of variance (ANOVA) and Duncan's multiple range test was used for the comparison of treatment means at $p < 0.05$. The correlation coefficients were between biochar ratios with individual parameters tested by Pearson's correlation test.

3. Results

The physicochemical growing media characteristics before the seedling transplanting are presented in Table 1. Biochar ≥10% increased growing media pH (up to 1.4 pH units) while biochar ≥15% decreased EC values compared to the control (peat) or lower biochar levels. Biochar addition increased organic matter content, organic C levels, and the C/N ratio compared to peat, with more profound effects at the high biochar levels. Nitrogen, Ca, P, and Na content were decreased but K content increased at the high biochar ratios. Magnesium content was varied among the treatments, revealing the highest levels at the biochar 15% treatment. Biochar presence at ≥10% decreased the total pore space, the air-filled porosity, and the available water-holding capacity, but increased the bulk density of the growing media.

Table 1. Growing media (peat and biochar-B), showing its physicochemical properties before plant transplantation.

	Peat 100%	**B 5%**	**B 10%**	**B 15%**	**B 20%**
pH	6.32 ± 0.18 c	6.37 ± 0.11 c	6.98 ± 0.02 b	7.47 ± 0.03 a	7.76 ± 0.03 a
EC (mS/cm)	0.84 ± 0.05 b	0.96 ± 0.03 a	0.84 ± 0.02 b	0.68 ± 0.05 c	0.69 ± 0.02 c
Organic matter (%)	72.39 ± 1.29 c	87.73 ± 0.06 b	90.29 ± 1.08 ab	87.82 ± 0.73 b	90.85 ± 0.30 a
Organic C (%)	41.99 ± 0.74 c	50.89 ± 0.03 b	52.37 ± 0.63 ab	50.94 ± 0.42 b	52.70 ± 0.17 a
C/N ratio	50.37 ± 2.13 c	79.37 ± 3.50 b	95.87 ± 4.25 a	84.51 ± 3.86 b	97.43 ± 2.75 a
N (g/kg)	8.35 ± 0.20 a	6.44 ± 0.29 b	5.48 ± 0.19 c	6.05 ± 0.24 bc	5.42 ± 0.14 c
K (g/kg)	2.04 ± 0.05 c	2.70 ± 0.12 b	2.25 ± 0.05 c	3.40 ± 0.20 a	3.13 ± 0.09 a
P (g/kg)	1.13 ± 0.04 ab	1.25 ± 0.07 a	1.02 ± 0.04 b	0.87 ± 0.04 c	0.83 ± 0.03 c
Ca (g/kg)	15.02 ± 0.57 a	13.19 ± 0.43 b	10.85 ± 0.28 c	12.80 ± 0.49 b	11.48 ± 0.18 c
Mg (g/kg)	0.8 ± 0.05 ab	0.74 ± 0.04 bc	0.66 ± 0.02 c	0.86 ± 0.03 a	0.79 ± 0.02 ab
Na (g/kg)	0.97 ± 0.03 a	0.76 ± 0.03 bc	0.70 ± 0.03 c	0.81 ± 0.03 b	0.78 ± 0.02 bc
Total porosity (% *v*/*v*)	84.97 ± 0.76 ab	95.89 ± 6.52 a	76.28 ± 4.56 b	72.89 ± 3.77 b	76.97 ± 3.42 b
Air filled porosity (% *v*/*v*)	18.43 ± 1.00 ab	26.19 ± 3.47 a	16.38 ± 3.55 b	14.47 ± 1.69 b	16.95 ± 1.96 ab
Bulk density (g/cm^3)	0.15 ± 0.000 c	0.17 ± 0.010 c	0.20 ± 0.009 b	0.22 ± 0.009 a	0.25 ± 0.003 a
Container capacity (% *v*/*v*)	66.55 ± 1.76 ab	69.7 ± 3.42 a	59.9 ± 1.84 bc	58.42 ± 2.09 c	60.02 ± 1.5 bc

Total porosity (TP), available water-holding capacity (AWHC-container capacity), air-filled porosity (AFP), and bulk density (BD) by volume. Values are mean ± standard error ($n = 4$). In each row, values followed by the same letter do not differ significantly at $p < 0.05$.

Samples of the growing media were taken after the end of the plant cultivation in order to determine the changes in the media properties (Table 2). The growing media pH was raised as the biochar levels were increased in the media. Organic matter and organic C were decreased at 20% biochar in comparison to peat (control) or lower biochar levels.

The content of N, P, Ca, Mg, and Na was reduced after the biochar addition in the growing media, and this reflected the decreased EC of the media, while K levels were unchanged among the tested growing media after the end of the cultivation.

Table 2. Growing media (peat and biochar-B), showing its physicochemical properties after plant transplantation.

	Peat 100%	**B 5%**	**B 10%**	**B 15%**	**B 20%**
pH	6.59 ± 0.02 d	7.02 ± 0.02 c	7.07 ± 0.04 c	7.45 ± 0.02 b	7.54 ± 0.03 a
EC (mS/cm)	1.32 ± 0.09 a	0.91 ± 0.09 b	1.06 ± 0.01 b	0.97 ± 0.01 b	1.04 ± 0.04 b
Organic matter (%)	74.14 ± 1.02 a	78.30 ± 2.51 a	82.75 ± 0.78 a	82.02 ± 2.13 a	65.49 ± 4.58 b
Organic C (%)	43.00 ± 0.59 a	45.42 ± 1.45 a	47.99 ± 0.45 a	47.57 ± 1.23 a	37.98 ± 2.66 b
C/N ratio	47.11 ± 3.85 c	59.88 ± 5.69 bc	68.78 ± 3.76 b	85.84 ± 5.88 a	68.06 ± 2.17 b
N (g/kg)	9.22 ± 0.59 a	7.68 ± 0.49 b	7.05 ± 0.47 b	5.58 ± 0.26 c	5.57 ± 0.22 c
K (g/kg)	2.47 ± 0.30 a	3.28 ± 0.43 a	3.14 ± 0.19 a	3.03 ± 0.29 a	3.06 ± 0.10 a
P (g/kg)	1.83 ± 0.08 a	1.49 ± 0.04 bc	1.57 ± 0.02 b	1.33 ± 0.06 c	1.14 ± 0.07 d
Ca (g/kg)	20.48 ± 0.31 a	18.90 ± 0.70 ab	18.49 ± 0.72 bc	16.98 ± 0.47 cd	15.59 ± 0.39 d
Mg (g/kg)	1.66 ± 0.03 a	1.53 ± 0.05 ab	1.53 ± 0.10 ab	1.34 ± 0.06 bc	1.30 ± 0.05 c
Na (g/kg)	2.48 ± 0.16 a	2.55 ± 0.23 a	2.46 ± 0.10 a	1.84 ± 0.12 b	1.73 ± 0.05 b

Total porosity (TP), available water-holding capacity (AWHC-container capacity), air-filled porosity (AFP), and bulk density (BD) by volume. Values are the mean ± standard error (n = 4). In each row, values followed by the same letter do not differ significantly at $p < 0.05$.

The main variations in the attributes from the beginning to the end of the crop duration are given in Figure S1. Phosphorus, Mg, Ca, and Na were accumulated in all treatments with biochar and in peat too, at the end of the cultivation, most likely owing to mineral release from the medium, and/or the accumulations of Ca and Na from the irrigation water. This release was also reflected in the increased EC values after the end of the cultivation period. At the end of the cultivation period, the high ratios of the biochar at 15–20% had less K and less N in case of biochar 15%, which can be attributed to the lower N levels at the initial material (Table 1). This can also be reflected in the decreased organic matter levels and C/N ratio after the decomposition process of the organic matter to produce a more stable material (decreased C/N). The final pH was increased at growing media of ≤15% biochar (Figure S1).

Biochar at ≥15% stimulated plant height and prevented the production of flowers, as plant continued the vegetative growth rather than the reproductive one, compared to the plants grown in peat (control) (Table 3). Interestingly, even at low biochar levels of 5–10%, the production of flowers was decreased almost to 50% compared to the control. No differences were found between fresh and dry plant biomass and, consequently, to the dry matter content among the treatments.

Biochar presence in the growing media affected plant photosynthetic performance, as biochar ≥10% decreased the chlorophyll fluorescence and SPAD, compared to the plants grown in biochar free growing media (peat) (Table 4). Indeed, no major effects were found on the Chlorophyll a and b content and as a result on the total chlorophylls levels, neither on the total carotenoids among the biochar-based and control treatments. The ratios of Chla/Chlb were above 1 (averaged in 2.74), while the ratios of carotenoids/total Chls were below 1 (averaged in 0.17).

Table 3. The effect of growing media (peat, biochar-B) on the snapdragon seedlings' height (cm/plant), flower number, upper part fresh weight (Fw; g/plant), dry weight (Dw; g/plant), and dry matter (DM) content (%) on plants grown in the greenhouse/nursery.

	Height	Flowers	Fresh Weight	Dry Weight	DM(%)
Peat 100%	16.75 ± 0.48 b	3.00 ± 0.00 a	11.40 ± 0.37	1.23 ± 0.02	11.13 ± 0.13
B 5%	17.75 ± 0.52 ab	1.50 ± 0.50 b	12.98 ± 0.65	1.60 ± 0.03	12.33 ± 0.33
B 10%	18.50 ± 0.94 ab	1.50 ± 0.50 b	10.23 ± 1.21	1.39 ± 0.43	12.03 ± 0.29
B 15%	19.17 ± 0.60 a	0.00 ± 0.00 c	10.71 ± 1.10	1.49 ± 0.10	11.57 ± 0.43
B 20%	19.25 ± 0.78 a	0.00 ± 0.00 c	10.25 ± 1.22	1.45 ± 0.37	15.63 ± 2.66

Values are mean ± SE (n = 6). Values in the columns that are followed by the same letter are not significantly different ($p < 0.05$).

Table 4. Effect of growing media (peat, biochar-B) on the snapdragon seedlings' chlorophyll fluorescence (Fv/Fm), SPAD values, chlorophyll levels (Chl a, Chl b, total Chls; mg/g Fw), carotenoid (mg/g Fw) content, and on ratios of Chl a/Chl b and carotenoids/total Chls on plants grown in a greenhouse/nursery.

	Chlorophyll Fluorescence	SPAD	Chl a	Chl b	Total Chls	Carotenoids	Chla/Chlb	Carotenoids/Total Chls
Peat 100%	0.85 ± 0.027 a	47.10 ± 0.38 a	0.57 ± 0.12	0.20 ± 0.03 ab	0.77 ± 0.15	0.13 ± 0.03	2.77 ± 0.162	0.17 ± 0.003
B 5%	0.81 ± 0.003 ab	44.18 ± 0.74 ab	0.77 ± 0.04	0.29 ± 0.01 a	1.06 ± 0.05	0.17 ± 0.01	2.66 ± 0.001	0.16 ± 0.007
B 10%	0.80 ± 0.006 b	39.92 ± 1.55 c	0.47 ± 0.12	0.16 ± 0.03 b	0.63 ± 0.15	0.11 ± 0.02	2.88 ± 0.135	0.17 ± 0.008
B 15%	0.81 ± 0.006 b	40.60 ± 1.62 bc	0.59 ± 0.10	0.21 ± 0.04 ab	0.80 ± 0.14	0.14 ± 0.02	2.78 ± 0.006	0.17 ± 0.006
B 20%	0.81 ± 0.007 b	41.48 ± 1.52 bc	0.58 ± 0.11	0.23 ± 0.05 ab	0.80 ± 0.15	0.14 ± 0.02	2.62 ± 0.052	0.18 ± 0.007

Values are mean ± standard error (n = 6). Values in the columns that are followed by the same letter are not significantly different ($p < 0.05$).

Mineral accumulation in snapdragon grown in peat or biochar-based media is presented in Table 5. Nitrogen content decreased up to 10% in plants grown in ≥10% biochar compared to the plants grown in peat. Similarly, P content was decreased with the biochar presence, reaching the lowest accumulation at biochar 20% treatment. The opposite was evident for the K accumulation, revealing increased values at the higher biochar ratios in the growing media (i.e., 20% biochar) compared the lower levels of 5–10% biochar and/or control treatments. Magnesium content was increased (up to 22%) with the biochar application as to plants grown in peat. Calcium and Na were accumulated the highest at the 5% biochar application compared to higher biochar levels or control treatments.

Table 5. Effect of growing media (peat, biochar-B) on mineral element contents (g/kg dry weight) in the snapdragon plants grown in the greenhouse/nursery.

	N	K	P	Na	Ca	Mg
Peat 100%	31.40 ± 1.58 a	22.13 ± 0.52 c	6.08 ± 0.09 a	9.06 ± 0.29 c	10.92 ± 0.53 b	3.68 ± 0.24 b
B 5%	28.75 ± 0.53 ab	22.48 ± 0.68 c	4.96 ± 0.31 bc	11.00 ± 0.29 a	12.64 ± 0.46 a	4.48 ± 0.18 a
B 10%	28.28 ± 0.34 b	27.34 ± 0.42 b	5.37 ± 0.20 b	10.57 ± 0.16 ab	11.41 ± 0.23 b	4.49 ± 0.09 a
B 15%	28.10 ± 0.38 b	29.00 ± 0.35 ab	4.39 ± 0.18 cd	9.27 ± 0.12 c	10.42 ± 0.38 b	4.30 ± 0.11 a
B 20%	26.13 ± 0.05 b	29.76 ± 0.57 a	3.98 ± 0.07 d	10.05 ± 0.12 b	10.87 ± 0.10 b	4.40 ± 0.09 a

Values are mean ± standard error (n = 4). Values in the columns that are followed by the same letter are not significantly different ($p < 0.05$).

Total phenols, total flavonoids, total flavanols content, and antioxidant status of snapdragon grown in peat or biochar-based media are illustrated in Figure 1. The content of total phenols increased (varying from 16.9% to 44.6%) in plants grown in biochar-based media, revealing the greatest content at 20% biochar, in comparison to the peat-grown

plants (Figure 1A). Moreover, biochar stimulated the production of total flavanols, with an increase up to 75% in plants grown at 5–15% biochar-based and up to 2.5-folds in plants grown at 20% biochar-based media (0.14 mg catechin/g fresh weight) compared to the plants grown in peat (0.04 mg catechin/g fresh weight) (Figure 1F). However, no differences were found between the total flavonoids content among the treatments, which was averaged at 0.15 mg rutin/g fresh weight (Figure 1E). The antioxidant capacity of the plants was stimulated with the presence of biochar in the growing media, with significant increases at the high biochar ratios. Therefore, plant DPPH was increased at ≥5% biochar (Figure 1B), and plant FRAP and ABTS were increased at 20% biochar in the growing media (Figure 1C,D).

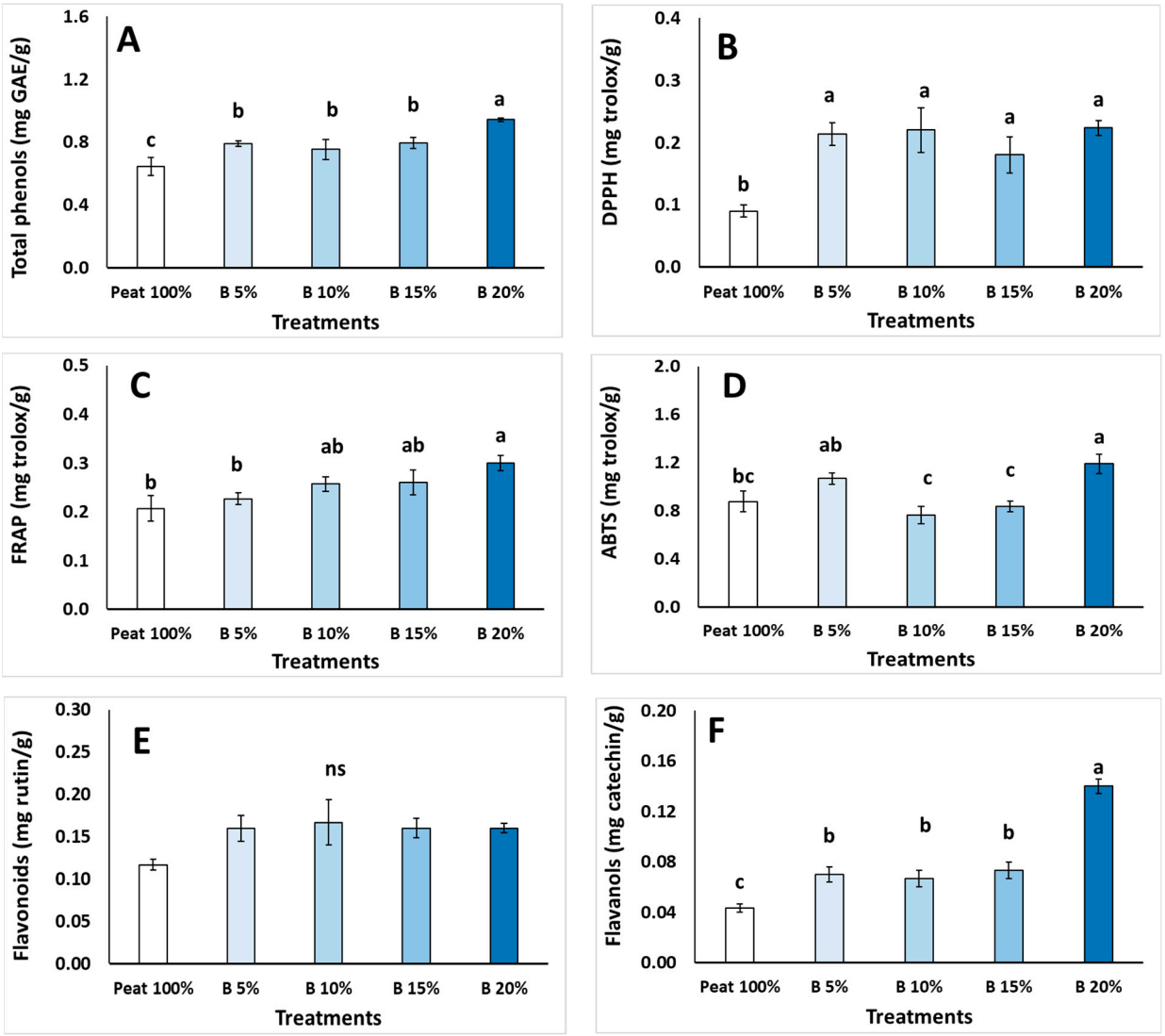

Figure 1. Effects of growing media (peat, biochar-B) on (**A**) total phenols (mg GAE/g Fw), (**B**–**D**) antioxidant activity (DPPH, FRAP, ABTS: mg trolox/g Fw), (**E**) flavonoids (mg rutin/g Fw), and (**F**) flavanols (mg catechin/g Fw) in the snapdragon. Values are mean ± standard error ($n = 6$). Mean values followed by the same letter do not differ significantly at $p \geq 0.05$, according to Duncan's Multiple Range Test.

Plants subjected to stress conditions were evaluated through stress indices, such as MDA and H_2O_2 production, as presented in Figure 2. The MDA content increased up to 41.1% in plants grown at ≥15% biochar compared to the plant grown in peat material

(control) (Figure 2A). This increase was combined with the increased H_2O_2 levels in plants grown in biochar-based media (Figure 2B).

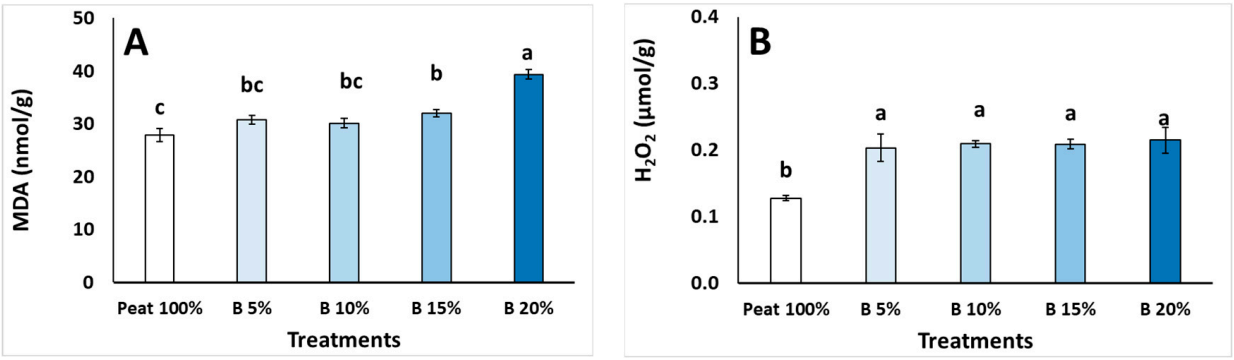

Figure 2. Effect of growing media (peat, biochar-B) on (**A**) lipid peroxidation per MDA (nmol/g) and (**B**) hydrogen peroxide—H_2O_2 (µmol/g) in the snapdragon plants grown in the greenhouse/nursery. Values are mean ± standard error (n = 6). Mean values followed by the same letter do not differ significantly at $p \geq 0.05$ according to Duncan's Multiple Range Test.

Linear correlation coefficients were computed and presented in Tables S1–S3 to demonstrate the contribution of the biochar ratio in the growing media for each individual factor. Therefore, biochar level correlated with the physicochemical characteristics of the growing media before transplanting and revealed a positive correlation with organic matter and organic carbon content, C/N, pH, bulk density, and K content of the growing media, and a negative correlation with the growing media EC, total porosity, container capacity, and N, P, and Ca content (Table S1). Regarding the correlation of biochar level and the growing media physicochemical attributes at the end of the experiment, biochar level was positively correlated with C/N and pH, and negatively correlated with the growing media N, P, Na, Mg, and Ca content (Table S2). Regarding biochar levels in the growing media and plant-related attributes, there was a positive correlation with plant height, leaf K content, total phenols, antioxidant capacity (FRAP), total flavanols, MDA, and H_2O_2 content, and a negative correlation with SPAD, chlorophyll fluorescence, number of flowers, leaf N, and leaf P content (Table S3).

4. Discussion

The current study was driven by the hypothesis that biochar could affect the growth and mineral uptake of snapdragon during a short period of plant growth under nursery conditions, before transplanting to the final place, usually in pots or in soil. Therefore, to test the effects of biochar on plant growth, development, and mineral content, increasing quantities of biochar were added to the peat-based growing media. The selected biochar ratio of up to 20% was based on previous reports as higher ratio often causes stress conditions with negative effects on plant development and quality of the products [17,19,39,40]. In the current research, biochar ≥10% increased the pH of the growing media, ameliorating the partial peat acidity, having liming potential [1,21]. However, the high pH values that biochar usually has, make unfavorable conditions for several plants if biochar is used as a sole material or in high levels in the growing media [41]. Considering the high temperature (700 °C) that biochar was produced with pyrolysis, the high pH of 9.57 that biochar had in the present study, agreed with prior reports. The pyrolysis temperature affects biochar properties with increased pH values at high temperatures (i.e., 650 °C) and lower pH values at lower temperatures (i.e., 450 °C) [42]. Indeed, there are reports that biochar use of 100% in the growing media improved plant growth metabolites [20]. When biochar is used at higher rates, i.e., up to 70% in peat, it does not require pH adjustment due to the strong alkaline pH of biochar, which is of interest for growing plants under nursery conditions [1]. The EC of the growing media was decreased with the increase in biochar application rates,

and this is related to the specific surface area as previously reported [43]. Considering that the EC values of the tested growing media, both before plant establishment but also after the experiment completion, exhibited the recommended threshold of 0.5 dS/m [3] for growing media, it could increase the salt content in the growing media. However, this is more related to the increased mineral levels, i.e., K, in the media rather that the increased Na, as the latter could result to adequate values of salinity.

The increase in pH and K content, and the decrease in P content of the examined growing media with the addition of biochar are similar to the results of Prasad et al. [44] and Fascella et al. [45]. It has been suggested that the high pH levels of biochar is related to the high K content measured in the original feedstocks prior to pyrolysis [46]. Commonly, the bulk density of a growing media should be lower than 0.4 g/cm^3 while the air space should be from 10 to 30% [3], as it was observed in all the tested biochar ratios in the growing media. The biochar addition affected the physical properties of the growing media as a negative correlation of biochar ratio with the total porosity and water filled capacity of the growing media was found. This is in contrast with previous reports [22], most probably due to the particle size distribution of the tested biochar, as more than 64.1% of particle size was <1 mm and considered as finer material.

The increased biochar levels in the growing media were positively correlated with the increased pH values of the media, but no effect was observed on the plant biomass produced, indicating plant tolerance to the increased pH. Alkaline amendments like biochar might reduce nutrient availability, particularly for P, iron (Fe), and manganese (Mn), leading to reduced yields [47]. In the present study, P and N availability in the growing media were decreased, but not to deficiency levels that cause biomass decreases, instead at levels where accumulation was less in the plant tissue. The similar biomass obtained in the present work in plants grown in increased biochar levels might be attributed to the enriched K content in the growing media which was mirrored to the K accumulation in the plant tissue. It was suggested that biochar can reduce cation activity (Ca^{2+}, Al^{3+}, Fe^{3+}) that interacts with phosphate in soil, by adsorbing them on the biochar surface and delaying phosphate adsorption; however, the peat material has low Al^{3+} and Fe^{3+} levels [44].

After 4 weeks of plant growth, the substrate pH was still increased in the biochar-based growing media, as reported previously [1]. Moreover, the biochar-based media increased mineral content at the end of the crop growing cycle compared to the initial phase (before plants placement), indicating the role of biochar in buffering minerals and releasing them upon plant request. However, this mineral buffering might have caused some nutrient shortage, indicated with the decreased N and P accumulation in snapdragon plants during the short period of crop growth. The role of biochar particle size to buffer nutrients and water availability has been reported previously [16]. It was found that Barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and maize (*Zea mays*) plants grown for one month under different biochar-based growing media had less available nutrients in the media compared to the initial levels [48], which is the opposite of the present findings. This can possibly be related to the different biochar material, rates, and crops used. The pH shift of biochar throughout incubation could be due to both opposing procedures: carbon oxidation and alkaline mineral dissolution, with larger alkaline metals released, including Na, K, Mg, and Ca [49]. The higher EC values obtained after the end of the cultivation period might be related with the higher soluble salt quantities in biochar, wherein after the dissolution process of biochar mineral matter the EC is increased [49]. However, this did not really affect snapdragon biomass as it is considered as a moderate salt-tolerant species [50]. Biochar at 20% decreased organic matter and organic C content after plant transplantation as the organic matter degradation was delayed due to the unfavorable conditions of available air-oxygen [48].

Biochar at 15% and 20% ratios in the growing media prioritize the increase in plant height rather than flower production when compared to peat. However, the nutrient content in plants was decreased, except for K content at 15% and 20% biochar application, which was related with the decreased EC values in the biochar-based growing media and/or

the use on nutrients for secondary metabolism procedures, i.e., polyphenol production. *Lavandula angustifolia* potted plants grown in increased biochar levels were taller than the ones grown in peat [45]. The *Tagetes erecta* plant had increased height at low biochar levels and decreased height at high (50–70%) biochar ratios when the pH was adjusted to pH 5.8 [1]. In the same study, it was reported that the pH adjustment to 5.8 negatively affected the plant height compared with the non-pH adjusted treatment at early plant growth stages, but this was not evident at the end of the experiment (9 weeks). Increased biochar levels have negatively affected flower abundance and the time of flowering in the snapdragon, as biochar might prevent/delay flowering formation due to the lower mineral presence (N, P, Ca, Mg) compared to the control treatment. The performance and flowering of pansy (*Viola* × *Wittrockiana* Gams) plants were significantly affected by the essential minerals available in the substrate [51]. Additionally, the flowering of pansy plants was enhanced in plants grown under 10% and 25% in oak-derived hardwood biochar but decreased at the 50% biochar rates, not only due to the lower mineral presence but due to the increased pH > 7 as it was observed in the present study [52].

Plant biomass was unaffected under the tested biochar ratios, which is in agreement with previous reports [45,53], while other studies showed increased biomass with biochar use in peat-based media [49,54]. Indeed, plants were grown for a short period of 4 weeks under nursery conditions in the present study, whereas a longer duration might show variation in the observed findings. Utilization of co-composted biochar did not improve the lettuce (*Lactuca sativa* L.) yield significantly compared to plants grown in soil; however, nitrate and copper accumulation were decreased in plant tissue compared to the control treatment, having a phytoremediation role [53]. Marigold (*T. erecta*) biomass was similar across all treatments [1], in accordance with the present findings. Notably, the use of biochar produced from low temperature pyrolysis of green waste could improve the plant growth and biomass production even at rates of 50% when mixed with peat for *Calathea rotundifola* compared to peat alone [55]. Apart from the positive correlation between snapdragon biomass and P content in biochar, crop biomass did not correlate with biochar nutrient concentrations, and biochar effects were more possibly related to the physicochemical changes on growing media characteristics such as the pH, the total porosity, and the water holding capacity and not only to the nutrient content, i.e., enriched K or decreased Ca. Kadota and Niimi [56] found that adding 10% or 30% biochar to the growing media boosted zinnia (*Zinnia linearis* Benth) shoot growth but had no effect on marigold (*T. patula*) or scarlet sage (*Salvia splendens* Ker Gawl.). However, increased biochar rates promoted plant growth of *T. patula* [49] and *Cichorium endivia* [57].

SPAD values, an indication of chlorophyll content, revealed sensitivity to increased biochar rates; however, biochar at the end of the experiment had little or no effect on chlorophylls and carotenoids content. Similarly, *T. patula* that grown in increased biochar levels revealed SPAD decrease at the first week only but not at the ninth week of plant development, highlighting some biochar effects on plant physiology initially, but then the plant was able to overcome that situation of possible stress conditions [1].

Nitrogen and P accumulation was decreased but K and Mg accumulation was increased with biochar addition in the growing media, whereas previous reports indicated a relevant increase in the N and P accumulation on tomato and pepper plants and highlighted the important role of the microbial community in the growing media and interactions with plant's roots [54]. Moreover, it was reported that Mg content was raised and Ca content declined in *T. patula* plants grown in low biochar levels [49]. Biochar treatment may reduce N availability by increasing the C/N ratio in growth medium, leading to greater N immobilization and slower net N mineralization [58], which is confirmed by the present findings. Awad et al. [59] suggested that the enhanced nutrient uptake by plants grown under biochar-based media might be related to the induction of beneficial microorganism growth on its surfaces. However, in the present study, the beneficial microorganisms and microbiome community diversity were not studied, which can be a task for future research. Moreover, the decreased N content in the growing media, which resulted in decreased

N accumulation in plant leaves, could be avoided if additional N fertigation was added during the crop development period. In such a case, fertilization will counteract some limitations of biochar application in snapdragon.

As reported previously, the higher biochar ratios in the growing media and the low temperature of biochar production (450 vs. 700 °C) significantly increased the content of total phenolics on *C. endivia* plants [57]. Total phenolics increased in plants grown in biochar-based media compared to the plants grown in peat, which is in agreement with previous reports [60]. The increased total phenols and antioxidant capacity of snapdragon plants, especially at high biochar ratios used in the growing media in the present study, reflects some unfavorable conditions of the selected growing media. Unfavorable growing conditions may have driven plants to accumulate various phenolic components [61,62], as indicated by the increased stress indicators (MDA and H_2O_2) in the present study. Such stress conditions resulted in the activation of various enzymatic and non-enzymatic antioxidant activities to scavenge reactive oxygen species [62]. The increase in the total phenolic content and the antioxidant capacity of the edible plant parts is of great importance as it is related to the increased nutritive value and higher medicinal properties. Total cannabinoid content (group of phenolic constituents) was unaffected in *Cannabis sativa* when plants were grown in biochar-based media, which was critical to define the potential use of biochar for the production of the crop and the possibility of partial peat substitution [63]. Moreover, in the case of plants with edible flowers, the amount of polyphenols should be considered for the actual inflorescences as well, as previous reports have indicated that biochar levels and fertilization practices affected total phenolic and flavonoids content in *Viola cornuta* flowers [64].

5. Conclusions

In conclusion, biochar affected physicochemical properties in the growing media. Biochar $\geq$ 10% increased the pH, K, and Mg content but decreased P and N content in the growing media. The biochar addition affected the physical properties as a negative correlation of biochar ratio with the total porosity and water filled capacity of the growing media was found. The addition of $\geq$15% biochar increased plant height and decreased flowering, but no effect was observed on the plant biomass produced. The increased biochar levels negatively affected leaf chlorophyll levels and plant photosynthetic performance. The content of total phenols, flavanols, and the antioxidant capacity of the plant exposed to the higher biochar levels increased compared to the plants grown in peat. This resulted in lipid peroxidation and a hydrogen peroxide content increase, causing oxidative stress. Adding biochar in the growing media increased the K and Mg content in plants, because the plants could absorb more available nutrients. The present findings clearly demonstrated the potential of biochar to lower EC depending on the rate employed and biochar surface area. This observation is relevant, since most materials utilized to dilute peat, such as composted green waste, have high EC, which limits the rate of peat dilution. Furthermore, bringing nutrients to the desirable level, as in peat-based media, may not be sufficient since biochar may interact with N and P availability, as evidenced by our growing media analysis and plant nutrient accumulation results. Further research is required in that direction considering the different crop needs in minerals too.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14111860/s1, Figure S1: Changes in growing media (peat, biochar-B) chemical properties (% of changes from initial to final material) after plant harvesting (at the end of the growing period). Table S1: Correlations coefficients and (*p*-values) between the biochar levels in the growing media and the physicochemical properties of the growing media before plant placement. Table S2: Correlations coefficients and (*p*-values) between the biochar levels in the growing media and the physicochemical properties of the growing media after plant growth. Table S3: Correlations coefficients and (*p*-values) between the biochar levels in the growing media and the plant related tested parameters.

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