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Biochar Improves the Properties of Poultry Manure Compost as Growing Media for Rosemary Production

Fernando Fornes [1,*], Luisa Liu-Xu [2], Antonio Lidón [3], María Sánchez-García [4], María Luz Cayuela [4], Miguel A. Sánchez-Monedero [4] and Rosa María Belda [1]

1 Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, 46022 Valencia, Spain; rmbelda@bvg.upv.es
2 Departamento de Ciencias Agrárias y del Medio Natural, Grupo de Bioquímica y Biotecnología, Universitat Jaume I, 12071 Castellón de La Plana, Spain; lliu@uji.es
3 Instituto Universitario de Ingeniería del Agua y del Medio Ambiente, Universitat Politècnica de València, 46022 Valencia, Spain; alidon@qim.upv.es
4 Department of Soil and Water Conservation and Organic Waste Management, CEBAS-CSIC, P.O. Box 4195, 30080 Murcia, Spain; msgarcia@cebas.csic.es (M.S.-G.); mlcayuela@cebas.csic.es (M.L.C.); monedero@cebas.csic.es (M.A.S.-M.)
* Correspondence: ffornes@bvg.upv.es; Tel.: +34-963877413

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Abstract: Compost represents a sustainable alternative for peat (P) replacement in soilless plant cultivation, but its use can be limited by several inadequate physical and physicochemical properties. Biochar can alleviate some of the limitations of compost for its use as growth media by improving the physical properties, decreasing salinity and making the phytotoxic compounds unavailable for plants. We studied the physical and physicochemical properties of holm oak biochar (B), poultry manure compost (PMC), poultry manure composted with biochar (PMBC), a commercial peat (P) and multiple combinations of these materials as growth media, and their effect on the rooting and growth of rosemary. PMBC and PMC showed similar physical and physicochemical properties as growing media, and they both were phytotoxic when used in a rate above 50% (by volume) in the growing medium. However, when used at proportion of 25%, PMBC was less phytotoxic than PMC and enhanced the percentage of rosemary cutting rooting. The incorporation of B in the growing medium instead of P (either at 50% or 75% in volume) increased the stability of the growing media and the percentage of rooted cuttings, but it did not affect plant growth significantly. Our results demonstrate the potential of substituting peat by a combination of poultry manure compost and biochar for the formulation of growth media.

Keywords: biochar; growth media; manure reclaim; peat alternatives; phytotoxicity alleviation; rooting media; *Rosmarinus officinalis*

1. Introduction

Seedling production for horticultural, ornamental or forestry purposes often uses organic materials as growth media. From these materials, peat has been the most widely used in the last decades [1]. However, there is a growing environmental concern regarding the use of peat in horticulture, since peat is a non-renewable resource and, additionally, the drainage of peat bogs leads to increased emissions of greenhouse gases such as CO_2, CH_4, and N_2O [2]. These factors encourage the search for sustainable organic materials alternative to peat, such as compost, pine bark, coir, wood and fiber.

High-grade composts (mature, fully stabilized and well structured) are widely accepted as suitable peat replacement in growing media [3]. Additionally, its horticultural use has the environmental

advantage of reclaiming a wide spectrum of organic wastes [4,5]. However, only few composts meet the standards of premium quality composts. Usually, the use of large percentages of composts in growth media is restricted due to several inadequate characteristics such as poor physical properties, low stabilization degree [3], or the presence of substances that might eventually be phytotoxic. In particular, compost phytotoxicity could have several origins: (1) High pH and EC due to high salt concentration, which is a characteristic of composts produced from agricultural wastes or from manures [6]; (2) Accumulation of phenolic compounds [7]; (3) High concentration of NH_3 and NH_4^+, which is characteristic of manure composts [8]; (4) High content of heavy metals, mainly Cu and Zn, which is characteristic of sewage sludge and pig slurry composts [9]. Despite that, mixing compost with peat is a good method to reduce peat consumption and improve plant performance than using peat alone, as has been indicated in many studies. The proportion of compost that can be successfully used in mixes with peat (or with other high-quality materials such as coconut coir) depends on the quality of the compost and on the plant species to be grown in it (for a review, see [10]). The case of poultry manure compost, which allows the reclamation of large amounts of wastes from intensive livestock production [11], is not different from that of other composts. Poultry manure compost has been successfully used as growth medium constituent for soilless plant growth [12]. This type of compost can only be used at low percentages in combination with high quality substrates due to its high pH, salinity, and NH_4^+ concentration [13].

Biochar has recently attracted the attention of researchers as co-constituent in compost-based growth media [14]. Biochar is the product of the controlled pyrolysis of biomass and it is mainly used as soil improver for agricultural, horticultural, and environmental aims [15]. Biochar has attracted attention due to its capacity to sequester carbon and reduce greenhouse gas emissions [16].

Recently, biochar has been tested as growth medium constituent to grow plants for ornamental [17,18], vegetable [19,20], forest [21,22], and energy or restoration [9] purposes, but generally in mixes with peat or coir. The combination of biochar with compost [23] or vermicompost [24] has also been examined, but to a lesser extent. However, the role of biochar as additive in organic waste composting has been well documented [25,26], but there is less information regarding the agronomical use of co-composted biochar [27]. Sánchez-García et al. [28] found no differences between the nutritional value of poultry manure composts produced with biochar and the nutritional value of poultry manure composts produced without biochar, but they did not study other physicochemical properties that may be relevant for the use of these composts as growing media.

Biochar is a light and highly porous material which is expected to improve the physical properties of composts [29] either when added to the composting mix or when added to the mature compost. Moreover, the physical properties of a biochar containing substrate could be easily adapted to different aims (production of seedlings in small containers; growth of plants in big containers; etc.) by sieving the biochar to obtain the 'ideal' particle size, as it is done for peat or coir [30]. Additionally, biochar reduces the risk of shrinkage and prevents the decomposition of the growth medium [18]. Biochar is rich in surface anionic charge sites which strongly retain cations such as NH_4^+ [31] having the potential of reducing the NH_4^+ phytotoxicity of composts. Additionally, some capacity to decrease plant disease incidence by affecting the presence and activity of soilborne pathogens [32] or by inducing plant systemic resistance to pathogens [33] has been attributed to biochar.

There is previous evidence of the positive impacts in root development and plant growth in substrates mixes containing biochar [34,35]. However, the use of biochar in the formulation of growth media for clonal plant propagation by rooting cuttings has been scarcely studied. Induced rooting of cuttings is a standard procedure to propagate shrubs such as rosemary [36]. Specifically studying the rooting of cuttings is relevant because the adequate physical and physicochemical characteristics of the substrate for potted plant growth [37] are not the same as for cutting rooting [38].

This study aimed at assessing the potential of biochar as peat replacement in compost based growth media for two different horticultural purposes relative to rosemary cultivation: Cutting rooting and plant growth. Our hypothesis is that the use of biochar, either as an additive for the preparation

of compost or as growing media constituent, would reduce compost phytotoxicity, improve its physicochemical properties as growing medium and enhance its performance for rosemary cultivation. To reach our objective, a full characterization of growth media containing poultry manure compost (PMC), poultry manure composted with biochar (PMBC), biochar (B), peat (P), and mixes of them at different ratios was performed. In addition, two experiments were conducted. In the first one, we studied the impact of using biochar as composting additive by comparing the horticultural performance of PMC and PMBC as growing media constituents. In the second one, we studied the impact of using biochar as constituent in compost-based growing media by comparing the horticultural performance of mixes of PMC and B with mixes of PMC and P at different ratios.

2. Materials and Methods

2.1. Characteristics of the Materials

Biochar (B; particle size < 6 mm) was purchased from Piroeco Bioenergy S.L. (Malaga, Spain). It was produced from holm oak by slow pyrolysis at 650 °C at atmospheric pressure and the residence time in the reactor chamber was 12–18 h. Composts were prepared from a mixture of poultry manure (78% dry weight basis) and barley straw (22% d.w.) (PMC) or from a mixture of poultry manure (76% dry weight basis), barley straw (21% d.w.), and biochar (3% d.w.) (PMBC). A full description of the composting process and the main characteristics of the raw materials have been previously described by Sánchez-García et al. [28]. Peat (P) (Kekkilä Ornamental Plant Mix 410, Kekkilä Oy) was purchased from Projar (Valencia, Spain). Cuttings of rosemary (*Rosmarinus officinalis* L.), about 5 cm in length, obtained from lateral or terminal buds of mother plants, were used in the rooting experiment, and seedlings of rosemary of similar age and size with a developed root ball were used in the pot experiment.

2.2. Physical and Chemical Characterization of the Growth Media

Characterization of growth media was carried out following the European Standards (EN) for soil improvers and growing media. Bulk density, water capacity and total water-holding capacity were determined using loosely-packed cores and methods described in EN 13041 [39], using steel cylinders of 40 mm height and 82.3 mm internal diameter (approx. 210 mL). Shrinkage was calculated as the percentage loss of bulk volume after drying the material contained in the cylinder at 105 °C. Total pore space is the percentage of the material volume that can be filled with water. Air capacity is the difference—in percentage by volume—between total pore space and moisture content at a suction of 1 kPa [39]. For a more detailed description see Abad et al. [30].

For the characterization of the physico-chemical and chemical characteristics, pH (EN 13037) [39], electrical conductivity (EC) (EN 13038) [39], and water soluble mineral element concentration (EN 13652) [39] in the substrates were determined on a 1:5 (*v*:*v*) substrate:water suspension. pH was measured using a Crison model 2000 pH meter. EC was determined with a Crison model 522 conductimeter. Water-soluble N (NO_3^- + NH_4^+), P, K, Ca, and Mg contents in the substrates were determined using reflectoquant technology (Merck®; Darmstadt, Germany): Analyses were conducted with a reflectometer RQflex 10 Reflectoquant using the corresponding bar-code strips for calibration and test strips for nutrient quantification, following manufacturer's instructions. Water-soluble mineral concentrations were expressed on a volume basis for the growth media. Organic matter (OM) was estimated by loss-on-ignition. The material was dried at 105 °C and ashed at 450 °C for 12 h and OM was calculated as the percentage of weight loss. All determinations were performed three times.

2.3. Phytotoxicity of the Growth Media

Seed germination assays were performed to determine the potential phytotoxicity of the growth media using seeds of cress (*Lepidium sativum* cv. Alenois), which are considered sensitive to toxic organic compounds such as polyphenols [40], and seeds of lettuce (*Lactuca sativa* cv. Romana Bionda Degli

Ortolani), which are considered especially sensitive to salinity [41]. To conduct these bioassays, 1:5 (*v:v*) water extracts were used. Seeds were germinated in Petri dishes, covered with filter paper on both sides, which had been wetted with the corresponding extract or with distilled water (control). Seeds were kept in the darkness in a growth chamber at 22 °C during 3 days for cress and at 17 °C during 5 days for lettuce. Results were expressed as percentage of the control (distilled water). The germination index was calculated according to Zucconi's [40]. These determinations were repeated five times.

2.4. Stability to Microbial Degradation of the Growth Media

The microbial stability of selected growing media (PMC, PMBC, B, P, and the mixtures of PMC:B, PMC:P, and PMBC:P at 50% (v:v)) was determined by CO_2 respiration activity and N mineralization assays.

For microbial respiration measurements, a method adapted from Fornes et al. [42] was followed. Three 250 mL glass flasks (three replicates), equipped with a septum plug, containing 10 g of each substrate, were incubated during 120 days at 25 °C and 60% of their water holding capacity (WHC; equivalent to container capacity in soils). CO_2 concentration inside the flasks was measured periodically with a CheckPoint portable gas analyzer (MOCON Europe Dansensor®; Ringsted, Denmark). When necessary flasks were opened to allow for aeration and to adjust the humidity. Cumulative released CO_2 was calculated from the periodical records. Results are expressed as g of CO_2 released per kg of substrate.

N mineralization was measured by monitoring ammonium dynamics in the substrates following a methodology adapted from Fornes et al. [42]. Eighteen 250 mL-flasks per growth media, each containing 10 g of material, were incubated in the same conditions as for the respiration measurements. Three flasks (3 replicates; n = 3) were removed from the set and analyzed at each of the following incubation periods (days): 0, 3, 7, 28, 45, and 60. For analysis, NH_4^+-N was extracted with 2 mol L^{-1} KCl (1:10 v:v), filtered through Whatman nº 42 filter paper and quantified using a FIAstar 5000 Analyser (FOSS Tekator, Hilleroed, Denmark).

2.5. Experimental Design, Plant Growing Conditions, and Plant Analysis

The assays described below were conducted in a glasshouse in a commercial nursery (TENISPLANT, S.L.) located in Picassent, Spain (39°33′ N, 0°44′ W). The management of plant material followed nursery standards. Irrigation water was chemically characterized and gave the following results: pH 7.95, EC 1.8 dS m^{-1}, N-NH_4^+ non-detectable, N-NO_3^- 23 mg L^{-1}, P non-detectable, K^+ 8 mg L^{-1}, Ca^{2+} 178 mg L^{-1}, Mg^{2+} 39.4 mg L^{-1}, HCO_3^- 220 mg L^{-1}, SO_4^{2-} 345 mg L^{-1}, and Na^+ 64 mg L^{-1}.

Two experiments (Exp. I and Exp. II) were conducted simultaneously. Each of the experiments consisted of two assays, one to study the effect of substrates on the rooting of cuttings (CR) and the other to study the effect of substrates on plant growth (PG).

In Exp. I growth media formulated with PMC or PMBC were compared. The treatments consisted of mixtures of the two composts with P in different proportions. The proportions (% v:v) assayed in the CR assay were 100:0 (100% composts), 75:25, 50:50, 25:75 and 0:100 (100% P). In the PG assay, the proportions assayed were 50:50, 25:75, and 0:100.

In Exp. II, B and P were compared as growth media constituents in mixes with PMC. The treatments consisted of mixtures of PMC with B and PMC with P in different proportions. The proportions (% v:v) assayed in the CR assay were 100:0 (100% compost), 75:25, 50:50, 25:75, and 0:100 (100% B or P). In the PG assay, the proportions assayed were 50:50, 25:75, and 0:100.

A diagram of the experimental design is shown in Table 1. For the cutting rooting assays (CR) three replicates consisting of 24-cell plastic rooting trays (cell volume = 20 mL) were filled with each of the substrates (with no additional fertilization) and distributed in a random block design. One cutting per cell was plugged in the substrate. No hormonal treatment (auxin) was applied for rooting stimulation. Cuttings were irrigated using a microsprinkler system (36 L h^{-1} m^{-2}) for 5 min once a day, resulting in

0.6 L tray^{-1} day^{-1}. Rooting (% of rooted cuttings) and growth (shoot and root dry weight) results were recorded two months after planting.

Table 1. Experimental design for the two assays (cutting rooting (CR) and plant growth (PG)) conducted in experiments I and II. In this diagram, 'R' means replicate. A factorial design, were two factors were crossed: Materials constituting the substrates and ratio at which they were present in the substrates, was applied. In the CR assays, each treatment was replicated three times (n = 3). Each replicate consisted in a 24-cell plastic rooting tray containing 24 cuttings (72 cuttings per treatment; 720 cuttings in total in each experiment). In the PG assays, each treatment was replicated three times (n = 3). Each replicate consisted of four pots with one plant each (12 plants per treatment; 72 plants in total in each experiment).

Experiment I								
	Cutting rooting (CR) assay					**Plant growth (PG) assay**		
	Ratio (%v:v)					Ratio (%v:v)		
Substrate	100:0	75:25	50:50	25:75	0:100	50:50	25:75	0:100
PMC:P	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3
PMBC:P	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3
Experiment II								
	Cutting rooting (CR) assay					**Plant growth (PG) assay**		
	Ratio (%v:v)					Ratio (%v:v)		
Substrate	100:0	75:25	50:50	25:75	0:100	50:50	25:75	0:100
PMC:P	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3
PMC:B	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3	R_1 R_2 R_3

For the plant growth assays (PG), rooted seedlings of about 10 cm shoot length were transplanted in 500 mL plastic pots, which were filled with each of the substrates. Three replicates consisting of four pots each (12 plants per treatment) were distributed in a random block design. Plants were irrigated with sprinklers (25 L h^{-1} m^{-2}) for15 min once a day. Fertilizers were applied by fertigation twice a week with an 8-1-10-1 ratio (N-P_2O_5-K_2O-MgO) at a rate of 150g m^{-3} of water. Plants were grown for five months. At the end of the assay, shoot length and a visual rating of the root ball size (root ball-VR) were obtained. In order to obtain the root ball-VR the root ball was taken out of the pot and the expansion of the root system was evaluated with an arbitrary scale were the root ball-VR was scored from 1 to 4: Value 1 representing roots that had not reached the surface of the substrate and value 4 representing a root system that had formed a compact mesh and colonized the whole substrate [43]. To reduce subjectivity, this estimation was performed by five independent individuals and the mean value was calculated. Additionally, fresh leaves were frozen (−40 °C) for chlorophyll analysis. The remaining shoot was oven dried (72 h at 70 °C) to obtain shoot dry weight and to carry out nutrient analyses. Chlorophyll content was determined following the Moran method [44] after extraction with N,N-dimethylformamide. Oven-dried leaf tissue was finely ground for the nutritional analysis. Leaf P, K, Ca, and Mg were analyzed by Atomic Emission Spectrophotometry with Inductively Coupled Plasma (ICP-AES; ICAP 6500 DUO/IRIS INTREPID II XDL; SpectraLab Scientific Inc., Markham, Ontario Canada). Total N was determined with the Kjeldahl method. All analytical determinations were repeated three times.

2.6. Data Analyses

Factorial analyses of variance (ANOVA) were performed to determine significant effects of the substrate composition on the physical, physico-chemical, and chemical characteristics, and on the phytotoxicity of the substrates. Two factors were analyzed: Constituent type and ratio of constituents in the media (Tables 2 and 3). Similarly, factorial ANOVAs were conducted to determine

significant differences of cutting rooting and of plant growth parameters between substrates (Tables 4 and 5, Tables S1 and S2). In the case of Figures 1 and 2, one-way ANOVAs were conducted on the data corresponding to the final sampling day. Data were tested for normal distribution using the Kolmogorov–Smirnov test. In order to ensure uniformity of the variance several transformations of the data were used as appropriate. In the analyses, when significant differences were found, the Tukey test (Tables 2–5, Tables S1 and S2) or the LSD test (Figures 1 and 2) at $P \leq 0.05$ were carried out to establish significant differences between means. Only statistically significant effects are reported and discussed throughout the text. Statistical analyses were performed using the Statgraphics Centurion XVII statistical package (2020 Statgraphics Technologies, Inc., The Plains, Virginia, USA).

Table 2. Physical properties of growth media containing mixes of poultry manure composted without biochar (PMC), poultry manure composted with biochar (PMBC), biochar (B), and peat (P) at different ratios. Main effects and statistical significance according to factorial analysis of variance. Three replicates ($n = 3$) were used for each substrate and ratio.

Substrate	Ratios (% v:v)	D_B (kg m^{-3})	WHC (%)	P_T (%)	Vair (%)	Vwater (%)	Shrinkage (%)
PMC:B	100:0	396b	169e	80ef	14fg	65cd	18bcd
	75:25	380bc	159e	80ef	20de	60de	10de
	50:50	370c	155e	79ef	21cd	58e	5ef
	25:75	377c	153e	78ef	20cde	58e	4ef
	0:100	323e	147e	81de	33a	47f	1f
PMC:P	100:0	393b	167e	79de	14fg	65cd	18bcd
	75:25	318e	233d	84d	11g	73a	28a
	50:50	290f	282c	84d	14fg	70abc	27a
	25:75	184h	368b	89b	17ef	72ab	24ab
	0:100	115i	585a	93a	26b	66bcd	19bc
PMBC:P	100:0	440a	163e	77f	6h	71abc	17bcd
	75:25	350d	211d	81de	7h	74a	16bcd
	50:50	267g	275c	85cd	13fg	72abc	15cd
	25:75	200h	363b	88bc	16ef	72ab	17bcd
	0:100	111i	581a	91ab	24bc	68abc	19bc
Main effects							
Mix	PMC:B	369A	156C	80C	22A	58B	8C
	PMC:P	260C	327A	86A	17B	69A	23A
	PMBC:P	273B	319B	84B	13C	71A	17B
Ratio	100:0	409A	166E	79D	11D	67A	18A
	75:25	349B	201D	82C	13CD	69A	18A
	50:50	309C	237C	83C	16BC	66A	16AB
	25:75	253D	295B	85B	18B	67A	15AB
	0:100	183E	438A	88A	28A	60B	13B
Significance							
Mix		***	***	***	***	***	***
Ratio		***	***	***	***	**	***
M × R		***	***	***	*	*	***

D_B: bulk density; WHC: water holding capacity; P_T: total pore space; Vair: air capacity; Vwater: water capacity; *, **, *** indicate statistically significant differences at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. Values in the same column with different letters are statistically different at $P \leq 0.05$ (Tukey test).

Table 3. Physico-chemical characteristics (pH and electrical conductivity, EC), total organic matter (OM), available (water extractable) nutrient content, and potential phytotoxicity measured by the cress and lettuce seed germination bioassays (germination index (GI), [40]) of growth media containing mixes of poultry manure composted without biochar (PMC), poultry manure composted with biochar (PMBC), biochar (B) and peat (P) at different ratios. Main effects and statistical significance according to factorial analysis of variance. Three replicates (n = 3) were used for each substrate and ratio for all parameters with the exception of Cress GI and Lettuce GI in which five (n = 5) replicates were used.

Substrate	Ratios (% v:v)	pH	EC (dS m^{-1})	OM (%)	NO_3—N (mg L^{-1})	NH_4^+-N (mg L^{-1})	P (mg L^{-1})	K (g L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})	Cress GI (%)	Lettuce GI (%)
PMC:B	100:0	9.2ef	10.6ab	45h	198b	233a	978b	17.7b	277b	156b	21d	25d
	75:25	9.5cd	8.6c	54g	171cd	175b	704d	13.1d	212c	120c	40cd	40cd
	50:50	9.7bc	7.0e	63e	144e	117d	528f	9.3f	159d	86d	60bc	55bc
	25:75	9.8ab	3.3gh	71d	116f	58e	266g	4.6h	86e	52e	100a	110a
	0:100	10.0a	0.8i	79c	89g	1g	20h	0.3i	32f	3f	110a	120a
PMC:P	100:0	9.2def	10.8a	44h	195b	230a	1000b	17.8b	280b	160ab	21d	25d
	75:25	8.6g	8.7c	58f	152de	173b	705d	13.1d	212c	120c	45bcd	43cd
	50:50	7.0j	6.9e	72d	110f	115d	530f	9.3f	164d	85d	65b	60bc
	25:75	6.8j	3.6g	85b	67h	58e	260g	4.6h	90e	51e	120a	110a
	0:100	4.2k	0.1j	98a	24i	1g	1i	0.01i	2g	5f	125a	125a
PMBC:P	100:0	9.5cde	10.3b	45h	316a	150c	1100a	19.1a	308a	176a	21d	55bc
	75:25	9.1f	7.7d	57fg	185bc	110d	770c	14.3c	227c	130c	50bc	65bc
	50:50	8.2h	5.7f	73d	153de	70e	575e	10.1e	172d	89d	70b	80b
	25:75	7.5i	3.2h	86b	82gh	32f	283g	5.0g	98e	64e	120a	110a
	0:100	4.2k	0.1j	97a	25i	1g	1i	0.01i	3g	4f	125a	125a
Main effects												
Mix	PMC:B	9.7A	6.1A	62B	144B	117A	499B	9.0B	154B	83B	75A	70B
	PMC:P	7.2C	6.1A	71A	111C	115A	499B	9.0B	150B	84B	66A	73B
	PMBC:P	7.7B	5.4B	71A	152A	73B	546A	9.7A	162A	93A	77A	87A
Ratio	100:0	9.3A	10.6A	44E	237A	204A	1026A	18.2A	288A	164A	21D	35C
	75:25	9.1B	8.4B	56D	171B	153B	726B	13.5B	218B	123B	45C	49BC
	50:50	8.3C	6.5C	69C	136C	101C	544C	9.6C	165C	87C	65B	65B
	25:75	8.1D	3.4D	80B	88D	50D	270D	4.7D	91D	56D	113A	110A
	0:100	6.1E	0.3E	91A	46E	1E	7E	0.1E	12E	4E	120A	123A
Significance												
Mix		***	***	***	***	***	***	***	***	***	Ns	**
Ratio		***	***	***	***	***	***	***	***	***	***	***
M × R		***	***	***	***	***	***	***	***	Ns	Ns	Ns

Ns, **, *** indicate not significant, statistically significant differences at $P \leq 0.01$, $P \leq 0.001$, respectively. Values in the same column with different letters are statistically different at $P \leq 0.05$ (Tukey test).

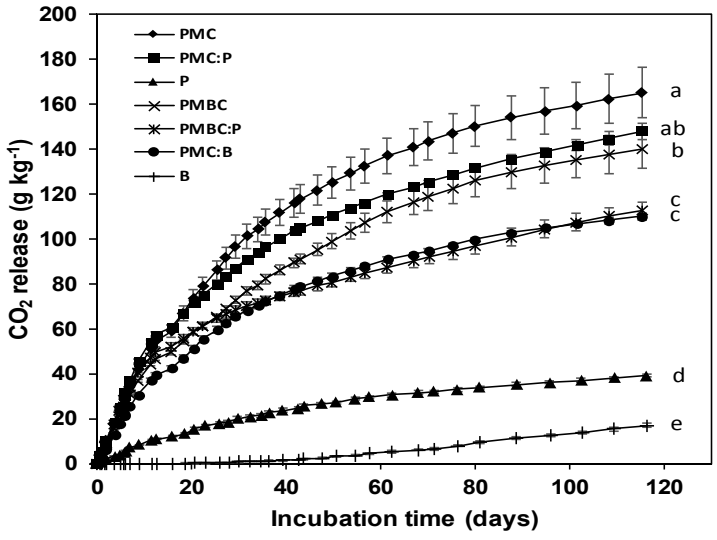

Figure 1. Cumulative CO_2 released from the materials (PMC = poultry manure composted without biochar; PMBC = poultry manure composted with biochar; P = peat; B = biochar) and their mixes (PMC:P, PMC:B and PMBC:P; 50%:50% by volume) during the incubation experiment. Three replicates (n = 3) were used for each substrate. Vertical bars in individual data indicate the standard error of the mean. Different letters indicate significant differences ($P < 0.05$) between means for the accumulated CO_2 released at the end of the experiment according to one-way ANOVA and LSD test.

Table 4. Cutting rooting (experiment I.CR) and plant growth (experiment I.PG) of *Rosmarinus officinalis* as affected by poultry manure composted without biochar (PMC), poultry manure composted with biochar (PMBC), and peat (P) containing growth media. Main effects and statistical significance according to factorial analysis of variance. Three replicates (n = 3) were used for each substrate and ratio.

Substrate	Ratios (% v:v)	Experiment I.CR Cutting Rooting			Experiment I.PG Plant Growth		
		Rooted Cuttings (%)	Shoot Dry Weight (mg)	Root Dry Weight (mg)	Shoot Length (cm)	Shoot Dry Weight (mg)	Root Size (Visual Rating Score; 1–4)
PMC:P	100:0	9cd	70fg	3cd			
	75:25	10cd	85de	1d			
	50:50	53b	112bc	20b	29a	2000a	2.1b
	25:75	89a	110c	35a	29a	2180a	2.7ab
	0:100	67ab	98cd	20b	21bc	540b	1.2c
PMBC:P	100:0	4d	60g	1d			
	75:25	22c	79ef	7c			
	50:50	78ab	125ab	36a	26ab	1870a	2.2b
	25:75	100a	128a	35a	24abc	1890a	2.9a
	0:100	69ab	100c	22b	19c	500b	1.3c
Main effects							
Material	PMC	46B	95A	16B	26A	1573A	2.0A
	PMBC	55A	98A	20A	23A	1420A	2.1A
Ratio	100:0	7C	65D	2C			
	75:25	16C	82C	4C			
	50:50	66B	119A	28AB	28A	1935A	2.1B
	25:75	95A	119A	35A	27A	2035A	2.8A
	0:100	68B	99B	21B	20B	520B	1.3C
Significance							
Material		**	Ns	*	Ns	Ns	Ns
Ratio		***	***	***	**	***	***
M × R		*	*	*	Ns	Ns	Ns

Ns, *, **, *** indicate not significant, statistically significant differences at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. Values in the same column with different letter are statistically different at $P \leq 0.05$ (Tukey test).

Table 5. Cutting rooting (experiment II.CR) and plant growth (experiment II.PG) of *Rosmarinus officinalis* as affected by growth media containing poultry manure compost mixed with peat (P) or biochar (B) at different ratios. Main effects and statistical significance according to factorial analysis of variance. Three replicates (n = 3) were used for each substrate and ratio.

Substrate	Ratios (% v:v)	Experiment II.CR Cutting Rooting			Experiment II.PG Plant growth		
		Rooted Cuttings (%)	Shoot Dry Weight (mg)	Root Dry Weight (mg)	Shoot Length (cm)	Shoot Dry Weight (mg)	Root Size (visual rating Score; 1–4)
PMC:P	100:0	9e	72d	4d			
	75:25	11e	87cd	1d			
	50:50	55d	115ab	22bc	30a	2020a	2.0ab
	25:75	90a	111abc	33ab	28a	2100a	2.6a
	0:100	68bc	99bc	22bc	20bc	520b	1.3b
PMC:B	100:0	10e	71d	4d			
	75:25	51cd	96bcd	19c			
	50:50	50d	106abc	28abc	26ab	1580a	2.0ab
	25:75	85ab	130a	36a	26ab	1530a	2.3a
	0:100	88a	118ab	37a	14c	320b	2.0ab
Main effects							
Material	P	47B	97B	16B	26A	1547A	2.0A
	B	57A	104A	25A	22B	1143B	2.1A
Ratio	100:0	10D	72C	4C			
	75:25	31C	91B	10C			
	50:50	53B	111A	25B	28A	1800A	2.0AB
	25:75	87A	121A	35A	27A	1815A	2.5A
	0:100	78A	109AB	29AB	17B	420B	1.7B
Significance							
Material		*	*	***	**	*	Ns
Ratio		***	***	***	***	***	**
M × R		**	Ns	**	Ns	Ns	Ns

Ns, *, **, *** indicate not significant, statistically significant differences at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. Values in the same column with different letter are statistically different at $P \leq 0.05$ (Tukey test).

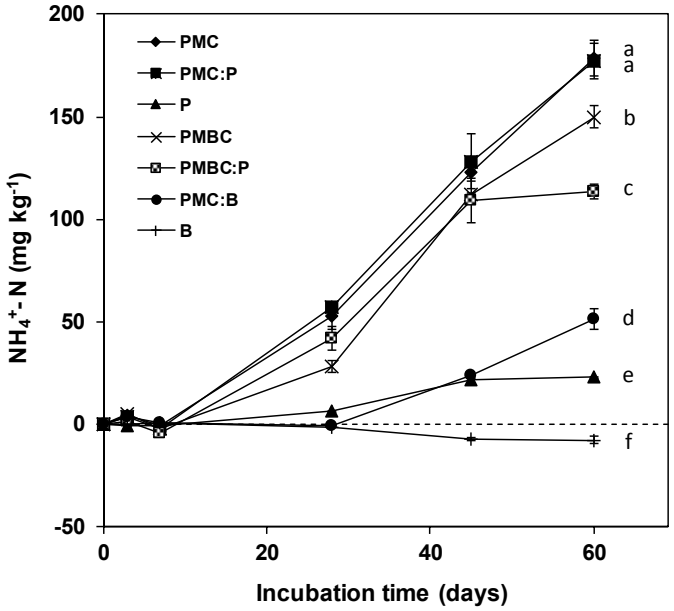

Figure 2. Concentrations of KCl- extracted ammonium (NH_4^+-N) from the materials (PMC = poultry manure composted without biochar; PMBC = poultry manure composted with biochar; P = peat; B = biochar) and their mixes (PMC:P, PMC:B and PMBC:P; 50%:50% by volume) during the incubation experiment. Three replicates (n = 3) were used for each substrate and date of analysis. Vertical bars in individual data indicate the standard error of the mean. Different letters indicate significant differences ($P < 0.05$) between means for NH_4^+-N released at the end of the experiment according to one-way ANOVA and LSD test.

3. Results

3.1. Physical Properties of the Growth Media

The main physical properties of the growth media are shown in Table 2. B had larger density (D_B) and aeration capacity (Vair), and lower porosity (P_T), water retention capacity (Vwater and WHC) and shrinkage than P. In the case of composts, PMBC had larger D_B and lower Vair than PMC, being both composts similar as for the other properties. Consequently, mixes of PMC with B had larger D_B and Vair, and lower WHC, Vwater, P_T and shrinkage than mixes of PMC with P, whilst mixes of PMBC with P had intermediate values for D_B, WHC, P_T and shrinkage, and the lowest values for Vair. Besides, the ratio at which each of the materials was present in the mixes affected the physical properties of the growth media significantly.

3.2. Chemical Characteristics and Germination Index of the Growth Media

Table 3 gathers the results of physicochemical and chemical characteristics of the growth media.

The pH was alkaline for B and for both composts and acidic for P. The mixes of PMC with B had an alkaline pH, whereas only some mixes with high proportion of P showed a pH close to neutrality. Both B and mainly P showed low ECs (0.1 to 0.8 dS m^{-1}) due to their low content in soluble minerals. On the contrary, composts had large amounts of minerals which accounted for their high EC (10.3 to 10.8 dS m^{-1}). In the mixtures, EC and the concentration of nutrients decreased with increasing proportions of P and B. The mixes of PMBC:P had more NO_3^--N, P, K, Ca, and Mg, and less NH_4^+-N than the mixes of PMC:B and PMC:P. Organic matter was 97% for P, 79% for B, and 45% for the composts. Accordingly, the mixes containing P had more OM than the others and OM increased in the mixes as B and mostly P increased in the mix.

Both cress and lettuce bioassays showed the largest GI for B, P and their 25:75 mixes with compost, but GI decreased progressively as the proportion of PMC increased in the mixes, proving phytotoxic

when the proportion of PMC was larger than 50%. Nevertheless, the lettuce bioassay showed that PMBC was less phytotoxic than PMC, especially at high percentages (above 75% of compost in the mix).

3.3. Stability to Microbial Degradation of the Growth Media

The microbial stability of the different growth media was assessed by their cumulative CO_2 release (Figure 1; C mineralization) and the changes in NH_4^+ concentration (Figure 2; N mineralization) of the selected materials (B, PMC, PMBC, P) and their mixes (PMC:P, PMBC:P, and PMC:B (50:50, % v:v)) during an incubation experiment.

The largest CO_2 respiration was found in both composts and the mix of PMC with P, reflecting the lower stability of the composts compared to P and B. The presence of B, both as part of PMBC or combined with PMC, led to significantly lower respiration rates compared to PMC and PMC:P, respectively. With respect to N mineralization, the largest release of NH_4^+ was observed in PMC and the mix PMC:P. NH_4^+ release was low in P and negligible in B. Media containing B had intermediate values in this order: PMBC released more NH_4^+ than PMBC:P and both released more NH_4^+ than PMC:B.

3.4. Experiment I: Comparison of Poultry Compost (PMC) Versus Poultry Manure Compost Co-Composted with Biochar (PMBC)

Table 4 shows the results of cutting rooting and plant growth assays in experiment I, where different mixes of PMC or PMBC with P were compared. In the cutting rooting assay (Experiment I. CR), mixes containing from 0% to 100% of either compost were assayed. In the plant growth assay (Experiment I, PG), only mixes containing 50% or less of PMC or PMBC were considered, since both germination indices (Table 3) and the cutting rooting assay (Experiment I, CR) showed phytotoxicity for mixes containing more than 50% compost.

In the CR assay, the percentage of rooted cuttings and the growth of adventitious roots were greater for PMBC than for PMC. However, shoot development was similarly affected by both composts. In the PG assay, shoot and root growth showed no difference between composts. The compost:peat ratio affected shoot and root growth both in the CR and in the PG experiments. The ratios 25:75 and 50:50 compost:peat produced the best results. In the case of the CR experiment, cuttings growing with high proportions of composts (75% to 100%) were virtually unable to develop adventitious roots, which made these media unacceptable for plant cultivation.

Table S1 shows the results of chlorophyll and nutrient contents of rosemary shoots. The presence of compost in the growth media increased the contents of P, K, and Mg, and decreased that of N and chlorophyll in shoots. The type of compost only affected the amount of chlorophyll (lower in PMBC than in PMC) and the K content (larger in PMBC than in PMC).

3.5. Experiment II. Comparison of Biochar (B) versus Peat (P) in Mixes with Poultry Manure Compost (PMC)

Table 5 shows the results of cutting rooting and plant growth assays in experiment II, where mixes of PMC with either B or P were compared. In the CR assay, the largest percentage of rooting and the largest shoot and adventitious root growth were found in B and in 25:75 mixes of both B and P. At high proportion of PMC (75%), mixes with B performed better than the mixes with P for these three parameters. The 100% PMC thwarted the rooting of cuttings almost completely, giving low shoot and adventitious root weight. Consequently, mixes with biochar yielded better results in the CR assay than mixes with peat. However, in the PG assay shoot growth benefited from having P in the mixes rather than B, although the root ball VR was equivalent in both types of mixes. The presence of PMC in either 25:75 or 50:50 mixes increased shoot length, shoot dry weight, and root size.

Table S2 shows the results of nutrient contents of rosemary shoots in experiment II. The presence of compost in the mix increased the concentration of P, K, and Mg in tissues and reduced that of Ca. The concentration of chlorophyll decreased when compost was present at 50%. When comparing the effect of biochar to that of peat in the substrate, P and K concentrations were enhanced by peat whereas

chlorophyll, Ca and Mg increased in the rosemary shoots grown in the substrates containing biochar. N was lower in B and in the 50% PMC:P mix than in the other treatments.

4. Discussion

Our initial hypothesis proposed that the use of biochar, either as an additive for the preparation of compost or as growing media constituent, might reduce compost phytotoxicity, improve its physicochemical properties as growing media and enhance its performance for rosemary cultivation. Our results proved most of this hypothesis.

On the one hand, the use of biochar as composting additive only had a minor impact on the properties of the compost. This may be related to the low percentage of biochar (3%) in the starting composting mix. PMBC had significantly larger D_B and pH, and lower P_T, Vair, and EC than PMC. However, the differences between both composts were small and did not have agronomic relevance. The characteristics of both composts indicated low quality when compared to the adequate ranges (AR) recommended for potted plant cultivation. The AR recommended by Maronek et al. [38] for cutting rooting media were Vair between 15% and 40% (ideally 20–25%), Vwater between 20% and 60%, and EC about 0.2 dS m^{-1}, and these parameters in both composts had values outside these ranges. Additionally, for plant growth, Bunt [37] recommended values for Vair between 20–30%, Vwater between 55–70%, D_B < 400 kg m^{-3}, P_T > 85% and EC from 0.75 to 3.5 dS m^{-1}, and, in our case, these parameters for both composts had values outside or close to AR, PMBC presenting worse indicators than PMC. Another negative characteristic of both composts was the shrinkage that they suffer when the growth medium is subjected to the wetting and drying cycles typical of xerophyte species cultivation.

As expected, both composts were phytotoxic as shown by the low GI values (Table 3) [40] and the rosemary cutting rooting assays (Tables 4 and 5), although the lettuce seed germination bioassay showed less phytotoxicity in PMBC than in PMC. The phytotoxicity of these composts could be due to a single factor or to several factors acting together. One of these factors may well be their remarkably high salinity (Table 3) which values were well above the AR recommended for rooting or plant growth. In fact, a negative correlation between EC and the percentage of rooted cuttings of *Rosmarinus* [45], *Euonymus*, and *Lavandula* [46] has been demonstrated. Another factor that probably contributed to phytotoxicity was the large amount of NH_4^+ in the composts, which was even larger than that of NO_3^- (Table 3). High amounts of NH_4^+ lead to the so-called ammonium syndrome, which shows through several stress symptoms (leaf chlorosis, growth reduction, ionic imbalances, oxidative stress, metabolic alterations, etc.) [47]. Although the threshold for NH_4^+ depends on the plant species [48], the amounts recorded in both composts fully justify the occurrence of phytotoxicity [49]. Besides, in the incubation experiment (Figure 2) the amount of NH_4^+ in the composts increased over time, probably due to bacterial ammonification activity. Related to this, both composts showed an intense microbial activity (CO_2 emitted through respiration; Figure 1). In this sense, it was remarkable that PMBC had lower initial ammonium content (Table 3), produced less ammonium (Figure 2) and emitted less CO_2 (Figure 1) in the incubation experiments than PMC. The possible presence of other phytotoxic elements in the composts has not been determined in this study. However, after analyzing heavy metal content of these composts, Sánchez-García et al. [28] classified them as class 2 due to the content of Zn, which was beyond the limit for class 1 composts [50]. This means that these composts cannot be use as the sole material to grow edible plants but may be used in mixes with other materials. In relation to this, biochar has been found to decrease the availability of Zn [9].

As peat had adequate D_B, P_T, and Vair, and an acid pH, the mixes of both composts with P resulted in improved values of these characteristics in comparison with the pure composts. However, EC was excessive even in the mixes containing as little as 25% of compost. The effects of mixing P with the composts were of similar magnitude for both composts. Nevertheless, a differential element between the PMBC:P and the PMC:P mixes was related to their ammonium content.

PMBC:P had lower initial ammonium content (Table 3), produced less ammonium (Figure 2) and emitted less CO_2 (Figure 1) in the incubation experiments than PMC:P. This agrees with the cutting rooting results, in which PMBC-based growth media performed better than PMC-based media (Table 4), in accordance with the fact that ammonium at high concentration inhibits primary root growth [49].

With respect to the plant growth assay (Table 4 and Table S1), no differential effect between the mixes of both composts with peat was found. Both composts supplied extra nutrients (Table 3) to the mixes and plants grew more in them than in the pure peat medium. Plants in the PMC mixes contained, on average, more chlorophyll and less K than those in PMBC mixes. Nevertheless, these differences were small and non-significant when we compared the same ratios for both composts. In any case, our results indicated that neither of the two composts ought to be used as growth media constituents at high ratio (larger than 50% in volume).

On the other hand, when we compared B with P as constituents of mixes with PMC (Table 5 and Table S2), we obtained contrasting results. While B improved the rooting of cuttings and the early growth of shoots and of new adventitious roots in comparison with P, P was more efficient than B for growing adult plants as shoots, although not roots, were larger in the P-containing media than in the B-containing ones. The physical properties of the growth medium are relevant for containerized soilless plant cultivation. Both B and P produced light growth media (decreased bulk density; D_B), although P did it to a larger extent than B. This has a practical repercussion because the lighter the substrate the easier the handling of containerized plants. Total porosity (P_T) and D_B are usually inversely related [30]. This was the situation for the P-containing media, which showed larger P_T than the B-containing media. As important as P_T is the pore distribution between those occupied by water (Vwater) and those occupied by air (Vair) [37]. Pore distribution correlates with pore size, which is dependent on particle size [30]. The amount of small particles within the range which has negative effect on aeration and favors water retention (0.125 to 1 mm diameter [30]) was larger for P than for B (data not shown). This might explain why P-containing media had larger Vwater and lower Vair than B-containing ones. Remarkable was also the fact that B reduced the shrinkage of the media whilst P did not. This might be related to the different nature, origin and characteristics of B and P. Biochar from hard wood is an organic material (79% OM; Table 3) yet, it is hard and acts like a non-deformable rocky material. Peat, on the contrary, is a boggy, spongy, and deformable organic material (98% OM; Table 3). From the physical point of view, biochar is a recalcitrant, hard to decompose and stable material. Contrary to biochar, peat decomposes during cultivation due to its non-stable physical properties [18]. Specifically, rooting media must provide the appropriate physical conditions for proper adventitious root formation [51]. In this sense, maintaining the correct moisture whilst permitting aeration is crucial. Based on Maronek et al. [38]'s recommendations, B and the B-containing media had adequate values for those parameters related to aeration (Vair) and water availability to plants (Vwater) (Table 2) whilst media containing P had too large Vwater and too low Vair. These physical factors might have contributed to the better performance of the B-containing media in comparison with the P-containing ones, and to the poor results of media containing high proportions of compost. In our experiment, the EC decrease in media containing both B and P might be related to the improvement of rooting but this cannot be the differential effect of both materials on rooting because both decreased the EC of the growth media similarly. Moreover, the most relevant element contributing to EC was K (Table 3) and this element is rapidly leached from the medium with irrigation, as has been previously shown [52]. Neither was pH the cause of the better performance of the B-containing media since they had inadequate pH values whilst P-containing media had adequate values for this parameter. However, it was relevant that the mix of PMC with B reduced the release and accumulation of NH_4^+ in the incubation experiment in comparison with PMC and the mix of PMC with P (Figure 2). This effect of B might be due to a decrease in microbial activity (decrease in the ammonification activity of bacteria) as the decrease in microbial respiration caused by B suggests (Figure 1), or might be due to the sequestration of NH_4^+ by biochar as indicated by Laird et al. [31]. With respect to the better growth (larger shoot) of adult plants in the P-containing media than in

the B-containing ones, the cause does not appear to rely on the physical properties of the media. In fact, a relevant parameter for containerized plant production, such as aeration, was closer to the AR indicated by Bunt [37] (Vair = 20–30%) for the B-containing media than for the P-containing ones. Other physical parameters were also inside or close to AR in all the growth media assayed. The reason for the difference might lie in the nutritional factors. pH was higher in B-containing media than in P-containing ones. This factor stands out as an element that might affect plant growth through its role on nutrient solubility and availability [53], which determines plant nutrient status. In this sense, *Rosmarinus officinalis* has been described as a non-strict calcicole species [54]. This means that its growth is favored in calcareous soils but it is also able to grow well in slightly acidic soils. In fact, Fornes and Belda [22] reported that an acidified biochar (pH = 7.0) performed better than a raw alkaline biochar (pH = 9.3) for the growth of rosemary. The comparison of the nutritional status of the plants in our experiment (Table S2) with the sufficient range (SR) reported for rosemary (2.09–2.52% for N; 0.26–0.35% for P; 2.36–2.55% for K; 0.48–0.69% for Ca; 0.17–0.40% for Mg) [55] indicates that the supply of nutrients by the growth media was sufficient for the majority of elements. The only exception was N which was below the SR in all cases. Our argument that plants were fed better by the P-containing media than by the B-containing ones was based on the fact that the amount of nutrients taken by plants in absolute terms (nutrient concentration (Table S2) x biomass (Table 5)) was larger in the P-containing media than in the B-containing ones. Moreover, the absolute amount of nutrients did not involve a dilution of nutrients (concentration reduction) in the tissues due to increased growth.

5. Conclusions

The most relevant conclusion of this study is that oak biochar performed better than peat for the rooting of cuttings for clonal propagation of rosemary. According to our results, the best option in designing peat-free substrates for rosemary clonal propagation would be to use compost based substrates containing 25% biochar. For some horticultural purposes, this opens the possibility to substitute peat, which is a non-renewable material, in the formulation of growth media. It is also noteworthy that the amendment of poultry manure with the small amount of biochar (3%) used in the preparation of PMBC, though not affecting the physico-chemical quality of the compost, enhanced rosemary cutting performance. In this sense, it would be advisable to try larger ratios of biochar in the composting pile of poultry manure. Both biochar and peat allowed the use of large amounts of poultry manure compost in the substrate (up to 50% v:v), which would otherwise be phytotoxic. This enables a means to reclaim this waste and to recover significant amounts of nutrients for plants.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/2/261/s1, Table S1: Chlorophyll and nutrient concentrations in shoots of *Rosmarinus officinalis* as affected by the proportions of poultry manure composted without biochar (PMC), poultry manure composted with biochar (PMBC), and peat (P) in the growth media. Main effects and statistical significance according to factorial analysis of variance. Three replicates (n = 3) were used for each substrate and ratio, Table S2: Chlorophyll and nutrient concentrations in shoots of *Rosmarinus officinalis* as affected by the proportions of poultry manure compost (PMC), peat (P), and biochar (B) in the growth media. Main effects and statistical significance according to factorial analysis of variance. Three replicates (n = 3) were used for each substrate and ratio.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used: Conceptualization, F.F. and R.M.B.; Methodology, F.F., R.M.B., and A.L.; Validation, F.F., R.M.B., A.L., M.S-G., M.L.C., and M.A.S.-M.; Formal Analysis, F.F., R.M.B., A.L., and L.L.-X.; Investigation, L.L.-X. and A.L.; Resources, M.S.-G., M.A.S.-M., and M.L.C.; Data Curation, F.F., R.M.B., A.L., and L.L.-X.; Writing—Original Draft Preparation, F.F., R.M.B., and A.L.; Writing—Review and Editing, F.F., R.M.B., A.L., M.S.-G., M.L.C., and M.A.S.-M.; Visualization, F.F., R.M.B., A.L., and L.L; Supervision, F.F. and R.M.B.; Project Administration, F.F. and R.M.B.; Funding Acquisition, M.A.S.-M. and M.L.C. All authors have read and agreed to the published version of the manuscript.

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