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RESEARCH ARTICLE

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ABSTRACT

The objective was to develop efficient substrates, with increasing proportions of biochar derived from anaerobic sewage sludge (ASS-biochar) and regionally available raw materials, to produce tobacco seedlings (*Nicotiana tabacum*) in floating systems. Physical, hydraulic and chemical variables and the effects on tobacco plants were evaluated through an indoor experiment with seedling production. The experiment was conducted on trays in a floating hydroponic system in a randomized block design with four replications and eight plants per plot. The ASS-biochar showed environmental safety and agronomic efficiency in substrate composition, in particular with 50% to 90% ASS-biochar in the formulation. The best formulations positively affected the growth-related variables, showing similar or superior performance to the three commercial substrates (references). The ideal ranges of the substrate characteristics were: dry density: 420–520 kg m^{-3}; total porosity: 60–75%; aeration space: 25–35%; easily available water: 9–12%; water buffering capacity: 3–5%; available water: 12–15%; remaining water: 20–25%; pH: 6.0–6.5; electrical conductivity: 1.4–2.3 mS cm^{-1}. The development of substrates considering ASS-biochar, regional raw materials and ideal physical-chemical properties would prevent environmental pollution by generating value-added products to improve tobacco seedlings and expand the productivity and quality of tobacco worldwide.

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Substrate formulation; *Nicotiana tabacum* seedlings; agronomic performance; environmental safety; waste management in agriculture

Introduction

Tobacco cultivation plays a significant role in generating income for smallholder farmers. Asia and the Americas are the world's largest tobacco producers, with 64.4% and 21.0% of the production registered in 2018, respectively. Among the producing countries, China, Brazil and

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India stand out with 36.8%, 12.6% and 12.3% of the total produced in 2019 (FAO 2020). With a cultivated area of 297,000 ha and an annual production of 664,000 tons in 2019, Brazil is the world's largest tobacco exporter (FAO 2020). In the 2018/2019 cropping season, this sector generated approximately 1 billion USD in Brazil, highlighting the importance of the tobacco chain for the agricultural sector.

Like other horticultural crops, the tobacco farming requires specific techniques, such as the pre-cultivation of seedlings in indoor floating systems, meaning the cultivation of seedlings in hydroponic floating trays filled with an appropriate substrate containing nutrient solution for the initial phases of the culture (Monteiro et al. 2020). Bearing in mind that the successful establishment of tobacco plants depends on the seedlings and transplantation to field conditions, the quality of the seedlings is crucial for the development and high quality of leaves at harvest.

The substrate must ensure ideal conditions for plant development and biomass production. For that reason, the physical, chemical and biological properties of the raw materials are crucial. Problems with phytotoxicity of the components of the formulation, inadequate particle size, chemical-nutritional imbalance and sanitary aspects often limit the good development of tobacco seedlings. The formulation of substrates is generally performed through the combination of regionally available organic residues (Monteiro et al. 2021) with the ability to provide suitable physical and chemical conditions for good plant development (Costa et al. 2015). Carbonized rice husk has been used in the formulation of most commercial substrates in Southern Brazil. Studies have demonstrated the viability of this waste as a raw material for substrates (Monteiro et al. 2021). On the other hand, there are difficulties in finding raw materials to substitute peat, a key substrate component that is non-renewable and increasingly scarce in tropical and subtropical countries (Regni et al. 2020).

Recent studies have demonstrated the effectiveness of using the carbonized anaerobic sewage sludge (ASS, also known as ASS-biochar) in several promising applications, such as in pollutant retention (Xiao et al. 2021), as a phosphorus fertilizer (Frišták et al. 2018) as a growth media for forest seedlings (Silva et al. 2017), among others. Another possible application is the use of ASS-biochar as a component of plant substrates, as it has similar physical and chemical characteristics to peat and is suitable for the satisfactory development of seedlings (Siqueira et al. 2019; Silva et al. 2020; Monteiro et al. 2021).

Despite this, none of the aforementioned studies evaluated the performance of formulations based on ASS-biochar in floating systems. Moreover, optimal ranges for the physical and chemical properties of substrates would be important to guide new formulations for tobacco seedling production.

In this way, the objective of the study was to develop efficient substrate formulations based on optimal proportions of ASS-biochar and evaluate their agronomic performance in the production of tobacco seedlings (*Nicotiana tabacum*) in a floating system.

Materials and methods

The study was conducted in a greenhouse, located at Embrapa Clima Temperado – Terras Baixas Experimental Station (31° 49′ 13′′ S and 52° 27′ 50′′ W), Capão do Leão, State of RS, Brazil. The ASS was obtained from the Sewage Treatment Station of Passo Fundo-RS (28° 13′ 18.64′′ S and 52° 22′ 07.13′′ W). After collection, a 0.1 m thick layer was distributed in fiberglass boxes inside an agricultural greenhouse covered by translucent plastic film (0.2 mm) until reaching 20% moisture. After drying, the ASS was subjected to pyrolysis in a prototype of biomass carbonization with a static capacity of 150 L. The process took place with a partial supply of air and variable temperature (300–600°C) for 3 h. After pyrolysis, the biochar had its granulometry standardized in particles smaller than 2.0 mm through grinding and sieving. The ASS-biochar was characterized in relation to pathogenic, inorganic contaminants and chemical, physical and hydraulic characteristics. The resulted batches of

Table 1. Physical, hydraulic and chemical characterization, inorganic and pathogenic contaminants from the anaerobic sewage sludge biochar, standard mixture, carbonized rice husks, vermiculite and organic compost.

Parameters	Anaerobic sewage sludge-biochar	Standard mixture[a]	Carbonized rice husk	Vermiculite	Organic compost	Limits of Brazil (2006)[b]	Limits of Brazil (2016)[b]
			Physical and hydraulic attributes				
Wet density (kg m^{-3})	788.7	467.2	385.9	489.8	823.8	-	-
Dry density (kg m^{-3})	598.3	307.1	249.1	265.6	630.7	-	-
Total porosity (%)	58.2	63.6	70.9	77.3	66.9	-	-
Aeration space (%)	22.6	39.2	46.9	17.9	17.6	-	-
Easily available water (%)	7.8	6.2	10.4	9.1	16.7	-	-
Water buffering capacity (%)	4.0	2.3	3.8	3.0	5.3	-	-
Available water (%)	11.8	8.5	14.2	12.1	21.9	-	-
Remaining water (%)	24.7	16.6	10.7	48.2	28.2	-	-
			Chemical attributes				
pH	4.7	7. 6	7.8	6.6	7.2	-	-
EC (mS cm^{-1})	2.3	0.4	0.2	0.1	3.1	-	-
Total nitrogen (mg kg^{-1})	1,173	111	953	2,126	4,834	-	-
Total phosphorus (mg kg^{-1})	8,921	4,805	474	67	15,577	-	-
Total potassium (mg kg^{-1})	578	2,084	2,437	1,379	6,886	-	-
Total calcium (mg kg^{-1})	4,252	16,478	1,016	1,096	79,201	-	-
Total magnesium (mg kg^{-1})	1,073	15,020	724	123,865	5,717	-	-
Total sulfur (mg kg^{-1})	13,092.3	6.0	3.3	<0.5	11.6	-	-
Total copper (mg kg^{-1})	111.9	12.8	2.1	19.4	55.3	-	-
Total zinc (mg kg^{-1})	405.8	67.5	98.6	28.6	105.3	-	-
Total iron (mg kg^{-1})	51,726	5,564	419	6,965	9,556	-	-
Total manganese (mg kg^{-1})	92	183	170	412	387	-	-
Total sodium (mg kg^{-1})	60	424	278	151	1,862	-	-
Total boron (mg kg^{-1})	5.2	<6.1	<5.3	37.8	<6.6	-	-
Total aluminum (mg kg^{-1})	21,643	10,272	235	47,685	7,875	-	-
			Inorganic contaminants				
Total arsenic (mg kg^{-1})	10.0	3.5	<0.3	<0.3	3.3	41.0	20.0
Total barium (mg kg^{-1})	113.0	59.0	6.0	<0.3	161.6	1,300.0	-
Total cadmium (mg kg^{-1})	<0.2	<0.3	<0.2	<0.2	<0.2	39.0	8.0
Total lead (mg kg^{-1})	39.6	3.3	<0.3	<0.3	7.5	300.0	300.0
Hexavalent chromium (mg kg^{-1})	<0.2	5.1	5.5	0.8	2.0	-	-
Total mercury (mg kg^{-1})	<0.01	< 0.02	<0.01	<0.01	<0.01	17.00	2.50
Total molybdenum (mg kg^{-1})	4.5	<0.5	<0.3	<0.3	0.9	50.0	-
Total nickel (mg kg^{-1})	10.0	203.6	0.7	<0.3	8.0	420.0	175.0
Total selenium (mg kg^{-1})	<0.3	<0.5	<0.3	<0.3	<0.3	100.0	80.0
			Pathogenic contaminants				
Thermotolerant Coliforms (Most Probably Number g Solid $Matter^{-1}$)	Absent	Absent	Absent	Absent	Absent	<1,000	1,000
Viable helminth eggs (n° g of Total $Solids^{-1}$)	Absent	Absent	Absent	Absent	Absent	<0.25	1.00
Salmonella sp	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Enteric viruses (g Total $Solids^{-1}$)	Absent	Absent	Absent	Absent	Absent	<0.25	-

[a]Standard mixture = 33.3% compost + 43.3% carbonized rice husk + 23.3% vermiculite.
[b]Limits of the contaminants allowed by the Brazilian legislation for the destination of biosolids in soils (Brazil 2006) and for the plant substrates (Brazil 2016).

carbonized ASS (ASS-biochar) met all the environmental requisites, since the results were under the maximum limits allowed by the current Brazilian legislation related to biosolids (Brazil 2006) and plant substrates (Brazil 2016), as shown in Table 1.

Substrate formulations were made from ten increasing proportions of ASS-biochar (10%-100%; S1-S10) and compared with three commercial substrates (S11-S13), used as references. Except for S10, the formulations with ASS-biochar (S1-S9) were complemented with a 'standard mixture' (SM) formed of commercial organic compost (constitution: bark and tree pruning waste, wood shavings, food waste, pulp and bagasse of citrus fruits, hatchery waste, sludges from wastewater treatment plants of the beverage, dairy, cellulose and slaughterhouse industries, among others), carbonized rice husk and fine vermiculite (< 3.0 mm) in the proportion of 33.3: 43.3: 23.3 (mass: mass), respectively. A characterization of the physical, hydraulic, chemical attributes and inorganic and pathogenic contaminants of SM and its components are shown in Table 1.

The commercial substrates (references) had the following compositions: a mixture of agro-industrial organic wastes like seeds, grape stalks and bagasse, ash, peat and carbonized rice husk (commercial 1); pine bark, vermiculite, dolomitic limestone and macronutrients (commercial 2); and sphagnum peat, expanded vermiculite, carbonized rice husk, dolomitic limestone, gypsum, NPK fertilizer and micronutrients (commercial 3). The evaluated substrates were: S1- 10% anaerobic sewage sludge (ASS)-biochar + 90% 'standard mixture' (SM) (mass: mass); S2- 20% ASS-biochar + 80% SM; S3- 30% ASS-biochar + 70% SM; S4- 40% ASS-biochar + 60% SM; S5- 50% ASS-biochar + 50% SM; S6- 60% ASS-biochar + 40% SM; S7- 70% ASS-biochar + 30% SM; S8- 80% ASS-biochar + 20% SM; S9- 90% ASS-biochar + 10% SM; S10- 100% ASS-biochar; S11- commercial 1; S12- commercial 2 and S13- commercial 3.

The physical and hydraulic characterizations of the evaluated substrates were carried out at the Soil Physics Laboratory of Embrapa Clima Temperado, where the following variables were determined: wet density (WD), dry density (DD), total porosity (TP), aeration space (AS), easily available water (EAW), water buffering capacity (WBC), available water (AW) and remaining water (RW). Wet and dry densities of the substrates were determined by the self-compacting method as described by Brazil (2007). The variables TP, AS, EAW, WBC, AW, and RW were determined according to De Boodt and Verdonck (1972). TP corresponds to the volume of water retained at the saturation point (0 kPa), AS is represented by the volume of pores drained from 0 kPa to 1 kPa, EAW is the volume of pores drained between 1 and 5 kPa, WBC corresponds to the pore volume drained between 5 and 10 kPa, AW represents the volume of drained within the range of 1 to 10 kPa, and RW is the volume of pores at a suction of 10 kPa.

The pH and Electrical Conductivity (EC) of the substrates were determined according to Brazil (2007). The pH was measured with a pH meter, model HI 2221 (HANNA Instruments, São Paulo – Brazil), and the (EC) with a Tec-4MP bench meter (Tecnal Scientific Equipment, São Paulo – Brazil). To analyze the total content of macro and micro nutrients in the substrates, samples were sent to an accredited laboratory (NBR ABNT ISO/IEC 17025:2005; Alac-Eurofins Brazil). To do these analyses, the samples were submitted to acid digestion (USEPA 1996), followed by determinations by ICP-AES (USEPA 2007) for inorganic metals and by ion chromatography (USEPA 1997) for inorganic non-metallic anions. Total organic Nitrogen – Kjldahl was determined through the 4500-Norg A/C Method, described in APHA (2017).

The performance of the substrates was evaluated through a crop experiment with tobacco seedlings in hydroponic floating trays in a greenhouse between July and August 2017. The experimental design was the randomized block design, with four replications and eight plants per plot. The substrates were distributed in Styrofoam trays (128 cells of 110 cm^3 each). Sowing was carried out by introducing two commercial tobacco seeds of cultivar Virginia per cell, with subsequent thinning to acquire one standard plant per cell. From this moment onwards, the trays were kept in the floating system. The floating system consisted of the maintenance of all trays floating inside fiberglass boxes filled with deionized water (water level = 200 mm). A liquid fertilizer solution (a commercial formula designed specifically for tobacco seedlings) was applied to overcome main macro and micronutrient deficiencies and imbalances. All plants were evaluated 44 d after emergence, the ideal point for transplantation.

The evaluated response variables were: number of leaves, length of the root system (LRS), dry mass of the aerial parts (DMAP), dry mass of roots (DMR), total chlorophyll index and total leaf area. The number of leaves was counted manually, while LRS (mm) was measured with a graduated ruler in the eight seedlings in each plot. DMAP and DMR were determined in individual plants after they were divided, packed in paper bags and dried out at 65 °C ± 1°C for 72 h. The total chlorophyll index was evaluated in three leaves per plant, using a portable chlorophyll meter (ClorofiLOG). The total leaf area of the plants was obtained by scanning images of all leaves of each plant (72 dpi). Subsequently, the digital images were processed with the AFSoft program (Silva and Jorge 2009).

The data sets from the characterization of the substrates and from the agronomic experiment were evaluated for normality of their probability distributions and checked for outliers, then subjected to analysis of variance (ANOVA). The data of each response variable with significant treatment effects (F test, $p < 0.05$) were submitted to polynomial regression analysis. The statistical analyses were performed with SigmaPlot software v. 11.0.

Results

Physical and hydraulic characterization of the substrates

Increasing the proportion of biochar in the substrates caused a linear increase in wet (Figure 1(a)) and dry densities (Figure 1(b)) as well as a linear reduction in total porosity (Figure 1(c)) and aeration space (Figure 1(d)). This result was expected since sewage sludge biochar has a higher bulk density than plant-based biochar. Considering the variables related to water retention, only water buffering capacity (Figure 1(f)) was positively influenced by the rate of biochar in the formulation, while easily available water (Figure 1(e)), available water (Figure 1(g)) and remaining water (Figure 1(h)) were not influenced.

The increase in biochar reduced TP (Figure 1(c)). However, the substrate with 20% biochar and S13 showed values within the recommended range (80–90% – Figure 1(c)). In relation to AS, a linear decrease was also observed as the proportions of biochar were increased (Figure 1(d)), and only the formulations with between 70 and 100% biochar and S13 among the commercial substrates presented AS considered adequate (20–30%). Considering the hypothesis that a high amount of ASS-biochar results in a high dry density (DD), such substrate formulations would also have a low TP and AS. In this sense, a possible solution to overcome this problem is to increase the amount of vermiculite (soft material) in the standard mixture.

Both biochar-based (S1-S10) and commercial substrates (S11-S13) showed EAW values below the range considered ideal (20–30%) (Figure 1(e)). For WBC, the substrates with 70 and 90% biochar and commercial S11 and S12 showed values within the recommended range (4–5%) (Figure 1(f)). It was also observed that both biochar-based and commercial substrates showed AW values below the range considered ideal (24–35%).

The obtained RW values were within the recommended range (20–30%) among the biochar-based substrates (Figure 1(h)). On the other hand, among the three commercial substrates, only the substrate S11 showed RW values within the recommended range, while the others (S12 and S13) showed higher values (Figure 1(h)). However, RW is not easily accessible to plants, as it is retained at higher tensions (above 10 kPa).

Chemical composition of the substrates

The pH of the substrates showed a quadratic response with the increase in the proportions of biochar (Figure 2(a)). The substrates with 60 to 90% biochar, in addition to S13, showed pH values within the recommended range for most crops, including tobacco (pH 6.0). The substrates with the lowest proportions of biochar (10–50%) had pH values higher than regionally recommended (pH > 6.5), probably due to the components of the SM, mainly composed of organic compost, carbonized rice husk and vermiculite, with pH values of 7.2, 7.8 and 6.6, respectively (Table 1). On the other hand, the substrate with 100% biochar as well as the commercial S11 and S12 had pH values below the range considered ideal for most cultures (Figure 2(a)). The EC increased linearly as the proportion of ASS-biochar increased (Figure 2(b)).

Increasing the proportion of biochar had no significant effect on the N concentrations of the substrates S1 to S10 (Figure 2(c)), while P followed a quadratic response (Figure 2(d)). The proportion of biochar that allowed the highest P content was 77%. The P concentrations of all ASS-biochar-based substrates were higher than those obtained in the three commercial substrates (S11, S12 and S13) (Figure 2(d)), demonstrating that the biochar is an important source of P.

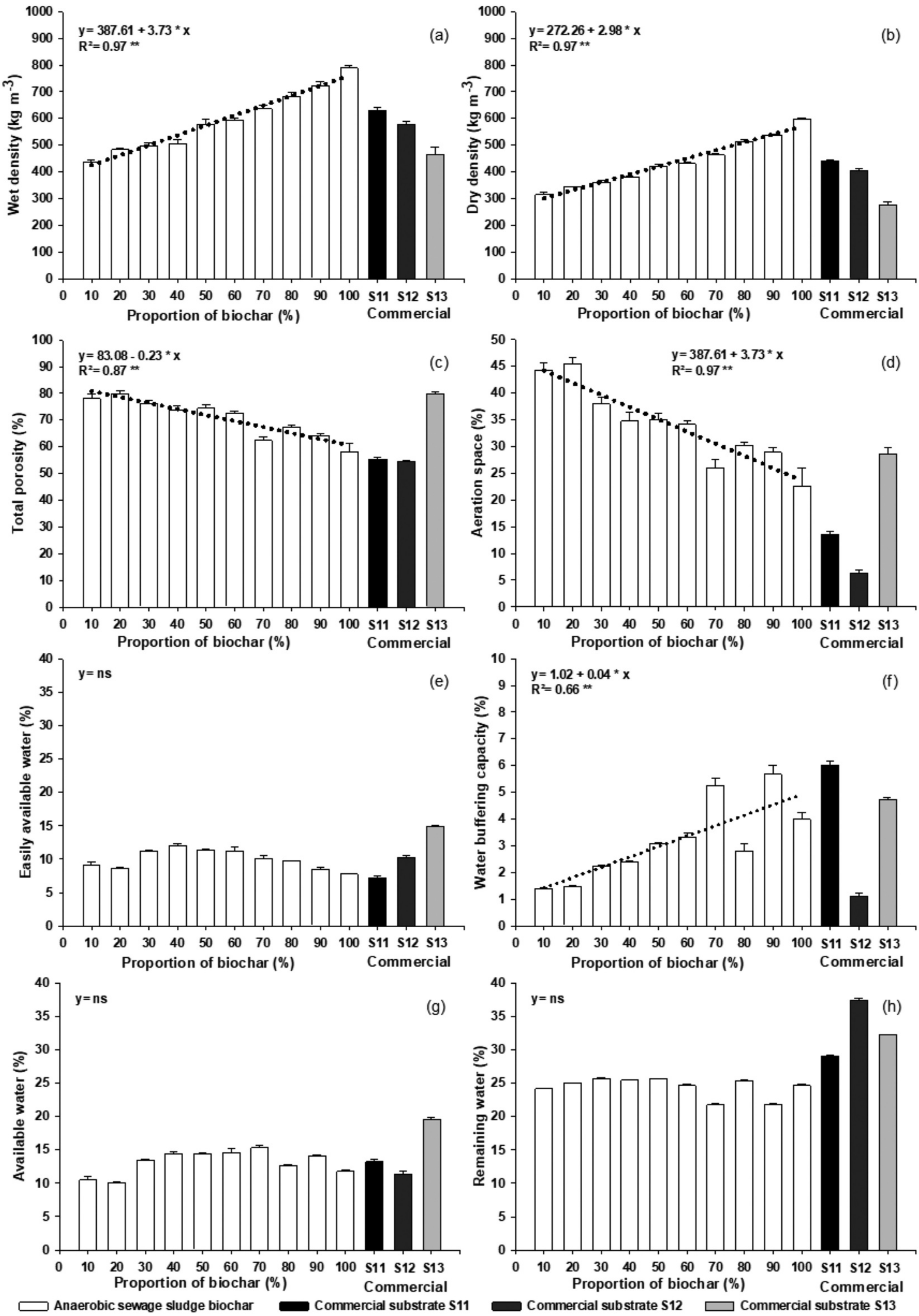

Figure 1. Physical and hydraulic parameters in the substrates with anaerobic sewage sludge biochar and three commercial substrates. ns: Polynomial regression not significant at $p < 0.01$ nor at $p < 0.05$; *: significant at $p < 0.05$; **: significant at $p < 0.01$.

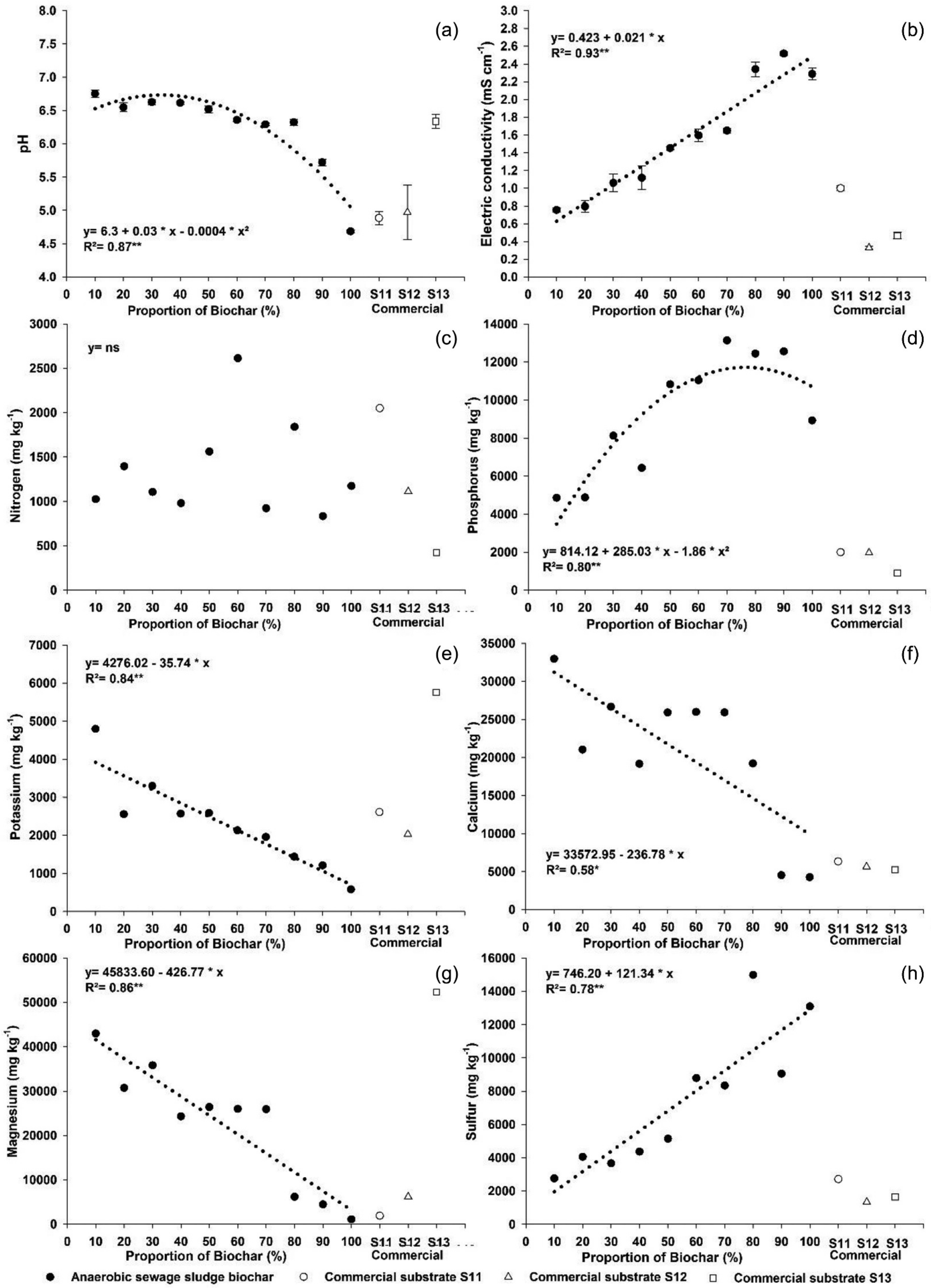

Figure 2. Values of pH, electrical conductivity (EC), and total content of macronutrients in the substrates with anaerobic sewage sludge biochar and three commercial substrates. ns: Polynomial regression not significant at $p < 0.01$ nor at $p < 0.05$; *: significant at $p < 0.05$; **: significant at $p < 0.01$.

The levels of K, Ca and Mg decreased linearly with increasing rates of ASS-biochar (Figure 2(e–g)). Therefore, ASS-biochar cannot be considered a relevant source of these elements, except for Ca (Table 1). With 10 to 80% ASS-biochar, Ca levels were three to six times higher than in S11, S12 and S13. The S13 stood out for the expressive contents of K and Mg compared to the other commercial formulations, probably due to the addition of conventional fertilizer.

Compared to the SM (Table 1), high levels of ASS-biochar resulted in a significant linear increase of S contents in the formulations with biochar. In the substrates with more than 20% biochar, the S content was already higher than in the commercial substrates. It was also found that S is one of the main factors responsible for the increase in EC (r = 0.71, $p < 0.01$).

The concentrations of Cu, Zn, Fe, Mn, Na and B were significantly influenced by ASS-biochar (Figure 3). Cu increased linearly when the proportion of ASS-biochar was increased (Figure 3(a)), always remaining higher than in S11, S12 and S13. For Zn and Fe, the response was quadratic (Figure 3(b,c)). The estimated proportions of ASS-biochar that provided the highest concentrations of Zn and Fe were 69 and 85%, respectively (Figure 3(b,c)). In general, the commercial substrates showed Cu, Zn, and Fe contents similar to the substrates with the lowest proportions of ASS-biochar (10 and 20%).

The concentrations of Mn, Na and B decreased linearly with higher proportions of biochar (Figure 3(d–f)). In the commercial substrates, the Mn content was similar to the substrates with biochar proportions between 20 and 80%. In relation to Na, the commercial substrates S11 and S12 showed similar contents to the substrates with 40% biochar, while S13 surpassed all other substrates in this regard (Figure 3(e)). In the case of B, the commercial substrates, especially S11 and S12, presented higher concentrations than those formulated with biochar.

The proportion of 72% biochar provided the highest concentration of Al in the substrate (Figure 3(g)). In addition, the substrates with biochar had a higher Al content than the commercial substrates, especially in the proportions between 50 and 90% (Figure 3(g)).

Agronomic response of the tobacco **(Nicotiana tabacum)** *seedlings*

The tested substrates significantly affected the growth variables of the tobacco seedlings (Figure 4). Apart from the length of the root system (LRS), increasing the proportions of biochar resulted in a cubic response (Figure 4). In general, there was a tendency of improvement of the growth-related variables with the proportions of 10–20% biochar, reaching a peak between 70 and 90%. On the other hand, the substrate with 100% biochar failed to provide the establishment of tobacco seedlings (Figure 4).

The increase in the proportion of biochar had a slight but significant effect on the total chlorophyll index and the number of leaves on the tobacco seedlings (Figure 4(a,b)). Both variables showed similar values in the biochar-based and commercial substrates (Figure 4(a,b)). Conversely, the tobacco seeds did not germinate in the formulation with 100% ASS-biochar.

In general, the proportions between 50 and 90% ASS-biochar induced a greater total leaf area and DMAP and DMR values, providing even higher values than the three commercial substrates, while the lowest proportions of biochar (< 50%) presented similar results to the commercial substrates (Figure 4 (c–e)). The increase in the proportion of biochar in the composition of the substrates had no significant effect on the LRS (Figure 4(f)). It should also be noted that the tobacco plants grown on substrates with up to 90% ASS-biochar obtained a LRS similar to those grown on commercial substrates.

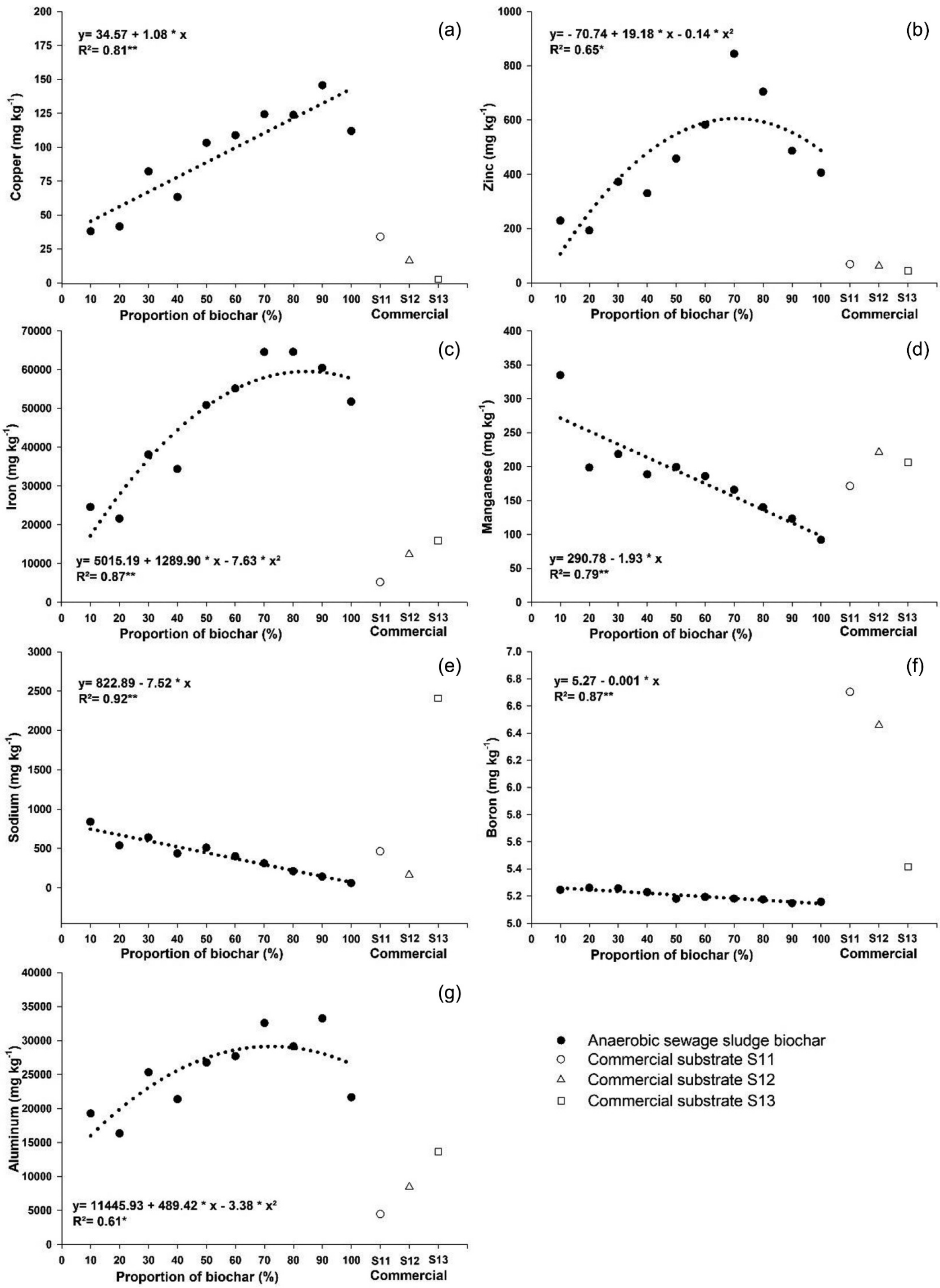

Figure 3. Total contents of micronutrients and aluminum in the substrates with anaerobic sewage sludge biochar and three commercial substrates. Polynomial regression not significant at $p < 0.01$ nor at $p < 0.05$; *: significant at $p < 0.05$; **: significant at $p < 0.01$.

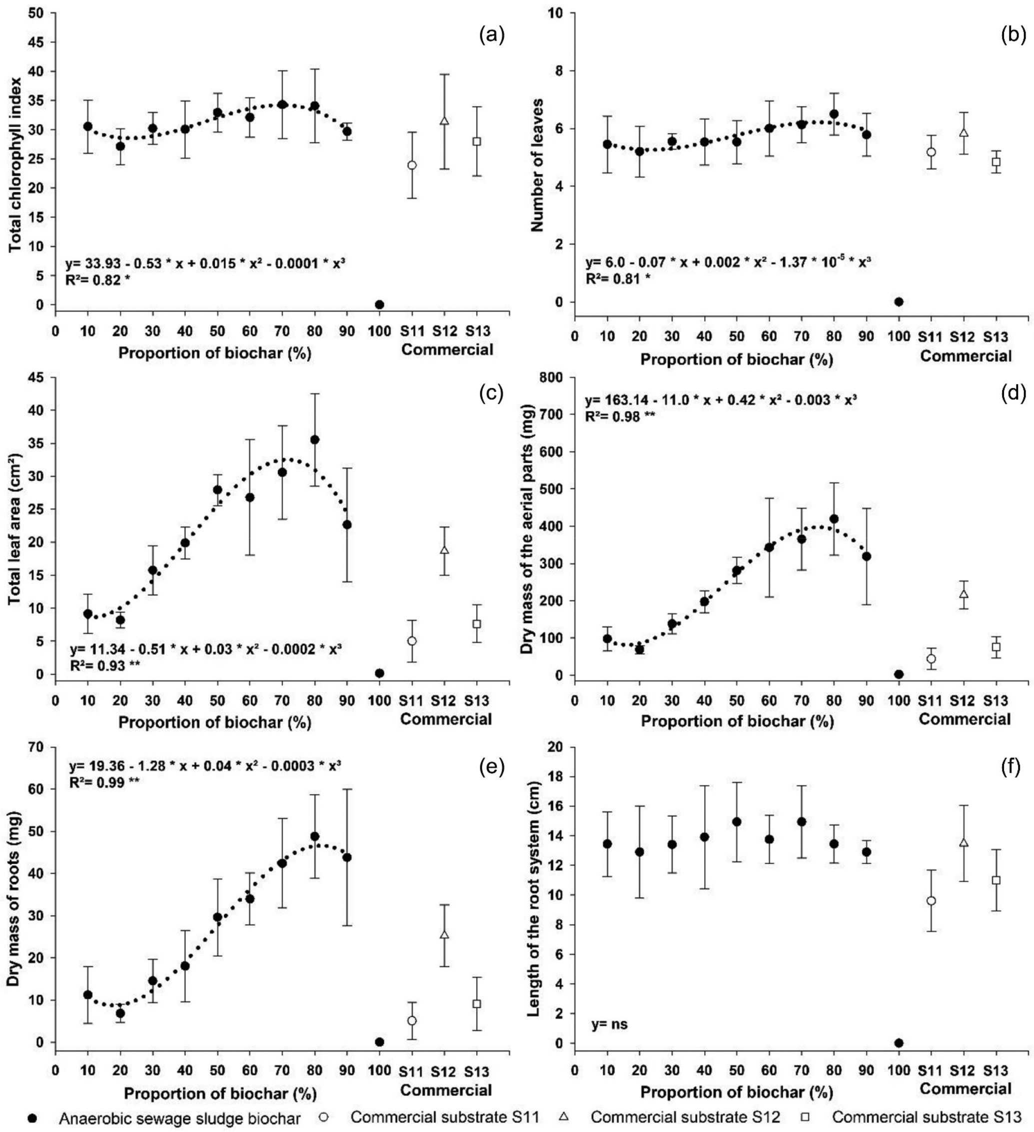

Figure 4. Total chlorophyll index, number of leaves, total leaf area, dry mass of the aerial parts, dry mass of root system, and length of the root system of tobacco plants grown on substrates with anaerobic sewage sludge biochar (ASS-biochar) and three commercial substrates. Polynomial regression not significant at $p < 0.01$ or $p < 0.05$; *: significant at $p < 0.05$; **: significant at $p < 0.01$. The polynomial regression analysis was performed only with the rates from 10 to 90% ASS-biochar, as the rate of 100% ASS-biochar resulted in insufficient germination of tobacco seeds.

Discussion

Substrate characteristics

The biochar-based substrates resulted in higher dry density (DD) values than from 100 to 300 kg m^{-3}, the range considered as ideal in cell trays. This resulted from the higher DD and lower TP of ASS-biochar in relation to the other raw materials (Table 1). In fact, even the commercial substrates struggled to keep within the recommended range, since only S13 reached the recommended range (Figure 1(b)).

Density and porosity are inversely proportional physical properties (De Barros et al. 2019), a relationship evidenced by the high correlation index between DD and TP (r = −0.91, $p < 0.01$), and DD and AS (r = −0.89, $p < 0.01$). TP is associated with macro and micropores, while AS is conditioned mainly by macropores, and these parameters directly affect the AW and RW (De Boodt and Verdonck 1972). In this regard, the results of the present study suggest that the pyrolysis process decreases the volume of macro and micropores in ASS-biochar.

AW is composed of EAW and WBC. Within this context, De Boodt and Verdonck (1972) showed that it is essential that the substrate retains water in the lowest possible energy state for full water absorption and aeration in the root zone. However, the values of EAW, WBC and, consequently, AW were below the values recommended by the authors (Figure 1). The low percentage of AW presented by the biochar-based substrates (Figure 1(g)) was probably related to the excess of macropores in the SM (Table 1), as expressed by the high AS (Figure 1(d)) of the substrates formulated with the lowest proportions of ASS-biochar.

Regarding the pH of the substrates, high values can induce P, Fe, Zn and Cu deficiency in plants. In the present study, the lowest concentrations of these nutrients were verified precisely in the treatments with the lowest proportions of biochar and, consequently, with the highest pH values (Figures 2 and 3).

According to Cavins et al. (2000), the EC of substrates can be classified as very low (0.00 to 0.11 mS cm^{-1}), low (0.12 to 0.35 mS cm^{-1}), normal (0.36 to 0.65 mS cm^{-1}), high (0.66 to 0.89 mS cm^{-1}), very high (0.90 to 1.10 mS cm^{-1}) and extremely high (>1.10 mS cm^{-1}). Considering this classification, the substrates formulated with 40% biochar or more presented an extremely high EC. Substrates with a high EC can damage the roots and reduce or even prevent absorption of water and nutrients. Thus, EC is one of the main indicators to be considered in the choice of substrate raw materials (Cavins et al. 2000). The high EC of biochar is directly linked to a high nutrient load in the raw material (Table 1). Higher proportions of such materials usually result in a saline media (Fornes et al. 2015), as observed by the strong correlation between EC and levels of P (r = 0.65, $p < 0.01$), S (r = 0.71, $p < 0.01$), Cu (r = 0.82, $p < 0.01$), Zn (r = 0.54, $p < 0.01$), Fe (r = 0.72, $p < 0{,}01$) and Al (r = 0.50, $p < 0.01$).

The limited effect of ASS-biochar on the N levels was owing to the low concentrations of N, since it was reduced from 5,000 to 1,172 mg kg^{-1} in the ASS-biochar due to the volatilization during pyrolysis (Zhang et al. 2012; Agrafioti et al. 2013; Yuan et al. 2015). However, in the commercial substrates, the N levels were within the same range as in the substrates with ASS-biochar (Figure 2(c)).

The high concentration of P in the ASS-biochar corroborates the results obtained by Yuan et al. (2015). These authors emphasize that a small fraction of P is lost during pyrolysis, resulting in an amount between 92 to 98% of the original found in sewage sludges. With regard to Cu, low rates of volatilization occur during the pyrolysis process (Hossain et al. 2010), with a tendency to concentrate in the biochar, since pyrolysis reduces the ASS mass, usually by more than 50%.

The total contents of Al, higher in the most enriched biochar formulations, were probably due to the presence of clay minerals from the soil in the sewage sludge, which commonly enter through infiltration in the raw sewage collection system. Moreover, the other raw materials involved in the present study are characterized by a low Al concentration.

Linear correlation between the substrate characteristics and the quality of the tobacco seedlings

The growth variables related to the aerial part of the plants indicate a positive effect of ASS-biochar when between 50 and 90%, which is probably related to a balance between the chemical and physical characteristics. A similar pattern was also observed by Gonzaga et al. (2018), who studied the effect of five doses of sewage sludge biochar (0, 20, 40, 80 and 100 Mg ha^{-1}) mixed with soil on the performance of eucalyptus seedlings. These authors found an increase in the quality, growth and morphological characteristics of the plants with the increase of biochar rates. On the other hand, the tobacco seeds did not germinate when 100% ASS-biochar was used. This probably occurred due to several unwanted characteristics such as high density, low porosity, extremely high EC and low pH.

For tobacco cultivation in a floating system, the parameters with the greatest effect on the growth of the seedlings were the physical and hydraulic variables: density (WD and DD), porosity (TP and AS) and the ability to provide water within 50 to 100 kPa (WBC), as well as the levels of several chemicals such as pH, EC and K, Mg, S and Ca (Table 2).

The reduction in TP and the increase in DD in S10 were likely determinant of the lack of tobacco germination and growth of seedlings. Peculiar characteristics of the substrates are essential in the floating system, in which the porous spaces for air storage and diffusion within the root zone are fundamental for the adequate development of roots and, consequently, of the aerial biomass. However, in the substrates with a proportion of 50 to 90% biochar, both the density and the pore spaces seemed adequate for the growth of tobacco seedlings, even though the density was higher and porosity lower than recommended (De Boodt and Verdonck 1972).

These results indicate the need for further studies aimed at updating the optimal ranges for the physical and hydraulic parameters. Cultivation systems have faced big evolutions in the last 40 years. For example, new sets of containers, irrigation equipment and fertigation systems, designed and fitted to specific plant requirements, are now available.

The correlation of the growth parameters with WBC (Table 2) suggests that the water availability, specifically at tensions within 50 to 100 kPa, is crucial for plant performance in a floating cultivation system. This result can also be connected to the negative correlation between WBC and RW. The RW is the quantity of water not accessible to plants and, if present in high proportions, porosity may be

Table 2. Pearson's correlation coefficient (R) between the physical, hydraulic and chemical characteristics of the anaerobic sewage sludge biochar-based substrates with agronomic parameters of tobacco seedlings.

		Agronomic parameters of tobacco seedlings					
		Number of leaves	LRS[a]	DMAP	DMR	Total chlorophyll index	Total leaf area
Physical and hydraulic characteristics of substrates	WD	0.45*	0.21^{ns}	0.89**	0.87**	0.47**	0.84**
	DD	0.43*	0.22^{ns}	0.89**	0.87**	0.48**	0.84**
	TP	−0.38*	−0.24^{ns}	−0.85**	−0.83**	−0.42*	−0.82**
	AS	−0.33^{ns}	−0.26^{ns}	−0.82**	−0.78**	−0.41*	−0.82**
	EAW	−0.35^{ns}	0.06^{ns}	−0.40*	−0.44*	−0.17^{ns}	−0.28^{ns}
	WBC	0.41*	0.21^{ns}	0.79**	0.68**	0.32^{ns}	0.82**
	AW	0.04^{ns}	0.21^{ns}	0.30^{ns}	0.18^{ns}	0.11^{ns}	0.43*
	RW	−0.28^{ns}	−0.15^{ns}	−0.50**	−0.41*	−0.17^{ns}	−0.53**
Chemical characteristics: macronutrients of substrates	pH	−0.47**	−0.12^{ns}	−0.76**	−0.78**	−0.47**	−0.69**
	EC	0.35^{ns}	0.27^{ns}	0.86**	0.85**	0.41*	0.80**
	N	−0.10^{ns}	0.03^{ns}	0.16^{ns}	0.10^{ns}	−0.23^{ns}	0.05^{ns}
	P	0.11^{ns}	0.18^{ns}	0.54**	0.47**	−0.02^{ns}	0.55**
	K	−0.24^{ns}	−0.34^{ns}	−0.73**	−0.78**	−0.37*	−0.71**
	Ca	−0.32^{ns}	−0.23^{ns}	−0.63**	−0.64**	−0.38*	−0.54**
	Mg	−0.30^{ns}	−0.25^{ns}	−0.77**	−0.80**	−0.39*	−0.68**
	S	0.06^{ns}	0.32^{ns}	0.62**	0.68**	0.22^{ns}	0.55**
Chemical characteristics: Microelements of substrates	Cu	0.26^{ns}	0.17^{ns}	0.70**	0.61**	0.19^{ns}	0.68**
	Zn	0.12^{ns}	0.22^{ns}	0.51**	0.47**	0.12^{ns}	0.59**
	Fe	0.13^{ns}	0.24^{ns}	0.65**	0.60**	0.04^{ns}	0.62**
	Mn	−0.38*	−0.29^{ns}	−0.70**	−0.73**	−0.52**	−0.69**
	Na	−0.45*	−0.31^{ns}	−0.83**	−0.84**	−0.55**	−0.78**
	Al	0.04^{ns}	0.13^{ns}	0.41*	0.31^{ns}	−0.11^{ns}	0.40*
	B	−0.45*	0.16^{ns}	−0.65**	−0.56**	−0.26^{ns}	−0.64**

^{ns} = not significant at $p < 0.01$ or $p < 0.05$; *: significant at $p < 0.05$; **: significant at $p < 0.01$.

The correlation analysis was performed with 10 to 90% ASS-biochar, as the treatment with 100% ASS-biochar resulted in insufficient seed germination.

[a]LRS – length of the root system; DMAP – dry mass of the aerial parts; DMR – dry mass of roots; WD – wet density; DD – dry density; TP – total porosity; AS – aeration space; EAW – easily available water; WBC – water buffering capacity; AW – available water; RW – remaining water; EC – electrical conductivity.

mostly composed of micropores. Therefore, it is essential to keep RW at a certain level to ensure the 'storage' and diffusion of air, which is a fundamental aspect for plant development in floating systems.

Several chemical variables also showed correlation with the growth of the tobacco plants (Table 2). The main effect of pH was on the availability of macro and micronutrients. The acid pH of ASS-biochar (pH 4.7) balanced the alkaline pH of the SM (pH 7.6) in formulations with 50 to 90% ASS-biochar (Figure 2). This was the range in which the tobacco presented the best agronomic performance (Figure 4). The EC, even though classified as 'extreme' in substrates with a high proportion of ASS-biochar, was positively correlated with the growth of the tobacco plants (Table 2). In the floating system, whose water availability is almost unrestricted, water uptake did not seem restrictive for plant development. This was evident when the treatments with the best seedling performance (80 and 90% of ASS-biochar), including the ones with the highest EC, were analyzed.

The best formulations – created with the mixture of ASS-biochar, carbonized rice husk, organic compost and vermiculite – showed efficiency similar or superior to the three commercial substrates. This indicates the potential of ASS-biochar as a raw material for substrates for a wide range of horticultural crops. Overall, ASS-biochar could be considered safe, since all living organisms and pathogens were inactivated after the pyrolysis, and no problems with inorganic contaminants were observed (Table 1), therefore corroborating with recent studies (Yue et al. 2017; Ren et al. 2018).

Even though several substrate formulations proposed and evaluated in the present study achieved similar or even superior performance to the commercial products taken as reference, there is an opportunity for further enhancements. New combinations with other wastes, raw materials and/or proportions of the same materials could be evaluated, as some parameters (pH, EC, Dry Density, TP, AS, EAW, WBC and AW) did not always reach the values considered ideal by literature, indicating that the results can still be maximized or extended to a greater number of species. Thus, further studies may also update the ranges considered ideal for plants.

Optimal ranges for the physical, hydraulic and chemical characteristics of substrates for tobacco seedling production in a floating system

The substrates with 50 to 80% ASS-biochar in their composition provided satisfactory seedling development. Considering these results, ranges for each physical, hydraulic and chemical parameter were indicated, recommending the ideal values for seedling production in a floating system, in particular for tobacco (Table 3). The recommended ranges for the physical, hydraulic and chemical parameters for tobacco cultivation in a floating system differed, for the most part, from the values

Table 3. Physical, hydraulic and chemical parameters recommended for the production of tobacco seedlings in the substrates based on ASS-biochar in the floating system.

Physical, hydraulic and chemical parameters of substrates	Optimal ranges
DD (kg m^{-3})[a]	420–520
TP (%)	60–75
AS (%)	25–35
EAW (%)	9–12
WBC (%)	3–5
AW (%)	12–15
RW (%)	20–25
pH	6.0–6.5
EC (mS cm^{-1})	1.4–2.3

[a]DD – dry density; TP – total porosity; AS – aeration space; EAW – easily available water; WBC – water buffering capacity; AW – available water; RW – remaining water; EC – electrical conductivity

suggested as ideals by the literature, especially for DD, TP, EAW, AW and EC (Table 3). The other parameters (AS, WBC, RW and pH), in spite of changing the limits, remained within the limits recommended by the literature (Table 3).

The DD values that showed the best crop performance ranged from 420 to 520 kg m^{-3}, higher than the values between 100 to 300 kg m^{-3}, considered optimal for cultivation in trays. The indicated values for TP, EAW and AW ranged from 60 to 75%, 9 to 12% and 12 to 15%, respectively. These values were lower than those recommended by De Boodt and Verdonck (1972), between 80 to 90% for TP and 20 to 30% for both EAW and AW (Table 3). For the EC that provided satisfactory development for the tobacco seedling in a floating system, the range varied from 1.4 to 2.3 mS cm^{-1}, which is higher than the values considered normal by Cavins et al. (2000), who recommend the range of 0.4 to 0.6 mS cm^{-1}. All these new ranges are in accordance with the best-fitted tobacco seedling performance and may, therefore, help substrate developers find out the best formulations based on locally available raw materials.

Conclusion and future thrust

Based on the results of the present study, it can be concluded that the anaerobic sewage sludge biochar (ASS-biochar) showed high agronomic efficiency for tobacco seedlings when used in a floating system. The proportions of 50–80% ASS-biochar provided the best agronomic efficiency. The ideal physical-chemical characteristics in a floating cultivation system were as follows: 420–520 kg m^{-3} (dry density); 60–75% (total porosity); 25–35% (aeration space); 9–12% (easily available water); 3–5% (water buffering capacity); 12–15% (available water); 20–25% (remaining water); 6.0–6.5 (pH); 1.4–2.3 mS cm^{-1} (electrical conductivity).

The pyrolysis is an effective process for sanitizing anaerobic sewage sludge, turning it into an environmentally safe and agronomically efficient raw material for substrates. The physical, hydraulic and chemical variables recommended by the present study for substrates used in the production of tobacco seedlings provided healthy and high-quality plants. The recommended ranges will also provide better establishment and early development of plants in the field. In addition, the pyrolysis process exempted ASS from pathogens, therefore preventing the proliferation of pests and diseases.

The development and commercialization of substrates combining sewage sludge-derived biochar with locally available residues is a promising strategy for the sustainable recycling of organic residues and, when also combined with the adherence to the optimal ranges for the physical and chemical properties, such substrates would certainly expand the productivity and quality of tobacco plants after transplantation to the soil.

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No potential conflict of interest was reported by the author(s).

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