



Substrates for seedlings with sewage sludge and biochar¹

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ABSTRACT

The objective of this study was to evaluate substrates formulated with proportions of sewage sludge (SS)-solarized and SS-biochar in the production of seedlings of two indicator crops: lettuce (*Lactuca sativa* L.) and black wattle (*Acacia decurrens* Willd). The study was composed of two experiments conducted in greenhouse, in randomized block designs. The treatments consisted of five doses of SS-solarized and SS-biochar, combined with rice husk ash and vermiculite in different proportions (20:40:40, 30:35:35, 40:30:30, 50:25:25 and 60:20:20) and two commercial substrates (controls). Morphophysiological characteristics of the seedlings and the physico-hydric characteristics, pH and electrical conductivity of the substrates were evaluated. The pyrolysis process enhanced the characteristics of SS as a component of substrates, where the substrate with 20% of SS-biochar, 40% of vermiculite and 40% of rice husk ash, presented the better performance.

Keywords: *Lactuca sativa* L.; *Acacia decurrens* Willd; biosolids; physical-water characteristics.

INTRODUCTION

Sewage sludge (SS) is a by-product of the domestic wastewater treatment, and its safe use is still a major challenge for today's society (Bai *et al.*, 2017). Currently, the agricultural use of SS has been indicated as the main alternative for its recycling role (Tessaro *et al.*, 2016), with positive effects when used as a fertilizer, a soil conditioner and in the recovery of land degraded areas (Caldeira *et al.*, 2012; Faria *et al.*, 2013). However, its use as a substrate component for seedling production is still poorly studied, although recent research indicates a high potential (Caldeira *et al.*, 2013; Freitas & Melo, 2013).

The use of SS as a component of substrates can also be a better destination when compared to its final disposal in landfills (Caldeira *et al.*, 2012). However, it is fundamental that SS has undergone a process that guarantees its innocuousness. Batches of SS must be monitored, in order to know that the levels of potentially toxic elements and pathogens for human health are in accordance with the limits established by current regional legislation.

Black wattle (*Acacia sp.*), timbó (*Ateleia glazioviana* Baill), teak (*Tectona grandis*) and eucalyptus (*Eucalyptus sp.*) are examples of forest species already tested with substrates formulated from SS (Caldeira *et al.*, 2013, Silva *et al.*, 2017). On the other hand, studies with substrates from SS in horticultural species are still scarce, perhaps due to the contamination risks of their consumable parts. Among them, we can mention the use of SS derived substrates in the production of tomato seedlings (*Solanum lycopersicum*) (Freitas & Melo, 2013). Another reason is the difficulty to formulate such substrates, since these species are more sensitive to chemical and physical characteristics of the growth medium.

Although positive results have been noticed by researchers regarding the agronomic efficiency of SS derived substrates, its practical application has been limited, particularly when inactivation of pathogenic contaminants is not properly carried out.

Solarization is the process by which sewage is subjected to heat and sun rays in containers covered by

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plastic film (Alves Filho *et al.*, 2016), or in agricultural greenhouses (Santos *et al.*, 2015; Pereira Lima *et al.*, 2009). When properly executed, this is a simple, effective and low-cost alternative for sanitizing SS batches (Ozdemir *et al.*, 2013; Alves Filho *et al.*, 2016).

Heat treatment is currently considered the most effective approach (Liu *et al.*, 2014), with pyrolysis being one of the most promising thermal methods for SS treatment worldwide (Agrafioti *et al.*, 2013; Méndez *et al.*, 2013; Yuan *et al.*, 2016; Agbna *et al.*, 2017).

The resulted product of the pyrolysis process of organic carbon sources is known as biochar, a stable carbon rich material with high amount of reactive sites. Biochar has been widely tested and used as a soil conditioner, since it benefits soil quality overall, in particular soil structure, porosity, bulk density, water storage, microbial activity and nutritional status (Silva *et al.*, 2017). Jin *et al.* (2016), evaluating SS pyrolysis temperatures ranging from 400 to 600 °C, performed during one hour, verified that with the increase in temperature there is a decrease in SS volume, an increase in pH levels and a decrease in the bioavailability of a wide set of potentially harmful elements, therefore resulting in a lower risk of SS-biochar application to the environment.

Production and use of SS based biochar in the formulation of substrates for seedling production in Brazil represent a new opportunity for this material, with potential economic gains, reduction of environmental impact and satisfactory agronomic performance. In this context, the objective of this work was to evaluate the performance of substrates formulated with SS-solarized and SS-biochar in the production of lettuce and black wattle.

MATERIAL AND METHODS

The study was composed of two experiments: lettuce (Experiment 1) and black wattle (Experiment 2). The experiments were conducted in greenhouse, with automated controlled temperature (15-30 °C), located at Embrapa Clima Temperado - Terras Baixas Experimental Station - Capão do Leão-RS (31°49'13" S 52°27'50" W).

SS-solarized and SS-biochar

The SS was collected at the Sewage Treatment Station of Rio Grande-RS, during the spring of 2015. After collect, SS-solarized was spreaded in 10 cm thick layer in fiberglass boxes, placed inside an agricultural greenhouse made of transparent plastic with automated control of aeration and heating. The heating system was activated every time the temperature reached 15 °C and turned off when it exceeded 19 °C, remaining in this condition for

approximately 45 days until the moisture content of SS reached less than 20%. After the solarization, part of the material was identified and stored and the other part was submitted to the pyrolysis process, with partial air supply and variable temperature (300-600 °C) for three hours. Finally, SS-solarized and SS-biochar used in this study was crushed and sieved until granulometry 100% < 2.0 mm.

Samples of SS-solarized were submitted to the characterization of the presence of pathogenic contaminants (thermotolerant coliforms, viable eggs of helminths, salmonella and enteric virus contents), inorganic contaminants (As, Ba, Cd, Pb, Cu, Cr, Cr⁶, Hg, Mo, Ni, Se and Zn) and chemical characteristics (pH, N, P, K, Ca, Mg, S, Cu, Zn, Fe, Mn, Na, Al, B and CTC) at the Eurofins ALAC Laboratory. This characterization was performed before the formulation of the substrates and the implantation of the experiments.

Treatments

The treatments (substrates) were the same for the two experiments, consisting of five treatments with increasing doses of SS-solarized (T1-T5), five treatments with increasing doses of SS-biochar (T6-T10) and two commercial substrates (T11 and T12): T1- 20% SS solarized (SS sol) + 40% vermiculite (VER) + 40% rice husk ash (RHA); T2- 30% SS sol + 35% VER + 35% RHA; T3- 40% SS sol + 30% VER + 30% RHA; T4- 50% SS sol + 25% VER + 25% RHA; T5- 60% SS sol + 20% VER + 20% RHA; T6- 20% biochar SS (Bioc SS) + 40% VER + 40% RHA; T7- 30% Bioc SS + 35% VER + 35% RHA; T8- 40% Bioc SS + 30% VER + 30% RHA; T9- 50% Bioc SS + 25% VER + 25% RHA; T10- 60% Bioc SS + 20% VER 20% RHA; T11- commercial substrate 1; T12- commercial substrate 2.

Experiment 1: Lettuce (Lactuca sativa L.)

The formulated substrates were homogenized and distributed in 110 cm³ cells (styrofoam trays containing 78 cells). Commercial seeds of lettuce, cv. Simpson, with germination power of 74%, were sown on 20 June 2016. From this moment, the trays were kept in a floating system during the experiment time. The plants were evaluated at 29 days after their emergence, when the crop stage ideal for transplantation was reached.

The response variables evaluated were: plant height, dry mass of aboveground parts (PDM) and dry mass of roots (RDM), total chlorophyll index and total leaf area. Plant height (cm) was measured in six plants of each plot with a graduated ruler. The PDM (g) and RDM (g) were determined in the same six plants of each plot, which were packed in paper bags and dried out at 65 °C until reaching a constant mass. The total chlorophyll index was evaluated in three leaves per plant, using a portable

chlorophyllometer (ClorofiLOG - Falker Automação Agrícola Ltda., Porto Alegre-RS). The total leaf area of the plants (cm^2) was obtained by scanning all leaves from the same six previously used plants using computer and scanner. Subsequently, the digital images were processed with the AFSOFT program (Silva & Jorge, 2009). The experimental design was a randomized block, with four replications, where each plot had six cells with one seedling per cell.

Experiment 2: Black wattle (*Acácia decurrens Willd*)

The substrates were disposed in polypropylene tubes with 53 cm^3 , then black wattle seeds (germination power of 72%) were sowed at 20 August 2016, with two seeds per tube, and then thinning when necessary. On 24 November 2016, when the plants reached the transplanting stage, the following variables were evaluated: plant height, dry mass of aboveground parts (PDM) and dry mass of roots (RDM). The plant height (cm) was measured in four plants of each plot with graded ruler, while the PDM (g) and RDM (g) were determined in the three plants of each plot, being conditioned in paper bags and dried at $65 \text{ }^\circ\text{C}$ until reaching a constant mass. The experimental design was randomized blocks, with three replications and three plants per plot.

Characterization of substrates

The following physico-hydric and chemical attributes were determined: granulometric fractionation, wet density (WD), dry density (DD), total porosity (TP), aeration space (AS), available water (AW), water retained at 10 kPa (WR_{10}), water retention capacity (WRC), hydrogenionic potential (pH) and electrical conductivity (EC).

The wet and dry densities of the substrates were determined by the self-compacting method described by Brasil (2007). The granulometric fractionation was performed with samples of 100 g of air dried substrate, with a set of sieves (4.76, 2.00, 1.00 and 0.50 mm) coupled to a mechanical stirrer, working for three minutes. The variables TP, AS, WA, WR_{10} and WRC were determined according to De Boodt & Verdonck (1972). The pH and CE values of the substrates were determined in 1:5 (substrate:water ratio) according to Brasil (2007).

Statistical analysis

The data sets obtained in experiments 1 and 2, and in the characterization of the substrates, were evaluated with relation to the normality of their distributions and about the presence of discrepant values. Each response variable was then submitted to analysis of variance and for those variables with significant treatment effect, the Scott-Knott test was applied at a 5% error probability level.

RESULTS AND DISCUSSION

Characteristics of SS-solarized

The concentrations of inorganic and pathogenic contaminants evaluated in SS-solarized were lower than the maximum limits posed by the Resolution N° 375/2006 (Brasil, 2006) for SS disposal in agricultural areas, and lower than the limits allowed in substrates for plants, posed by the Normative Instruction N° 07/2016 (Table 1). Therefore, the concentrations of these contaminants were not impeditive to the use of SS-solarized in the composition of plant substrates.

The concentrations of pathogenic contaminants found in SS after solarization demonstrate that this method is efficient in SS sanitation, being similar to observed by other studies (Pereira Lima *et al.*, 2009; Ozdemir *et al.*, 2013; Santos *et al.*, 2015; Alves Filho *et al.*, 2016). Pereira Lima *et al.* (2009) evaluating the chemical and microbiological characteristics of SS when subjected to hygienization in an agricultural greenhouse, observed that after 28 days of solarization, SS reached the limits of the current legislation for pathogenic contaminants. Thus, considering that SS-solarized meets the limits posed by the current legislation (Table 1), and SS-solarized is the raw material for biochar production, these contaminants were not evaluated in the SS-biochar.

For the SS-solarized, the chemical results are shown in Table 1. Based on agronomic aspects, SS-solarized resembles animal manures such as from cattles and equines both used in large scale in agriculture (Kiehl, 1985).

Characteristics of substrates

The proportion of SS-solarized or SS-biochar utilized in the mix influenced significantly the physico-hydric and chemical characteristics of the substrate. In general, there is a tendency to increase the values of wet density (WD), dry density (DD), total porosity (TP) and pH, and to decrease the electrical conductivity (EC) with the increase of the proportions of SS-solarized (T1 - T5) and SS-biochar (T6 - T10) in the substrates (Table 2). On the other hand, this trend was not detected for the aeration space (EA).

The tendency of increasing in the values of WD and DD as SS-solarized and SS-biochar increase in the substrates are in agreement with the results obtained in the characterization of these materials (SS-solarized: $\text{WD} = 888.50 \text{ kg m}^{-3}$ and $\text{DD} = 718.80 \text{ kg m}^{-3}$; SS-biochar: $\text{WD} = 897.52 \text{ kg m}^{-3}$ and $\text{DD} = 728.24 \text{ kg m}^{-3}$), which indicated higher densities compared to rice husk ash and vermiculite (rice husk ash: $\text{WD} = 385.87 \text{ kg m}^{-3}$ and $\text{DD} = 249.12 \text{ kg m}^{-3}$; vermiculite: $\text{WD} = 489.81 \text{ kg m}^{-3}$ and $\text{DD} = 265.60 \text{ kg m}^{-3}$). The high WD and DD values presented by SS-solarized and SS-biochar resulted in compacted substrates where these materials are predominant. This is a negative aspect

in comparison with the commercial substrates (T11 and T12), since the commercial substrates have 22% and 26% lower DD than substrates with SS-solarized and SS-biochar, respectively.

The TP was higher in the substrates with SS-solarized (T1 to T5), value 29% higher than the SS-biochar (T6 to T10) and 22% higher than commercial T11 and T12 (Table 2). The TP of substrates with SS-biochar did not differ statistically from commercial substrates T11 and T12. However, substrates with SS-solarized and SS-biochar showed, respectively, a TP of 17.84 and 32.81% below the reference value of 85%, established by De Boodt & Verdonck (1972) and Verdonck & Gabriels (1988).

The highest aeration space (AS = 13.62%) was found in T11 (Table 2), while the lowest AS (1.64%) was obtained by T10, which corresponds to the highest dose of SS-biochar (60%). According to Verdonck & Gabriels (1988), the ideal range for AS is 10 to 15%. For De Boodt & Verdonck (1972), the ideal AS is in the range of 20 to 30%. Thus, only Commercial substrate 1 (T11) presented AS in considered adequate by Verdonck & Gabriels (1988) (Table 2).

The highest pH values were found on substrates with 50% and 60% SS-solarized (T4 and T5, respectively). Among the SS-biochar substrates, the highest pH value (7.69 to 8.09) was obtained in T2 to T5 (20% to 50% SS-solarized) and T9 (substrate with 50% SS-biochar) (Table

Table 1: Inorganic and pathogenic contaminants in SS-solarized samples and maximum limits allowed by current legislation

Parameter	SS-solarized	CONAMA 375/2006 ¹	IN 7/2016 ²
Inorganic contaminants			
Arsenic (mg kg ⁻¹)	< 1.72	41.00	20.00
Barium (mg kg ⁻¹)	246.30	1300.00	-
Cadmium (mg kg ⁻¹)	1.37	39.00	3.00
Lead (mg kg ⁻¹)	26.50	300.00	150.00
Copper (mg kg ⁻¹)	147.80	1500.00	-
Chromium (mg kg ⁻¹)	85.90	1000.00	500.00
Hexavalent chromium (mg kg ⁻¹)	< 0.172	-	2.00
Mercury (mg kg ⁻¹)	< 0.115	17.00	1.00
Molybdenum (mg kg ⁻¹)	4.37	50.00	-
Nickel (mg kg ⁻¹)	25.90	420.00	70.00
Selenium (mg kg ⁻¹)	< 1.72	100.00	80.00
Zinc (mg kg ⁻¹)	963.20	2800.00	-
Pathogenic contaminants			
Thermotolerant coliforms (MLN/g de TS)	1.00	< 1.000	1.000
Viable eggs of helminths (n ^o /g de TS)	< 0.25	< 0.25	1.00
<i>Salmonella</i>	Absence	Absence	Absence
Enteric Viruses (g/TS)	Absence	< 0.25	-
Agromomic parameters			
pH	6.00		
Carbon (%)	35.00		
Nitrogen (%)	5.70		
Phosphorus (%)	2.40		
Potassium (%)	0.49		
Calcium (%)	1.60		
Magnesium (%)	0.56		
Sulphur (%)	0.99		
Copper (mg kg ⁻¹)	125.00		
Iron (%)	2.30		
Manganese (mg kg ⁻¹)	328.00		
Sodium (mg kg ⁻¹)	520.00		
Aluminum (%)	0.92		
Boron (mg kg ⁻¹)	56.00		
CTC – Cation exchangeable capacity (mmol kg ⁻¹)	556.00		

¹ CONAMA 375/2006: Resolution No. 375, of August 29, 2006, defines criteria and procedures for the agricultural use of sewage sludge generated in sewage treatment plants and their by-products; ² IN 7/2016: Normative Instruction SDA No. 7, of April 12, 2016, amends Annexes IV (maximum limits of contaminants allowed in substrate for plants) of Normative Instruction SDA no. 27, of June 5, 2006; MLN - Most Likely Number; TS - Total Solids.

2). The lowest pH values were found in commercial substrates 1 and 2 (pH = 5.24 in T11 and pH = 5.26 in T12). Thus, it is noted that both substrates with SS have higher pH than commercial ones. In addition, a positive correlation was found between the increase in the proportion of SS-solarized ($r = 0.88$, $p = 0.001$) and SS-biochar ($r = 0.92$, $p = 0.001$) and increase in pH of the substrates. Substrates having pH below 5.0 may cause deficiencies of nutrients such as nitrogen, potassium, calcium, magnesium and boron; on the other hand, deficiencies in phosphorus, iron, manganese, zinc and copper may happen in those with pH values above 6.5 (Valeri & Corradini, 2000). In this study, the substrates based on SS-solarized and SS-biochar presented an average pH of 7.64, being classified in the second case.

The highest EC values were found on the substrates T1 to T7 (1,230.83 $\mu\text{S cm}^{-1}$ to 1,629.67 $\mu\text{S cm}^{-1}$), followed by substrates SS-biochar (T8 to T10) and commercial T11 (Table 2), respectively. The lowest EC value was found on the substrate T12 (309.10 $\mu\text{S cm}^{-1}$). The highest percentage of available water (WA = 20%) was presented by T9 (50% SS-biochar) (Figure 1A), remaining at the lower limit of the range of 20% to 30% adopted as a reference by De Boodt & Verdonck (1972).

The substrates with 50% and 60% of SS-solarized (T4 and T5) had the lowest WA values (6.93% and 7.13%, respectively) (Figure 1A), a result that may be related to the higher water retention capacity (WRC) from SS-solarized when compared to SS-biochar. This fact is

evident when analyzing the WR_{100} values of T4 and T5 substrates (50% and 60% of SS-solarized), since in these substrates the highest values of WR_{100} were obtained (Figure 1A). This relationship can be corroborated by the positive correlation ($r = 0.94$, $p = 0.001$) between the increase of SS-solarized and WR_{100} in the substrate. The substrates containing SS-solarized presented, in general, a content of WR_{100} on average 39% higher than the substrates with SS-biochar and 54% higher than the commercial ones.

In general, the formulated substrates presented low percentages of particles larger than 4.76 mm (Figure 1B). On the other hand, expressive differences were verified in relation to particles lower than 0.50 mm, so that the substrates composed by the lower concentrations of SS-solarized (T1, T2 and T3) and SS-biochar (T6, T7, T8 and T9) formed the two highest percentage groups (Figure 1B).

Only the substrates T4, T5, T9 and T10 showed granulometry similar to the commercial substrates. The main granulometric differences of the substrates are verified in the granulometric bands between 4.76 and 2.00 mm and less than 0.50 mm. There was an increase in particles between 4.76 and 2.00 mm as the proportions of SS were increased in substrates, while lower particles than 0.50 mm in substrates formed by lower amounts of SS (solarized and biochar) were due to the greater proportion of rice husk ash, which has 86% of particles within this grain size range.

Table 2: Wet Density (WD), Dry Density (DD), Total Porosity (TP), Aeration Space (AS), Hydrogenionic potential (pH) and Electrical Conductivity (EC) of different substrates with sewage sludge (SS): SS-solarized (T1 to T5), SS-biochar (T6 to T10); and two commercial substrates (T11 and T12)

Treatments ¹	Physico-hydric characteristics			Chemical Characteristics		
	WD	DD	TP	AS	pH	EC
	kg m ⁻³		%			$\mu\text{S cm}^{-1}$
T1	667.84 c	508.78 e	63.04 a	8.37 b	7.01 c	1,629.67 a
T2	647.93 d	505.61 e	67.81 a	9.30 b	7.69 a	1,577.67 a
T3	696.75 c	534.61 d	67.55 a	5.67 c	7.87 a	1,230.83 a
T4	733.19 b	570.61 b	68.75 a	2.82 d	8.09 a	1,240.67 a
T5	816.73 a	614.71 a	68.63 a	5.02 c	8.01 a	1,431.67 a
T6	689.04 c	535.73 d	50.36 b	2.07 e	7.37 b	1,532.33 a
T7	705.05 c	555.03 c	52.82 b	3.29 d	7.53 b	1,332.67 a
T8	695.15 c	561.37 c	54.07 b	5.72 c	7.61 b	930.83 b
T9	720.45 b	585.24 b	53.53 b	1.75 e	7.70 a	999.07 b
T10	799.96 a	617.45 a	50.16 b	1.64 e	7.55 b	1,040.20 b
T11	630.92 d	440.64 f	55.33 b	13.62 a	5.24 d	805.33 b
T12	576.21 e	406.22 g	54.48 b	6.36 c	5.26 d	309.10 c
CV (%)	2.15	2.02	7.36	13.23	2.77	20.97

¹ Means followed by the same lowercase letter in the column belong to the same group by the Scott-Knott test at 5% error probability. T1- 20% SS solarized (SS sol) + 40% vermiculite (VER) + 40% rice husk ash (RHA); T2- 30% SS sol + 35% VER + 35% RHA; T3- 40% SS sol + 30% VER + 30% RHA; T4- 50% SS sol + 25% VER + 25% RHA; T5- 60% SS sol + 20% VER + 20% RHA; T6- 20% biochar SS (Bioc SS) + 40% VER + 40% RHA; T7- 30% Bioc SS + 35% VER + 35% RHA; T8- 40% Bioc SS + 30% VER + 30% RHA; T9- 50% Bioc SS + 25% VER + 25% RHA; T10- 60% Bioc SS + 20% VER 20% RHA; T11- commercial substrate 1; T12- commercial substrate 2.

There were significant differences between the studied substrates in relation to the water retention at the tensions of 0, 1, 5 and 10 kPa (Figure 1C). At the tensions of 0 and 1 kPa, the substrates composed with SS-solarized presented greater water retention. However, in the higher tensions (5 and 10 kPa), only the substrates with higher proportions of SS-solarized (T3, T4 and T5) formed the group of highest water retention (Figure 1C). Thus, introducing SS in substrates clearly increase their WRC.

In relation to water retention capacity at 1 kPa (WRC1), it was observed that SS-solarized at proportions of 30, 40, 50 and 60% (T2, T3, T4 and T5, respectively) formed the group of substrates with the highest WRC1, differing significantly from the other treatments (Figure 1D). The

T1, with the lowest proportion of SS-solarized (20%), did not differ from SS-biochar and commercial substrates (Figure 1D), indicating that the SS-solarized increased the water retention of the substrates, even in comparison to commercial substrates. Data from literature suggest that WRC1 between 20 and 80% is ideal for substrates to vegetables (De Boodt & Verdonck, 1972; Verdonck & Gabriels, 1988), confirming that the studied substrates are within the ideal values for this variable.

Experiment 1 - Lettuce (*Lactuca sativa L.*)

The plant height of lettuce had a significant response to the substrates, and three response groups were formed (Table 3). The group with highest plants was formed by T6, T7 and T10, composed of 20, 30 and 60% of SS-biochar,

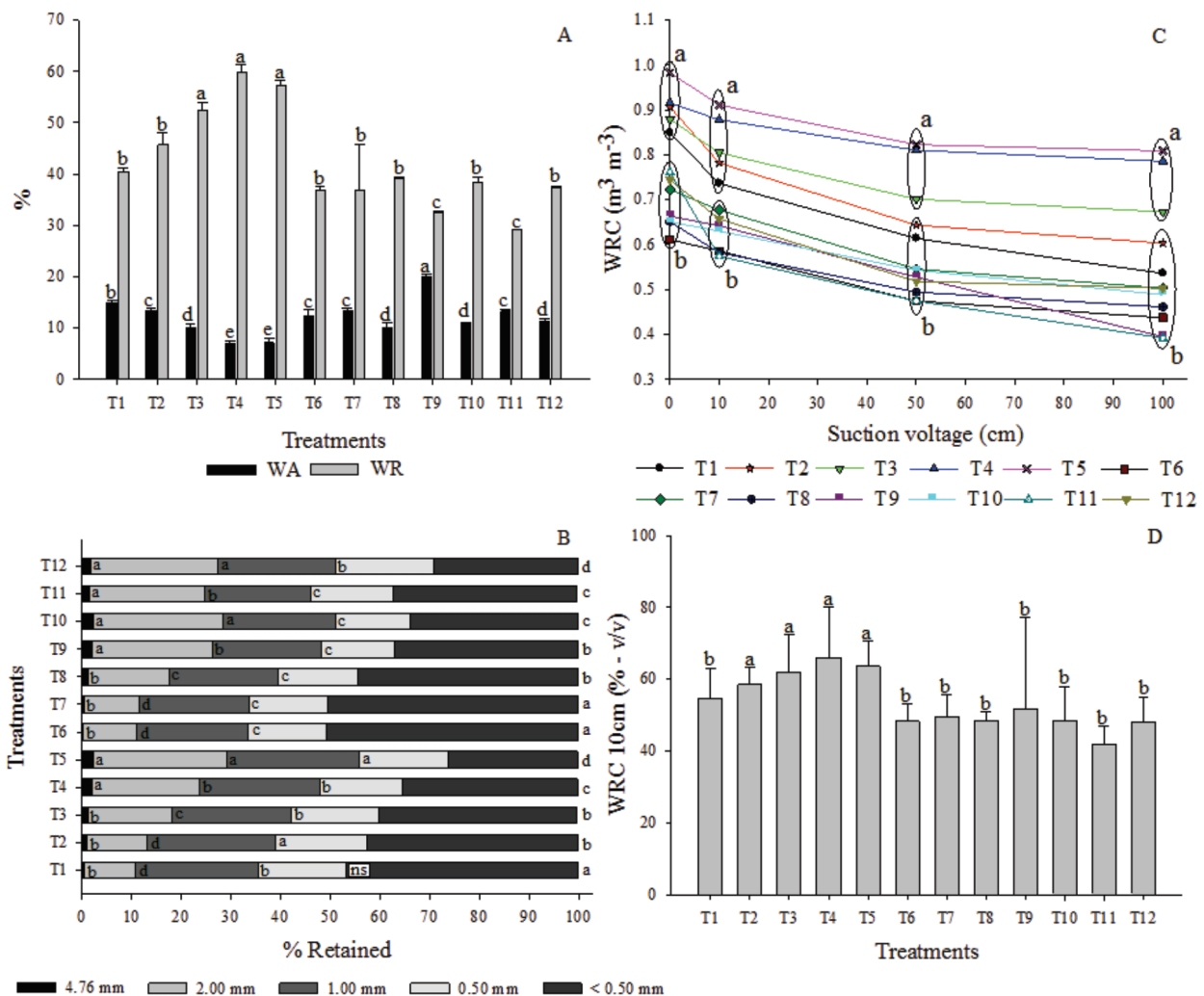


Figure 1: Water available (WA) and remaining water (WR) (A), grain size (B), water retention curve (C) and water retention capacity at 10cm water column (WRC10cm), in substrates with SS solarized, biochar SS and commercial. * Means followed by the same lowercase letter in the column belong to the same group by the Scott-Knott test at 5% error probability. T1- 20% SS solarized (SS sol) + 40% vermiculite (VER) + 40% rice husk ash (RHA); T2- 30% SS sol + 35% VER + 35% RHA; T3- 40% SS sol + 30% VER + 30% RHA; T4- 50% SS sol + 25% VER + 25% RHA; T5- 60% SS sol + 20% VER + 20% RHA; T6- 20% biochar SS (Bioc SS) + 40% VER + 40% RHA; T7- 30% Bioc SS + 35% VER + 35% RHA; T8- 40% Bioc SS + 30% VER + 30% RHA; T9- 50% Bioc SS + 25% VER + 25% RHA; T10- 60% Bioc SS + 20% VER 20% RHA; T11- commercial substrate 1; T12- commercial substrate 2.

Table 3: Plant height, plant dry mass, root dry mass, total chlorophyll and total leaf area of lettuce seedlings grown on different substrates (Experiment 1). Plant height, plant dry mass and root dry mass of black wattle seedlings grown on different substrates (Experiment 2)

Treatments ¹	Experiment 1 - Lettuce					Experiment 2 – Black wattle —		
	Plant height (cm)	Plant dry mass (g)	Root dry mass (g)	Clorofila total	Total leaf area (cm ²)	Plant height (cm)	Plant dry mass (g)	Root dry mass (g)
T1	6.798 b	0.154 c	0.025 c	12.749 ^{ns}	28.141 c	14.500 ^{ns}	1.480 a	0.265 a
T2	5.181 b	0.104 c	0.020 c	12.750	22.330 c	5.553	0.328 b	0.022 b
T3	1.494 c	0.007 d	0.001 d	-	4.001 d	-	-	-
T4	1.096 c	0.003 d	0.001 d	-	2.543 d	7.167	0.560 b	0.167 b
T5	0.958 c	0.003 d	0.001 d	-	1.256 d	5.000	0.150 b	0.006 b
T6	10.554 a	0.589 a	0.118 a	13.346	86.806 a	5.167	0.417 b	0.093 b
T7	9.717 a	0.413 b	0.071 b	12.997	63.885 b	4.333	0.201 b	0.033 b
T8	6.190 b	0.200 c	0.037 c	12.298	40.222 b	5.667	1.353 a	0.350 a
T9	5.574 b	0.162 c	0.024 c	12.494	21.492 c	10.000	1.240 a	0.343 a
T10	10.030 a	0.343 b	0.054 b	12.886	50.453 b	5.833	0.367 b	0.123 b
T11	10.359 a	0.430 b	0.077 b	13.008	54.504 b	5.611	0.440 b	0.090 b
T12	8.480 a	0.311 b	0.080 b	12.595	49.813 b	7.311	0.843 a	0.217 a
CV (%)	26.37	41.13	39.08	3.74	43.49	52.65	78.7	87.11

¹ Means followed by the same lowercase letter in the column belong to the same group by the Scott-Knott test at 5% error probability. T1- 20% SS solarized (SS sol) + 40% vermiculite (VER) + 40% rice husk ash (RHA); T2- 30% SS sol + 35% VER + 35% RHA; T3- 40% SS sol + 30% VER + 30% RHA; T4- 50% SS sol + 25% VER + 25% RHA; T5- 60% SS sol + 20% VER + 20% RHA; T6- 20% biochar SS (Bioc SS) + 40% VER + 40% RHA; T7- 30% Bioc SS + 35% VER + 35% RHA; T8- 40% Bioc SS + 30% VER + 30% RHA; T9- 50% Bioc SS + 25% VER + 25% RHA; T10- 60% Bioc SS + 20% VER + 20% RHA; T11- commercial substrate 1; T12- commercial substrate 2.

respectively, as well as commercial substrates (T11 and T12). On the other hand, the plants submitted to the substrates with the highest proportions of SS-solarized (T3, T4 and T5) had the lowest heights.

In relation to the dry mass of aboveground parts (PDM) and dry mass of roots (RDM), there was an identical effect of the substrates on these variables (Table 3), occurring four distinct groups for the two variables. The highest mass resulted from T6, the lower concentration of SS-biochar. The substrates T7 and T10, containing 30 and 60% SS-biochar, respectively, together with commercial substrates 1 and 2 (T11 and T12) also resulted in high PDM and RDM, being lower only to T6 treatment. On the other hand, treatments with SS-solarized, especially T3, T4 and T5, resulted in lower values of PDM and RDM among the evaluated substrates.

The total leaf area was significantly influenced by the substrates, with the formation of four response groups (Table 3). The largest leaf area was presented by the smallest amount of SS-biochar (T6). After T6, the better performance resulted from the commercial substrates and T7, T8 and T10, with 30, 40 and 60% of SS-biochar, respectively. On the other hand, the substrates that resulted in the smallest leaf areas were those with the highest proportions of SS-solarized (T3, T4 and T5). For total chlorophyll, there was no significant differences among all the substrates.

In general, SS-solarized resulted in plants with lower performance than SS-biochar for lettuce seedlings production (Table 3). This fact is possibly related to the higher content of WR_{100} presented by SS-solarized substrates (Figure 1A), being on average 39% higher than SS-biochar substrates, which caused water excess and therefore deficiency of root aeration.

This water excess was possibly aggravated by the production system of seedlings (floating). Considering that in the floating system the trays are suspended in a water table, water is largely available for plants. In floating systems it is strongly important to have substrates with high macroporosity and water free pores, to ensure gas exchange in the plant root system zone.

Experiment 2 - Black wattle (*Acacia decurrens*)

In the black wattle cropping, there was no significant effect of the studied substrates on plant height (Table 3). On the other hand, for PDM and RDM, the substrates T1, T8, T9 and T12 presented the largest mean values for both PDM and RDM variables. The low biomass production of black wattle crop on T2, T3, T4 and T5 substrates, based on SS-solarized, is probably related to the high WRC of the SS-solarized.

This high WRC provided by SS-solarized is related to the high WR_{100} content (Figure 1A), causing an oxygen

deficiency and less gas exchange in the root system, as observed in the lettuce crop. This decrease in gas exchange is aggravated by the fact that black wattle seedlings are produced in plastic tubes, which are impermeable and therefore avoid the connection of substrates with the free air environment.

Physico-hydric characteristics versus morphophysiological characteristics of lettuce and black wattle

In general, the substrates formulated with SS-biochar have shown similar results to commercial substrates in the production of lettuce and black wattle seedlings (Table 3). On the other hand, substrates containing SS-solarized have shown a poorer performance than others, particularly with the greater proportions of this material.

Correlation analysis indicates that the physico-hydric parameters that best explain the growth of lettuce seedlings are TP, WA, WR_{100} and WRC. This means that, for lettuce, physical aspects of substrates are of major importance during the early crop development days. It was observed that higher average values of TP, WRC and WR_{100} , verified in the substrates composed with SS-solarized, are related to the worse performance of these substrates.

The pore diameter distribution and the macro and micropores affect the physico-hydric characteristics of a substrate, so that it can retain more or less water. In the case of SS-solarized, it probably promotes the micropores and these are occupied in greater proportion by water, but in unavailable form for plants, being the main reason that the material showed a high WRC (Figure 1C and Figure 1D).

In addition to the higher WRC presented by substrates with SS-solarized, results from AR_{100} (Figure 1A) indicate that this water portion is harder to be accessed by plants. Therefore, the values of WA were low and those of WR_{100} were high in these substrates. This condition result in plant stress due to lack of aeration, and certainly contributed to the fact that the substrates composed of SS-solarized, especially in the higher proportions, have provided the worst conditions for lettuce plants development.

In the production of eucalyptus seedlings, Caldeira *et al.* (2013) observed in substrates with different doses of SS-solarized and vermiculite (100:0, 80:20, 60:40, 40:60 and 20:80) that higher microporosity were presented by substrates with higher doses of SS-solarized and, consequently, also increased WRC. These results are similar to those obtained in the present study. According to De Boodt & Verdonck (1972), it is essential that the substrate retain water in an energy state as low as possible to the plants, in addition to sufficient air in the root zone. However, some stress

symptoms due to water excess and lack of oxygen in pores of substrates with SS-solarized were identified in the two evaluated species.

The significant correlation observed between total chlorophyll from lettuce plants and physico-hydric parameters (TP, WA, WR_{100} and WRC), indicates that the balance of macro and micropores from substrates plays an important role in water availability and root aeration. Lettuce seedlings are more sensitive during the early stages of life, and a substantial part of water was not available to plants because of its stronger state of retention and, at the same time, this water occupied most of the pores, causing lack of aeration. Lenhard *et al.* (2010) reported a reduction in chlorophyll concentration in leaves of several species as a consequence of oxygen restrictions caused by inadequate substrate pore volume distribution or soil flooding.

The two evaluated species had different responses to the substrates (Table 3). The lettuce crop showed to be more sensitive to the different formulations, with the formation of several groups of response of the growth variables to the tested substrates, being able to differentiate substrates with better, similar or inferior performance to the commercial ones. Black wattle presented at most two response groups, one with similar performance and other poorer than the commercial substrates. These results can be related to several aspects, such as the great difference of rusticity between species. For example, tolerance to water stress, which is high in black wattle and small in lettuce (Attias *et al.*, 2013) may explain most of the effects on the morphophysiological variables of the evaluated plants.

The different responses obtained in the two species show the importance of performing multispecies studies while developing and evaluating alternative substrates. Although Abad (1991) points out that there is no substrate that is universally valid for all species, the evaluation of the same substrate for different species can broaden the market for this product. This study goes further, because the combination of raw materials for different crops can be the same, but the proportion of each material that returns the best substrate for each crop may vary substantially.

In relation to the materials used in the alternative substrates (T1-T10), vermiculite and rice husk ash are already widely tested materials (Kratz & Wendling, 2016). However, SS is a residue whose studies to this employment is still incipient, even though there is a great potential due to its chemical and physical characteristics (Nascimento *et al.*, 2014). It is clear from this study that the main reason for its low utilization is the lack of knowledge about safe and low-cost ways of eliminating the risk of contamination by pathogenic microorganisms

(Nascimento *et al.*, 2014) and the preconcept regarding the use of SS in agriculture.

However, biochar produced from SS, when mixed with other materials that complement its chemical and especially physical characteristics, has proved to become a viable alternative. From efficiency aspects, SS-biochar was superior to SS-solarized and similar to commercial substrates. In addition, it can be considered a safe material because after the pyrolysis process, the pathogenic contaminants are eliminated (Agrafioti *et al.*, 2013; Liu *et al.*, 2014) as well as a waste recycling process.

Although the results indicate that several proposed substrates have achieved similar performance and even higher than the commercials tested, further studies should be conducted. There is a need to test new combinations with other materials and/or proportions of the same materials, since some chemical and physical parameters (pH, WD, DD, TP, AS and WA) did not reach the values considered ideal by the literature, indicating that the results obtained in the present study could be maximized or extended to a greater number of species.

CONCLUSIONS

The biochar made from sewage sludge is more effective in comparison to the solarized sewage sludge as component of substrates for the studied species;

The formulated substrate composed by 20% sewage sludge biochar, 40% vermiculite and 40% rice husk ash presented better performance among the studied substrates;

Solarized sewage sludge increases the water retention capacity of the substrates, but negatively affects the development of lettuce and black wattle seedlings.

REFERENCES

- Abad M (1991) Los sustratos hortícolas y técnicas de cultivo sin suelo. In: Rallo Romero L & Nuez Vináls F (Eds.) La horticultura Española en la C.E. Reus, CECH. p.271-280.
- Agbna GHD, Dongli S, Zhipeng L, Elshaikh NA, Guangcheng S & Timm LC (2017) Effects of deficit irrigation and biochar addition on the growth, yield, and quality of tomato. *Scientia Horticulturae*, 222:90-101.
- Agrafioti E, Bouras G, Kalderis D & Diamadopoulos E (2013) Biochar production by sewage sludge pyrolysis. *Journal of Analytical and Applied Pyrolysis*, 101:72-78.
- Alves Filho A, Camargo R, Lana RMQ, Moraes MRB, Maldonado ACD & Atarasi RT (2016) Treatment of sewage sludge with the use of solarization and sanitizing products for agricultural purposes. *African Journal of Agricultural Research*, 11:184-191.
- Attias N, Siqueira MF & De Godoy BH (2013) Acácias australianas no Brasil: histórico, formas de uso e potencial de invasão. *Biodiversidade Brasileira*, 2:74-96.
- Bai Y, Zang C, Gu M, Gu C, Shao H, Guan Y, Wang X, Zhou X, Shan Y & Feng K (2017) Sewage sludge as an initial fertility driver for rapid improvement of mudflat salt-soils. *Science of The Total Environment*, 578:47-55.

- Brasil (2006) Conselho Nacional do Meio Ambiente (CONAMA) - Resolução N° 375, de 29 de agosto de 2006. Critérios e procedimentos, para o uso agrícola de lodos de esgoto gerados em estações de tratamento de esgoto sanitário e seus produtos derivados. DOU, 30/08/2006, Seção 1, p. 01.
- Brasil (2007) Instrução normativa n° 17, de 21 de maio de 2007. Métodos analíticos oficiais para análise de substratos para plantas e condicionadores de solo. DOU, 24/05/2007, Seção 1, p. 02.
- Caldeira MV, Delarmelina WM, Peroni L, Gonçalves EO & Silva AG (2013) Lodo de esgoto e vermiculita na produção de mudas de eucalipto. *Pesquisa Agropecuária Tropical*, 43:155-163.
- Caldeira MVW, Peroni L, Gomes DR, Delarmelina WM & Trazzi PA (2012) Diferentes proporções de biossólido na composição de substratos para a produção de mudas de timbó (*Ateleia glazioveana* Baill). *Scientia Forestalis*, 40:015-022.
- De Boodt M & Verdonck O (1972) The physical properties of the substrates in floriculture. *Acta Horticulturae*, 26:37-44.
- Faria JCT, Caldeira MVW, Delarmelina WM, Lacerda LC & Gonçalves EO (2013) Substratos à base de lodo de esgoto na produção de mudas de Senna alata. *Comunicata Scientiae*, 4:342-351.
- Freitas RXA & Melo GA (2013) Avaliação do uso de biocomposto de lodo de esgoto como substrato para produção de mudas. *Monografias Ambientais*, 12:2665-2673.
- Jin J, Li Y, Zhang J, Wu S, Cao Y, Liang P, Zhang J, Wong MH, Wang M, Shan S & Christie P (2016) Influence of pyrolysis temperature on properties and environmental safety of heavy metals in biochars derived from municipal sewage sludge. *Journal of Hazardous Materials*, 320:417-426.
- Kiehl EJ (1985) *Fertilizantes Orgânicos*. Piracicaba, Agronômica Ceres. 492p.
- Kratz D & Wendling I (2016) Crescimento de mudas de *Eucalyptus camaldulensis* em substratos à base de casca de arroz carbonizada. *Revista Ceres*, 63:348-354.
- Lenhard NR, Scalon SP & Novelino JO (2010) Crescimento inicial de mudas de pau ferro (*Caesalpinia ferrea* Mart. Ex Tul. Var. *Leiostachya* Benth.) sob diferentes regimes hídricos. *Ciência e Agrotecnologia*, 34:870-877.
- Liu T, Liu B & Zhang W (2014) Nutrients and heavy metals in biochar produced by sewage sludge pyrolysis: its application in soil amendment. *Polonian Journal of Environmental Studies*, 23:271-275.
- Méndez A, Terradillos M & Gascó G (2013) Physicochemical and agronomic properties of biochar from sewage sludge pyrolysed at different temperatures. *Journal of Analytical and Applied Pyrolysis*, 102:124-130.
- Nascimento AL, Sampaio RA, Zuba Junio GR, Fernandes LA, Crus SF, Carneiro JP, Barbosa CF & Lima NN (2014) Atributos químicos do solo adubado com lodo de esgoto estabilizado por diferentes processos e cultivado com girassol. *Bioscience Journal*, 30:146-153.
- Ozdemir S, Aslan T, Celebi A, Dede G & Dede OH (2013) Effect of solarization on the removal of indicator microorganisms from municipal sewage sludge. *Environmental Technology*, 34:1497-1502.
- Pereira Lima MR, Além Sobrinho P, Cunha ERQ, Vilela KS & Loureiro LF (2009) Avaliação da qualidade do lodo de esgoto quando submetido à secagem/higienização em estufa agrícola. *Revista AIDIS de Ingeniería y Ciencias Ambientales: investigación, desarrollo y práctica*, 2:41-48.
- Santos AGVA, Athiê AAR, Bueno RF & Seo ESM (2015) Secagem do biossólido em uma estufa agrícola. *Revista de Iniciação Científica, Tecnológica e Artística*, 7:87-102.
- Silva DJCB & Jorge LAC (2009) AFSOFT (r)-Software para análise foliar. In: *Jornada Científica-Embrapa São Carlos, São Carlos. Anais, Embrapa Pecuária Sudeste/Embrapa Instrumentação Agropecuária*. p. 53.
- Silva IS, Mackowiak C, Minogue P, Reis AF & Moline EFV (2017) Potential impacts of using sewage sludge biochar on the growth of plant forest seedlings. *Ciência Rural*, 47:01-05.
- Tessaro D, Sampaio SC & Castaldelli APA (2016) Wastewater use in agriculture and potential effects on meso and macrofauna soil. *Ciência Rural*, 46:976-983.
- Valeri SV & Corradini L (2000) Fertilização em viveiros para produção de mudas de *Eucalyptus* e *Pinus*. In: Gonçalves JLM & Benedetti V (Eds.) *Nutrição e fertilização florestal*. Piracicaba, IPEF. p.167-189.
- Verdonck O & Gabriels R (1988) Substrate requirements for plants. *Acta Horticulturae*, 221:19-23.
- Yuan H, Lu T, Wang Y, Chen Y & Lei T (2016) Sewage sludge biochar: Nutrient composition and its effect on the leaching of soil nutrients. *Geoderma*, 267:17-23.