

Original Research Article

Use of organic compost and residues as an alternative to commercial substrate for lettuce cultivation (*Lactuca sativa* L.)

ABSTRACT

Aims: The objective of this work was to evaluate the physical and biological characteristics of the substrates formulated from organic compost (OC), carbonized rice husk (CRH), sewage sludge (SS) and commercial substrate, as well as the vegetative development of lettuce seedlings cultivated in the compositions.

Study design: Eight treatments were studied in a completely randomized design: TO- commercial substrate (CS), T1- 50% OC & 50% CRH, T2 – 75% OC & 25% CRH, T3- 50% SS & 50% CRH, T4- 75% SS 25% CRH, T5- 100% SS, T6- 100% OC and T7- 30% SS, 30% OC & 40% CRH, being carried in box of expanded polystyrene (PEE) with four replicates.

Place and Duration of Study: The experiment was conducted in an experimental area of Campus Pelotas, Federal Institute Sul-rio-grandense, Pelotas, Brazil, period from November until December 2015.

Methodology: The physical characteristics evaluated were: total porosity, macroporosity, microporosity, water retention capacity and density. The microbiological analyses as carbon in the microbial biomass (Cmic), basal respiration (BR) and metabolic quotient (qCO₂). Also, organic matter, electrical conductivity and dry matter and plant growth.

Conclusion: The T2 (75% OC & 25% CRH) and T7 (30% SS, 30% OC & 40% CRH) treatments were the most promising in relation to physical characteristics resulting in good microporosity, total porosity, water retention capacity and density, but low macroporosity. Regarding the microbiological characteristics the T2 treatment presented greater microbial activity and ideal electrical conductivity. For vegetative growth and dry matter T2 and T7 presented similar results to the commercial substrate.

Keywords: *Residue; organic compost; rice husk; sewage sludge; microbial activity.*

1. INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most consumed and produced vegetables in the world, it represents an important source of mineral salts, mainly calcium and vitamins [1]. The intensive practices of lettuce cultivation demand large amounts of fertilizers and pesticides, which can lead to soil contamination and degradation [2]. Therefore, it is necessary to provide alternatives to improve the sustainability of such agricultural ecosystems without reducing their productivity.

An important factor in the production of lettuce seedlings is the substrate, which supports the plants and regulates the availability of nutrients to the roots. It must be sufficiently porous, have good water retention capacity, satisfactory drainage, good aeration, low resistance to root growth [3]. In addition to the appropriate physical and chemical properties, the substrates must be composed of materials of low cost and be of easy acquisition, long durability and recyclable [1].

Recent studies show that the cultivation of seedlings can be made from substrates produced by the farmer himself using materials found at the place of production and of low cost. In this sense, Caltoldi et al. [1] formulated alternative substrates based on vermicompost, sand,

carbonized rice rusk and basalt powder to grow lettuce seedlings and obtained a lower cycle of cultivation and better development of the plants. According to Bohm et al. [3] the use of alternative substrates is aimed at promoting the use of local resources and consequently reducing the use of chemical inputs, contributing to a better environmental balance, maintaining biodiversity, producing quality seedlings and seeking the viability of sustainable agriculture.

Although this system of production of seedlings has several advantages of production, some problems have been noticed in relation to the different characteristics of the substrates used, such as moisture conservation, aeration and the availability of nutrients [4].

The objective of this work was to evaluate the feasibility of using organic compounds, treated sewage sludge and carbonized rice husk in different combinations, as an alternative to inorganic fertilizers in lettuce cultivation, as well as the effect of these treatments on the physical properties, microbiological and the development of lettuce seedlings. In this sense, the hypotheses of this work were: 1) that substrates formulated from organic compounds, treated sewage sludge and carbonized rice husk, can provide appropriate physical characteristics equivalent to the commercial substrate required for seedling lettuce development; and 2) substrates formulated through organic compounds, treated sewage sludge and carbonized rice hulls improve the microbiological properties of the soil by replacing the organic matter in the soil and improving the physical characteristics of the soil.

2. MATERIAL AND METHODS

The experiment was conducted in an experimental area of Campus Pelotas, Federal Institute Sul-rio-grandense, geographic coordinates 31° 76' 68" S 52° 35' 35" W, Brazil, in the period from November until December 2015.

The following materials were used in the experiment: commercial substrate Plantmax HA® (composed of composted pine bark, peat, charcoal and vermiculite), carbonized rice husk-CRH (obtained from rice industry, located in Pelotas city/RS Brazil), sewage sludge- SS (obtained from the Pelotas Sewage Treatment Station - SANEP) and organic compound- OC (resulting from the decomposition of domestic organic waste, vegetable remains and fruit).

Eighth treatments were studied in a completely randomized design: TO- commercial substrate (CS), T1- 50% OC & 50% CRH, T2 – 75% OC & 25% CRH, T3- 50% SS & 50% CRH, T4- 75% SS & 25% CRH, T5- 100% SS, T6- 100% OC e T7- 30% SS, 30% OC & 40% CRH, being carried in box of expanded polystyrene (PEE) with four replicates.

As vegetable material were used lettuce seeds of the company Feltrin®, three seeds in each cell for sowing. Irrigated daily until germination, after this period irrigation was performed according to the agronomic need of the plants. Ten days after sowing (DAS) thinning was performed, leaving only one seedling per cell.

The chemical characteristics were determined before to cultivation under low humidity conditions: pH in water (pH), organic carbon (Corg), total nitrogen (N), total phosphorus (P), total potassium (K), total calcium (Ca), total magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and carbon/nitrogen ratio (C:N). The analysis of these characteristics was performed according to the method described by Tedesco [5]. The pH was determined by potentiometer in substrate: water suspensions (1:5, v:v). The Corg was determined by the moist combustion method Walkey Black and the N by the Kjeldahl method. The P and K were determined by sulfur digestion, with P analyzed in mass spectrometry and K in atomic absorption spectrometry. The analysis of metals trace (Cu, Zn and Fe) according to Abreu et al. [6] using atomic absorption spectrometry.

92 **Table 1- pH, macronutrients, micronutrients and metal trace provided in each**
 93 **treatment determined before the growing period.**
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	T0	T1	T2	T3	T4	T5	T6	T7
pH	6.80	8.71	8.61	3.84	3.59	3.41	7.96	5.57
Corg g kg ⁻¹	237.69	112.07	278.04	89.46	91.15	117.60	318.60	239.71
N g kg ⁻¹	14.77	17.37	10.17	6.25	7.12	7.29	12.86	8.16
P g kg ⁻¹	0.55	1.48	1.35	2.62	3.25	3.07	1.73	2.62
K g kg ⁻¹	2.16	4.87	4.60	2.97	1.89	1.35	3.24	3.24
Ca g kg ⁻¹	20.68	7.20	8.43	0.58	0.58	0.43	10.66	3.10
Mg g kg ⁻¹	3.40	1.03	1.21	0.11	0.08	0.24	1.23	0.22
Cu g kg ⁻¹	10.28	26.44	27.91	73.45	82.26	80.79	35.26	66.10
Zn g kg ⁻¹	17.07	95.78	111.45	63.74	69.66	66.52	137.92	80.80
Fe g kg ⁻¹	3983.25	4932.53	4550.95	11074.92	11298.28	11326.20	5807.35	10479.92
Mn g kg ⁻¹	120.83	284.75	245.40	104.91	74.00	45.90	242.59	149.87
C:N	36:1	12:1	27:1	14:1	13:1	16:1	25:1	29:1

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97 The Organic Matter (OM) and the Electrical Conductivity (EC) was determined at 30 DAS. In
 98 this same period, the dry matter was determined Microbial Biomass Carbon (Cmic) and the
 99 evolution of CO₂ released in the process of Basal Respiration (BR). The OM was determined
 100 by calcining 2 g of substrate sample, previously oven dried at 60°C, in a muffle at 550°C for
 101 4 hours, promoting the loss of volatiles from the sample [7].

102 The Cmic was determined by the method of Vance et al. [8], as modified by Ferreira et al.
 103 [9]. According to this method, soil microorganisms are killed by irradiation at 2450 MHz for 4
 104 min instead of fumigation with chloroform. The Cmic was determined by the difference
 105 between the irradiated and nonirradiated soil sample after K₂SO₄ extraction and C
 106 determination by dichromate oxidation and titration with ferrous ammonium sulphate. The
 107 value for Cmic was calculated by the following formula: Cmic= (Ci - Cni)/Kc, where Ci and
 108 Cni are the C content of the irradiated and nonirradiated samples, respectively; and Kc is the
 109 correction factor with a value of 0.33. The results were expressed in µg CO₂ g⁻¹ soil.

110 The Basal Respiration (BR) was determined by the quantification of CO₂ released in the
 111 microbial respiration process for 42 days, using the method used by Bohm et al. [10]. CO₂
 112 was quantified by titration with 1M HCl solution after the addition of BaCl₂ solution (25% w/v)
 113 and 3 drops of phenolphthalein (1%) as indicator. The amount of CO₂ released in each
 114 treatment and evaluation period was calculated by the formula: RB = (VPB-VA) x M acid x
 115 Eq. C-CO₂, where: VPB = volume of HCl spent in the blank; VA = Volume of HCl spent in the
 116 sample; M acid= HCl concentration; Eq. C-CO₂ = gram equivalent of C-CO₂. The results

were expressed as $\mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$. The rate of respiration per unit of biomass or metabolic quotient ($q\text{CO}_2$) was obtained by the relationship between the basal respiration rate and microbial biomass.

The Electrical Conductivity (EC) was determined with 50 ml of sample and 250 ml of deionized water in a 300 ml flask. After 30 minutes of rest, the samples were filtered and measurements in conductivity equipment Digimed.

The physical characteristics: macroporosity, microporosity, total porosity, water retention capacity and density, were evaluated according to the method described by Bohm et al [3]. To determine the physical attributes, the following equations were used:

Macroporosity (%) = $[(A-B) / C] \times 100$
 Microporosity (%) = $[(B-D-E) / C] \times 100$
 Total porosity (%) = macroporosity + microporosity
 Maximum water holding capacity ($\text{ml } 50\text{cm}^{-3}$) = $B-D-E$
 Density = $(D-E) / C$.

Where: A = weight of the soaked substrate; B = weight of substrate drained; C = volume of the tube; D = weight of dry substrate; E = tube weight.

The values found for physical properties in this study were compared with the respective values or ranges considered ideal in literature [11, 12, 13, 14] in the formulation of substrates for plant cultivation (Table 2).

Table 2. Ideal reference in literature range for the physical properties of substrates

Macroporosity (%)	35-45
Microporosity (%)	45-55
Total porosity (%)	> 85
Water holding capacity (%)	20-30
Density (g.cm^{-3})	0,10-0,35

The Dry Matter (DM) was determined at 42 DAS the plants were removed from the trays and then proceeded to the careful washing of the root system. The seedlings were cut at the height of the colon to separate aerial part and root. The aerial parts were dried at 60°C until constant weight to determine the dry matter, with the aerial part crushed in wiley type mill. At the same time, to evaluate the seedlings four plants of each replication were removed from the trays and washed in running water to remove the substrate from the roots. The number of leaves was obtained counting the true leaves, being those with a length superior to 1,0 cm. The height of the seedling (root collar at the tip of the highest leaf) was obtained using a ruler.

The results were submitted to the analysis of variance test with Tukey to 5% of probability. Statistical analyzes were performed using Statistix 8.0 (for Windows, Analytical Software Inc., Tallahassee, FL, USA).

3. RESULTS AND DISCUSSION

In relation to OM, the highest levels were obtained by the control treatment T0 (82.2%) and the lowest by the treatment T4 (14.5%) (Table 3). During the mineralization process, the OM releases nutrients to the plants [15], being an important variable in agricultural substrates. In

relation to OM optimal levels should be higher than 80% [3], value reached only by T0 (CS). According to Schmitz [11], suggest a minimum value of 50% of OM for substrates used in the production of seedlings, being within this range the T6 treatment. The treatments T3, T4, T5 and T7 presented the lowest levels for this variable, on average 16.3% (Table 3). The OM causes changes in the physical, chemical and biological characteristics of the soil, increasing the aeration and the retention of moisture [16]. OM is the main source of macro and micronutrients essential to plants, increase the nutrient retention capacity and increasing the activity of soil microorganisms [3]. According to Schmidt [4] the process of microbial decomposition of soil is controlled by substrate quality and the availability of carbon and nutrients.

For Cmic T2 treatment had the highest levels $242.5 \mu\text{g g}^{-1}$, being 74.3% higher than the control T0 treatment (Table 3). Thus, the highest Cmic contents may be associated to the use of organic compost with high organic carbon contents (278.04 g g^{-1}) (Table 1). The treatments with addition of SS (T3, T4 and T5) resulted in mean Cmic contents of $124.9 \mu\text{g g}^{-1}$, this lower result in relation to treatments with addition of organic compost may be associated the presence of sewage sludge in the composition of the substrates may have resulted in a lower content of Corg, as this decreases significantly during the process of stabilizing the sludge through microbiological respiration, converting it into CO_2 and also through mineralization [14]. However, when working with doses between 2.5 and 30 ton ha^{-1} , Sullivan et al. [17] did not observe changes in the Cmic as a result of the application of sewage sludge and suggest that, in this case, there was a rapid adaptation of the soil microorganism.

The microbial activity of the soil was determined by the evolution of CO_2 , the highest BR rates were obtained by treatments T6, presenting an average of $1.067 \mu\text{g C-CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ (Table 3). Thus, these values were associated to the presence of organic compost in their compositions, which provides a higher activity of soil microorganisms [10].

These results suggest beneficial effects of the organic compounds on the microbiological characteristics of the soil. The supply of Corg and biomass provided by the compound is probably the factor that most contributes to the increase of the Cmic, the stimulation of the microbial activity and the better soil physical conditions [18]. The higher values of BR ratio suggest that the effects of organic materials on soil microbial activity depend on the characteristics of organic wastes [19, 20].

As for $q\text{CO}_2$, the T1 treatment presented a higher quotient $6.85 \cdot 10^{-3}$. The lower quotients were obtained by treatments T3, T4, T5 and T7 indicating greater microbial efficiency. This result may be a consequence of higher microbial activity, with lower CO_2 release per unit of Cmic, caused by the presence of a readily assimilable substrate for the development of microbial activity, corroborating with the results obtained by Bohm et al. [21].

For Rosa et al. [22] the presence of sewage sludge provided stimulus in the production of Cmic, the addition of sludge in the soil can raise the microorganisms in the soil to respond differently depending on the physical and chemical properties and environmental conditions [20].

Table 3- Organic matter (OM), microbial biomass carbon (Cmic), basal respiration (BR), metabolic quotient ($q\text{CO}_2$), electrical conductivity (EC) and dry matter (DM).

Treatments	OM	Cmic	BR	$q\text{CO}_2$	EC	DM
	%	$\mu\text{g g}^{-1}$	$\mu\text{g C-CO}_2 \text{ g}^{-1} \text{ h}^{-1}$	$\times 10^{-3}$	mS cm^{-1}	g
T0- CS	82.2 ^a	062.1 ^e	0.073 ^d	1.17 ^c	1.63 ^c	2.47 ^c
T1-50% OC & 50% CRH	30.3 ^c	133.4 ^c	0.910 ^b	6.85 ^a	2.47 ^a	2.70 ^{bc}
T2-75% OC & 25% CRH	35.8 ^c	242.5 ^a	0.264 ^c	1.09 ^c	1.15 ^d	4.40 ^a

T3-50% SS & 50% CRH	14.6 ^d	125.0 ^c	0.025 ^d	0.20 ^d	1.99 ^b	3.60 ^{ab}
T4-75% SS & 25% CRH	14.5 ^d	106.2 ^d	0.016 ^d	0.18 ^d	1.24 ^d	3.22 ^{ab}
T5-100% SS	16.3 ^d	143.5 ^b	0.033 ^d	0.23 ^d	2.52 ^a	-
T6-100% OC	58.8 ^b	266.1 ^a	1.067 ^a	4.01 ^b	1.73 ^c	2.54 ^c
T7-30% SS, 30% OC & 40% CRH	19.9 ^d	092.9 ^d	0.086 ^d	0.93 ^c	2.08 ^b	3.11 ^{ab}

Means followed by the same letters, in the same column, did not differ significantly by the Tukey test at the 5% probability level.

The qCO₂ has been used as a biological indicator of soil balance, since as microbial biomass becomes more efficient, less carbon is released as CO₂ by respiration and a higher proportion of carbon is incorporated into microbial biomass [21]. It is known that stress factors (herbicides, heavy metals, pH, nutrient limitations) as well as disturbance factors (environmental conditions) induce microbial inefficiency. A potential effect of soil sludge application is the stimulation or inhibition of soil microorganisms with important functions such as mineralization and nutrient immobilization [3].

The ideal range for EC is between 0.76 and 1.25 mS cm⁻¹ [11]. Only the T2 e T4 treatment was within this range, all others resulted in higher levels. According to Martinez [23] electrical conductivity contents above 3.5 mS cm⁻¹ is excessive for most plants. Excess EC was not observed in any treatment. Bohm et al. [3] found significantly lower EC contents in substrates formulated from CRH and SS, on average 0.3 mS cm⁻¹. For some authors, such as Abad [24] the optimal electrical conductivity for substrates should be less than 0.5 mS cm⁻¹.

Regarding dry matter values, treatments T2, T3, T4 and T7 stood out in relation to the others, resulting in 4.4, 3.6, 3.22 and 3.11 respectively (Table 3). The presence of organic compost, sewage sludge and carbonized rice husk favored the development of the plants resulting in a greater amount of dry matter. Other authors also obtained positive results in the development of plants in crops with application of treated sewage sludge and carbonized rice husk [3, 11, 25].

Table 4 - Macroporosity (Macro), Microporosity (Micro), Total Porosity (Porosity), Water Retention Capability (Ret Cap.) and Density.

Treatments	Macro	Micro	Porosity	Ret Cap	Density
	%	%	%	ml 50cm ⁻³	g cm ⁻³
T0- CS	51.52 ^a	48.86 ^a	78.10b	22.20 ^a	0.24 ^b
T1-50% OC & 50% CRH	47.31 ^a	38.63 ^b	75.91b	22.62 ^a	0.13 ^c
T2-75% OC & 25% CRH	33.32 ^b	49.43 ^a	92.72a	23.32 ^a	0.26 ^b
T3-50% SS & 50% CRH	49.33 ^a	36.70 ^b	66.01b	17.13 ^b	0.15 ^c
T4-75% SS & 25% CRH	36.02 ^b	32.08 ^b	68.02b	24.60 ^a	0.23 ^b
T5-100% SS	19.01 ^c	23.20 ^c	42.20c	15.61 ^c	0.46 ^a

T6-100% OC	23.32 ^c	26.21 ^c	29.51d	12.20 ^c	0.50 ^a
T7-30% SS, 30% OC & 40% CRH	43.10 ^{ab}	46.90 ^a	81.91a	23.96 ^a	0.26 ^b

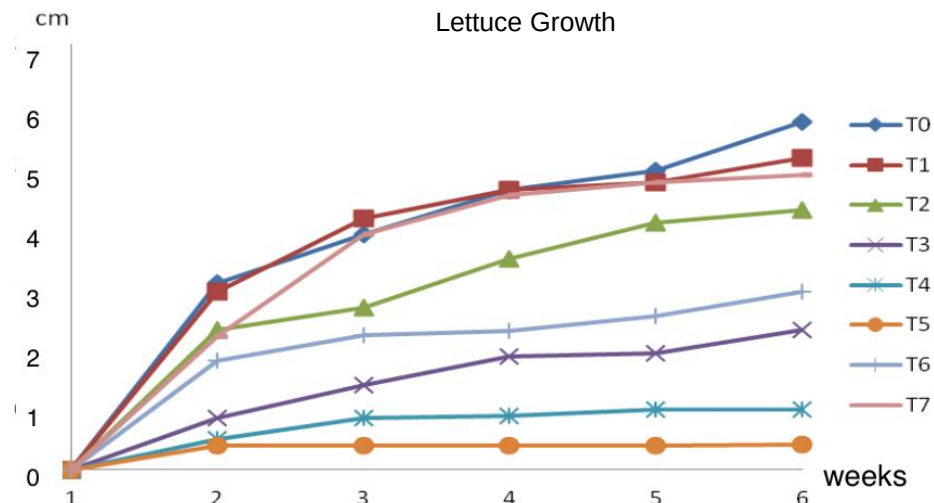
Means followed by the same letters, in the same column, did not differ significantly by the Tukey test at the 5% probability level.

For the macroporosity variable the treatments T2, T4 and T7 resulted in values within the range indicated for this variable, which according to Lopes et al. [12] ideal macroporosity values should be in the range of 35 to 45%. The treatments T0, T1 and T3 resulted in higher values than indicated. Because the CRH is a light and inert material to hydration, an increase in the porosity of the substrate can occur, mainly due to the increase in the percentage of macropores, this fact can be observed in the T1 and T3 treatments that have 50% CRH in composition.

As for microporosity, treatments T0, T2 and T7 presented percentages of 48.86, 49.43 and 46.90% respectively, being within the range indicated as ideal (Table 2). The remaining treatments T1, T3, T4, T5 and T6 resulted in levels below the recommended range. As for total porosity, treatments T2 and T7 resulted in percentages of porosity of 92.72 and 81.91%, respectively (Table 4), being these values within the range recommended for this variable (> 85%, Table 2), thus presenting better aeration, water infiltration and drainage. Costa et al. [26] obtained 88.18% porosity with the addition of 32.33% CRH. Bohm et al [3] obtained results below these, around 72% total porosity with addition of 30% CRH. According to Zorzeto [27] substrates with low porosity may impair the development of seedlings because they hinder root gas exchange and water drainage, while high porosity can result in low water retention causing water deficiency for plants. In the case of T5-100% SS and T6-100%, the substrate compaction may reflect a decrease in total porosity, especially in substrates with smaller particles and with greater particle size inequality [28]. This fact can be observed in the treatments T5- 100% SS and T6- 100% OC that resulted in low total porosity mainly due to the substrate compaction.

As for the water retention capacity, treatments T1, T2, T4 and T7 presented 22.62, 23.32, 24.06 and 23.96%, respectively, similar to the control treatment T0 (22.20%), being within the range indicated by Martínez [23], which considers an optimal water retention capacity between 20 and 30%. Substrates formulated with high percentages of CRH (T1 and T3) or with large amounts of sewage sludge (T5) or organic compound (T6) resulted in treatments with low water retention capacity which may result in poor rooting of plant. According to Costa et al. [26] low water retention generates possible water stress which leads to a higher energy expenditure by the plant to supply this need and impairs its development.

As for density, treatments T2, T4 and T7 resulted in densities similar to the T0 control treatment. Taking the recommended density of 0.10 to 0.35 g cm⁻³ [13], only the T5 and T6 treatments presented results above this range. The density of the substrate is directly related to porosity, root ventilation and moisture retention [1]. Among the substrates studied, formulations with mixtures of SS, OC and CRH resulted in density compatible with the commercial substrate (Table 3). Generally, higher densities are associated with less aeration and storage of water. This fact is visible in the results obtained by the T5 and T6 treatments studied because they presented high densities and low porosity and water retention in comparison to the other treatments, probably reflecting the addition of CRH that has a density much smaller than SS or OC [1] and adding them would probably reduce the density of the substrate. The good physical properties of the compounds, especially the porosity and the good proportion of air and water, are very important when the compound is used as a



culture medium for plant transplants [13].

Fig. 1 - Lettuce growth in centimeters over the six week period.

Regarding the vegetative development of the seedlings, treatments T1, T2 and T7 presented a shoot height similar to the control treatment T0 (Fig. 1), T3 and T4 treatments had a small growth in the first weeks and after the third week practically stabilized, being very low of the T0 control treatment. The T5 treatment was the one that presented worse development of the aerial part and did not resist until the end of the experiment. Another parameter that was analyzed in the development of seedlings is the period necessary to obtain five leaves that should be between 26 and 33 days. In the leaf count of the cultivated seedlings the amount of five to six leaves in the treatments T0, T1, T2 and T7 in the T5 treatment was verified in the 30 DAS, the development of only two leaves was below the required standard for the formation of seedlings.

In the process of seedling production, the substrate plays a primordial role, as it influences the initial development of the plant. Because of the limited volume of root growth, when using containers, substrates must be able to provide constant supply of water, oxygen, and nutrients to plants [29]. In a study by Lima et al. [30] with green mint plants observed that the highest number of leaves was obtained with the substrate based on carbonized rice husk and organic compound. Substrates that condition a smaller period of the seedlings in the production trays become an important tool in the area of lettuce seedlings production. This means the possibility of more cycles of production of seedlings in the nursery, lower costs and greater capital turnover to the seedlings producer.

Other authors such as Castoldi et al. [1], Schmitz et al. [4], Zorzeto [11] and Bohm et al. [3] also studied substrates formulated from organic compounds, carbonized rice husk, sewage sludge and other alternative materials and were able to obtain positive results in relation to the development of vegetable seedlings. In general carbonized rice husk has been reported as possible of application in the formulation of substrates. Other materials such as sewage

sludge have been reported as possible in improving the microbiological qualities of the substrate [3]. Organic compounds formulated from bark and fruit have been successful in improving the physical and chemical qualities of substrates [1, 4].

4. CONCLUSION

The addition of organic compost, treated sewage sludge and carbonized rice husk are suitable for the formation of alternative substrate for the cultivation of lettuce seedlings. The T2 (75% OC & 25% CRH) and T7 (30% SS, 30% OC & 40% CRH) treatments were the most promising in relation to physical characteristics resulting in good microporosity, total porosity, water retention capacity and density, but low macroporosity. Regarding the microbiological characteristics the T2 treatment presented greater microbial activity than all the others. For vegetative growth and dry matter T2 and T7 presented similar results to the commercial substrate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT (WHERE EVER APPLICABLE)

Not applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Not applicable

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