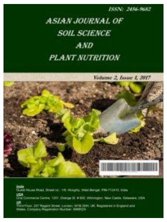

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Physical-chemical Characterization and Microbial Activity of Alternative Substrates for Arugula Cultivation (*Eruca sativa* Mill.)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GMBB and TM conceived and designed the experiment. Authors SMS and SLL conduced the experiment. Authors GMBB, SMS and EBM analyzed the data. All authors jointly wrote and approved the final manuscript.

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ABSTRACT

Aims: The objective of this work was to evaluate the physical, chemical and biological characteristics of the substrates formulated from carbonized rice husk, industrial sludge, vermiculite, soil and commercial substrate, as well as the development of arugula seedlings cultured in the compositions.

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Study Design: Eight treatments were studied in a completely randomized design: TO = commercial substrate (SC); T1= commercial substrate + soil (1SC:1S); T2= commercial substrate + soil + carbonized rice husk (1SC:1S:1CAC); T3= commercial substrate + vermiculite (1SC:1V); T4= commercial substrate + vermiculite + carbonized rice husk (1SC:1V:1CAC); T5= sludge + vermiculite (1L:1V); T6= sludge + vermiculite + carbonized rice husk (1L:1V:1CAC); T7= commercial substrate + sludge + vermiculite + carbonized rice husk (1SC:1L:1V:1CAC), 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 of Sul-rio-grandense, Pelotas, Brazil, period from October until December 2015.

Methodology: The chemical characteristics evaluated were: pH in water, organic carbon, total nitrogen, total phosphorus, total potassium and C/N ratio. The physical characteristics as total porosity, macroporosity, microporosity, water retention capacity and density. Also, organic matter, electrical conductivity and basal respiration were evaluated.

Conclusion: The SC:V and SC:V:CAC treatments present pH, C Org., N, OM and C/N characteristics close to the commercial substrate, but present P, K and EC levels below the ranges indicated in the literature as suitable for the formation of substrates. Regarding the physical characteristics, the formulation closest to the ideal was also SC:V, which results in good microporosity, total porosity, water retention capability, but low macroporosity and density.

Keywords: Residue; electrical conductivity; rice husk; industrial sludge; basal respiration.

1. INTRODUCTION

The cultivation of arugula (*Eruca sativa* Mill.) has been prominent in the world scenario due to its nutritional and phytotherapeutic properties, as they are rich in biologically active compounds including ascorbic acid, carotenoids, fibers, polyphenols and glucosinolates [1]. The most commercialized vegetables in Brazil, it occupies the 24th position, and among the hardwoods is in fifth place coming soon after the lettuce, chives and cabbage [2].

Alternative substrates has been widely used for obtain seedling cultivation in an environmentally safe and profitable way [3,4,5]. A number of residues, such as mixtures using peat and tree bark [6], sewage sludge [4,7,8,9] have been used over the last decades [10]. CAC as a component for the formulation of substrates has been used in regions that have rice industries due to their chemical and physical characteristics, low cost and high availability [10].

The evaluation of physico-chemical and microbiological parameters are necessary to monitor aspects related to soil composition, structure and microbial activity [11]. The physical characterization of substrates includes total porosity, macroporosity, microporosity, water retention capacity and density. For these physical properties already have been studied and defined standards and ranges of values that serve as reference to characterize the ideal conditions of the substrate to be used for the production of seedlings in box [3,6].

The objective of this study was to evaluate the physical, chemical characteristics and biological activity of substrates formulated from different mixtures involving commercial substrate, industrial sludge, carbonized rice husk, vermiculite and soil, as well as to compare the results with reference values cited in the literature, for the formulation of suitable substrates for the cultivation of vegetable seedlings in box.

2. MATERIALS AND METHODS

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

The following materials were used in the experiment: commercial substrate Hdecher®, vermiculite, soil is classified as Dystrophic Red Yellow Podzolic (U.S. soil taxonomy), carbonized rice husk and industrial sludge, the latter two obtained from rice industry, both located in the municipality of Pelotas/RS Brazil.

Eight treatments were studied in a completely randomized design: TO= commercial substrate (SC); T1= commercial substrate + soil (1SC:1S); T2 = commercial substrate + soil + carbonized rice husk (1SC:1S:1CAC); T3= commercial substrate + vermiculite (1SC:1V); T4= commercial substrate + vermiculite + carbonized rice husk (1SC:1V:1CAC); T5 = sludge + vermiculite (1L:1V); T6= sludge + vermiculite + carbonized rice husk (1L:1V:1CAC); T7= commercial substrate + sludge + vermiculite + carbonized rice husk (1SC:1L:1V:1CAC), being carried in box of expanded polystyrene (PEE) with four replicates.

As vegetable material were used arugula 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. Eight days after planting (DAP) thinning was performed, leaving only one seedling per cell.

The chemical characteristics evaluated were: pH in water (pH), organic carbon (C Org.), total nitrogen (N), total phosphorus (P), total potassium (K) and C/N ratio. The analysis of these characteristics was performed according to the method described by Tedesco [12]. The pH was determined by potentiometer in substrate: water suspensions (1:5, v:v). The C Org. 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 chemical characteristics were determined before to cultivation under low humidity conditions. The organic matter content and the electrical conductivity was determined at 20 DAP. In this same period, the dry matter was determined the evolution of CO_2 released in the process of microbial respiration.

The organic matter content (OM) was determined by calcining 2 g of substrate sample, previously oven dried at 60°C, in a muffle at 550°C for 4 hours, promoting the loss of volatiles from the sample [13].

The electrical conductivity 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 dm3.

The physical characteristics: total porosity, macroporosity, microporosity, water retention capacity and density, were evaluated according to the method described by Guerrini and Trigueiro [14]. For it, the polypropylene tubes with a volume of 50 cm^3 were identified, weighed and filled manually with substrate. The substrate was compacted, simulating the beating for particle densification, similar to that used for the production of commercial scale seedlings. After filling the tubes and densification, the substrate was submitted to water saturation. The initial waterlogging period was 1 h. The tubes were then drained for 30 min. The first weighing was performed with the substrate soaked. For the second weighing, the drainage was carried out in two stages, the first one with the drainage surface free for 1 h, and the second with the drainage surface in contact with sheets of newsprint and a plastic foam blade for 12 h. Afterwards, the drained substrate was transferred to capsules, which were taken to a regulated oven at 105°C for 24 h. After this time, the capsules were stored in desiccators for cooling, followed by weighing.

To determine the physical attributes, the following equations were used:

Macroporosity (%) = [(A-B) / C] x 100

Microporosity (%) = [(B-D-E) / C] x 100

Total porosity (%) = Macroporosity + Microporosity

Maximum water holding capacity (mL.50 cm^{-3}) = B-D-E

Apparent density of substrate = (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 chemical and physical properties in this study were compared with the respective values or ranges considered ideal in literature [3,6,15,16,17,18,19] in the formulation of substrates for plant cultivation (Tables 1 and 2).

Table 1. Ideal reference range for the chemical properties of substrates

pH	**5,2 - 7**
Electric conductivity (mS cm^{-1})	0,76 - 1,25
Organic matter (%)	> 80
Carbon / Nitrogen ratio (C:N)	20 - 40
Total Nitrogen(g kg^{-1})	0 - 20
Total phosphorus (g kg^{-1})	6 - 10
Total Potassium (g kg^{-1})	1,6 - 3,0

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

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

Basal respiration (RB) was determined by the quantification of CO_2 released in the microbial respiration process for 42 days, using the method adopted by Bohm [20]. CO_2 was quantified by titration with 1M HCl solution after the addition of $BaCl_2$ solution (25% w/v) and 3 drops of phenolphthalein (1%) as indicator. The amount of CO_2 released in each treatment and evaluation period was calculated by the formula: RB = (VPB-VA) x M acid x Eq. C-CO_2, where: VPB = volume of HCl spent in the blank; VA = Volume of HCl spent in the sample; M acid= HCl concentration; Eq. C-CO_2= gram equivalent of C-CO_2. The results were expressed as µg CO_2 g^{-1} h^{-1}.

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

Considering that the ideal pH range is between 6 and 7, for substrates of mineral origin and between 5.2 and 5.5, for organic-based substrates [17], the formulations mixtures from SC, soil, CAC, vermiculite and sludge present elements of organic and mineral base. The treatments T1, T2, T3 and T4 (1SC:1S: 6.15; 1SC:1S:1CAC: 6.24; 1SC:1V: 6.25; 1SC:1V:1CAC: 6.47) presented pH within the ideal range. On the other hand, the treatments T5, T6 and T7 (1L:1V: 8.93; 1L:1V:1CAC: 9.11 and 1SC:1L:1V: 1CAC: 8.7) presented pH above the ideal range, indicating the need for pH correction for use as a substrate for the production of most seedlings in box, this alkaline pH may be related to the presence of alkaline and alkaline earth metals present mainly in the sewage sludge. According to a study by Vieira [7], the sludge from the parboilization of rice presents high pH, being observed by the author a pH equal to 8.5. Thus, the increase in the contents of this attribute may be associated with the presence of sludge from the rice industry in the composition of the substrates.

Adopting the limits of 25% of C org. for substrates used in the production of seedlings, recommended by Schmitz [3], only treatments T0, T3, T4 and T7 present sufficient C org. contents to be used as suitable substrates for the cultivation of plants in box. The treatments T1, T2, T5 and T6 presented low levels of this variable (Table 3). T5 and T6 treatments had the lowest levels. In a study by Schmitz [3], CAC also presented low levels of C org. (17.3%), which has been justified due to the high silicon content and the carbonization process. The presence of sewage sludge in the composition of the substrates may have resulted in a lower content of C org., as this decreases significantly during the process of stabilizing the sludge through microbiological respiration, converting it into CO_2 and also through mineralization [20].

Regarding the N contents, considering the ideal range of 0-20 (Table 1), all treatments were within the indicated range, but the ideal P contents (6-10 g kg^{-1}) were not reached by any treatment, treatments T0, T1, T2, T3 and T4 present lower values than those recommended in the literature (Table 1), whereas treatments T5,

Table 3. pH, Organic Carbon (C Org.), Total Nitrogen (N), Total Phosphorus (P), Total Potassium (K), Organic Matter (OM), Electric Conductivity (EC) and Carbon / Nitrogen Ratio (C/N)

Tratamentos	pH	C Org. (%)	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	OM (%)	EC (mS cm^{-1})	C/N
T 0 - SC	6.3	48.61	12.65	1.19	1.29	83.03	0.295	38:1
T 1 - 1SC:1S	6.1	11.71	2.95	0.57	1.71	13.51	0.140	40:1
T 2 - 1SC:1S:1CAC	6.2	11.90	3.12	0.38	1.75	11.91	0.129	38:1
T 3 –1SC:1V	6.2	35.32	9.01	0.05	4.86	53.06	0.229	39:1
T 4 - 1SC:1V:1CAC	6.4	24.60	6.41	0.95	5.29	46.45	0.141	38:1
T 5 - 1L:1V	8.9	6.55	9.53	34.81	7.72	19.17	0.987	7:1
T 6 - 1L:1V:1CAC	9.1	6.75	7.10	23.21	8.36	36.94	0.348	9:1
T 7-1SC:1L:1V:1CAC	8.7	48.41	9.70	14.14	6.22	15.15	0.339	50:1

T6 and T7 present higher results than those indicated, thus evidencing the need for correction through fertilization suitable for use as substrates in plant cultures.

As for the contents of K for the cultivation of vegetables, the average contents are in the range of 1.6 - 3.0 [15]. The treatments T3, T4, T5, T6 and T7 present levels considered very high for this variable and the T0 treatment resulted in a value classified as low [15]. This increase in K concentration is possibly due to the addition of the sludge in the mixtures, which has high nutrient concentration, and with higher pH, has become more available [21].

The ideal C/N ratio for horticultural substrates is between 20-40 (Table 1) which was obtained by treatments T0, T1, T2, T3 and T4. The T7 treatment had a high C/N ratio. The treatments T5 and T6 presented the lowest C/N ratio, respectively, 7:1 and 9:1, which can be explained by the presence of sludge, since materials of easy decomposition have low values of this ratio [22].

During the mineralization process, the organic matter releases nutrients to the plants [23], being an important variable in agricultural substrates. In relation to OM optimal levels should be higher than 80% (Table 1), value reached only by T0 (SC). According to Schmitz [3], suggest a minimum value of 50% of OM for substrates used in the production of seedlings, being within this range the T3 treatment composed by SC and Vermiculite. The treatments T1, T2, T5 and T7 presented the lowest levels for this variable, on average 14.93% (Table 4). The OM causes changes in the physical, chemical and biological characteristics of the soil, increasing the aeration and the retention of moisture [7]. Chemically, OM is the main source of macro and micronutrients essential to plants, as well as indirectly acting on their availability, due to the elevation of pH, increase the nutrient retention capacity, avoiding losses. Biologically, OM is the source of energy and nutrients, thus increasing the activity of soil microorganisms [22]. According to Schmidt [24] the process of microbial decomposition of soil is controlled by substrate quality and the availability of carbon and nutrients.

The ideal range for electrical conductivity (EC) is between 0.76 and 1.25 mS cm^{-1} (Table 1). Only the T5 treatment (1L:1V) was within this range, all others resulted in lower values. According to Martinez [25] electrical conductivity (EC) contents above 3.5 mS cm^{-1} is excessive for most plants. Excess EC was not observed in any treatment. For some authors, such as Abad [16] the optimal electrical conductivity for substrates should be less than 0.5 mS cm^{-1}. This range was reached by the other substrates tested in this work.

For the macroporosity variable, all the treatments had lower levels than the T0 control treatment, which presented 42.80%. The treatments T1 and T3 resulted in values closer to the range indicated for this variable, which according to Lopes et al. [6] ideal macroporosity values should be in the range of 35 to 45%. According to Guerrini and Trigueiro [14], the carbonized rice husk is a light and inert material, an increase in the porosity of the substrate can occur, mainly due to the increase in the percentage of macropores.

Only T1 treatment had levels below the recommended range for microporosity, although without differing from the T0 treatment. The substrates formulated with the addition of vermiculite, CAC and sludge (T3, T4, T5, T6 and T7) presented higher percentages of

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

Tratamentos	Macro (%)	Micro (%)	Porosity (%)	Ret. Cap (%)	Density ($g.cm^{-3}$)
T 0 - SC	42.80 ^{a}	49.13 ^{bc}	91.93 ^{a}	24.56 ^{bc}	0.04 ^{b}
T 1 - 1SC:1S	32.13 ^{b}	39.06 ^{c}	71.19 ^{bc}	19.53 ^{c}	0.42 ^{a}
T 2 - 1SC:1S:1CAC	18.06 ^{cd}	51.80 ^{b}	69.86 ^{bc}	25.90 ^{abc}	0.31 ^{a}
T 3 –1SC:1V	24.00 ^{bc}	55.53 ^{ab}	79.53 ^{ab}	27.76 ^{ab}	0.03 ^{b}
T 4 - 1SC:1V:1CAC	11.53 ^{de}	59.86 ^{ab}	71.39 ^{bc}	29.93 ^{ab}	0.11 ^{b}
T 5 - 1L:1V	7.03 ^{e}	57.60 ^{ab}	64.63 ^{c}	28.80 ^{ab}	0.15 ^{b}
T 6 - 1L:1V:1CAC	9.66 ^{de}	63.66 ^{a}	73.33 ^{bc}	31.83 ^{a}	0.08 ^{b}
T 7-1SC:1L:1V:1CAC	12.60 ^{de}	60.60 ^{ab}	73.20 ^{bc}	31.96 ^{a}	0.10 ^{b}

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

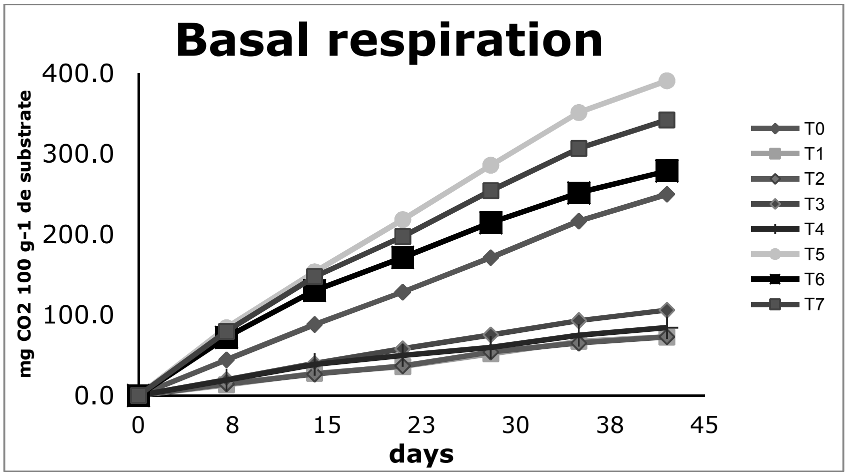

Fig. 1. Basal respiration of the treatments incubated for the period of 42 days

microporosity, being on average 21% higher than that presented by the control treatment T0, corroborating with the results obtained by Simões et al. [26], which observed higher percentages of microporosity in substrates formulated with carbonized rice husk, coconut fiber and vermiculite.

As for total porosity only the T0 treatment is within the recommended values for this variable (> 85%), although the T3 treatment did not differ from the T0 treatment, thus presenting better aeration, water infiltration and drainage [27]. Costa et al. [28] obtained 88.18% porosity with the addition of 32.33% CAC. Materials with low porosity may impair root gas exchange and water drainage, while high porosity may result in low water retention causing water deficiency for plants [29]. According to Costa et al. [28], the determination of total porosity does not distinguish between macro and micropores, since it does not specify pore size, so that the same total pore space can be occupied by different volumes of air and water. According to Pagliarin et al. [30], the substrate compaction reflects a decrease in total porosity, especially in the substrates with smaller particles and with greater particle size unequality.

As for the water retention capacity, treatments T6 and T7 presented 22.82% and 23.15%, respectively, higher than the control treatment T0, but these treatments did not differ from treatments T2, T3, T4 and T5. For this physical attribute, all treatments are within the range indicated by Martínez [25], which considers an optimal water retention capacity between 20 and 30%. The water retention capacity is an important attribute for the development and rooting of the plants, because the low retention of water generates possible water stress, which leads to a greater energy expenditure by the plant to supply this need [28].

For density treatments T1 and T2 presented adequate results for this variable, 0.42 and 0.31 g cm^{-3}, respectively, with a recommended density of 0.10 to 0.35 g cm^{-3} [17]. The other treatments resulted in a low density of 0.085 g cm^{-3}, with no significant difference between the other results.

The microbial activity of the soil was determined by the evolution of CO_2, the highest RB rates were obtained by treatments T5, T6 and T7, presenting an average of 2.6019 µg CO_2 g^{-1} h^{-1}, 31.47% higher than that presented by the control treatment (Fig. 1). Thus, these values were associated to the presence of sludge in their compositions, which provides a higher activity of soil microorganisms [20]. For Rosa et al. [8] the presence of stabilized sludge, used in the cultivation of arugula, provided stimulus in the production of microbial biomass. The addition of substrate in the soil can cause the microorganisms present in the soil to respond differently depending on the physical and chemical properties and environmental conditions [31].

4. CONCLUSION

The substrates and mixtures tested in this study allow to conclude that they have different properties of the references cited as ideal for the formulation of a substrate for the production of vegetable seedlings in trays.

Regarding the chemical attributes, the 1SC:1V and 1SC:1V:1CAC treatments present pH, C Org., N, OM and C/N characteristics close to the commercial substrate, but present P, K and EC levels below the ranges indicated in the literature as suitable for the formation of substrates.

Regarding the physical characteristics, the formulation closest to the ideal was also SC:V, which results in good microporosity, total porosity, water retention capability, but low macroporosity and density.

The presence of stabilized industrial residue sludge provides greater soil microbial activity, but does not result in improvements in the chemical and physical attributes of the substrates.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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