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Substrates Formulated with Biochar for Seedling Production of *Moringa oleifera* Lam.

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Abstract

Moringa oleifera is an allogamous plant that is propagated by seeds and cuttings, rich in vitamin A and C, phosphorus, calcium and proteins. However, its best propagation form for the large-scale production is still unknown, as well the most suitable substrate for seedling production. Production of forest species seedlings with a high quality is directly related to physical and chemical substrates properties. Using biochar as substrate for seedlings production is an economical and sustainable solution for this proposal. The aim of this study was to evaluate the influence of three biochar types, in two concentrations, for production of *Moringa oleifera* seedlings. Three types of substrates were formulated using residues of dry coconut shells, sewage sludge and orange bagasse. The experiment was carried out in a greenhouse, installed in a completely randomized design, consisting of seven treatments (substrates formulated with biochar in two doses, 1 and 2%) and the control, with four replicates, totalizing 28 seedlings. One month after sowing, the seedlings were evaluated biweekly for 60 days in relation to stem base diameter, shoot heigh, leaves number, shoot dry matter, root dry matter, total seedling dry matter and Dickson Quality Index. The substrate formulated using sewage sludge biochar at 2% provided a greater increasing in shoot dry matter. This concentration improved seedling height and stem base diameter in 10.5 and 0.83 mm, respectively, compared to the control. In general, biochar improved physical and chemical soil quality, promoting a better *M. oleifera* seedlings development.

Keywords: forest species, silviculture, diameter, height, soil properties

1. Introduction

Moringa oleifera Lam., belongs to Moringaceae family, composed of only one genus (Moringa). It is a native species from Northern India that grows normally in several tropical countries. In addition, *M. oleifera* grows rapidly and survives on poor soils. It is easily propagated and adapted to a wide range of soils. For being drought tolerant, requires less attention during long drought periods. This species is rich in vitamins A and C, phosphorus, calcium, and protein. It has also diversified uses, especially in parks and gardens ornamentation, animal feed, human food supplementation and medicine. Furthermore, highlights *M. oleifera* uses for biological decontamination of water supply (Vieira et al., 2008; Santos et al., 2011; Agustini et al., 2015).

During plant growth process, choosing an inadequate substrate may affect seed germination and seedling establishment, which can lead to reduction in plant production quality. Consequently, the material used for composing the substrate needs to considerate variations in physical, chemical and biological soil properties. So that, characteristics such as soil structure, aeration, water retention and pathogens contamination are important in order to determine the best materials mix to compose a substrate (Silva et al., 2011). Moreover, the ideal substrate should have a low cost and be available in large quantities. For this reason, using industrial waste is a sustainable agricultural practice that aims to minimize environmental impact and improve simultaneously seedling production quality (Neves et al., 2010).

The substrate compounds obtained from natural materials may not only supply soil nutrients properly, but also provide a reuse in subsequent plantations as an alternative to minimize production costs and reach gains in productivity (Petter et al., 2012). However, a higher degradation rates of these materials limit their beneficial effects over time, in which can be mitigated when the waste is treated through carbonization process before being used as a party of the substrate mix.

Biochar produced through pyrolysis process considered as a soil conditioner may provide increases in soil biomass, carbon fixation, nutritional balance and filtration of percolating soil water. In fact, its capacity of being highly stable in soil contribute to improvements in chemical, physical and biological soil properties (Trazzi et al., 2016; Petter & Madari, 2012). As a result, biochar can improve soil commercial substrates and contribute to reduction of chemical fertilizers uses, being a sustainable and low-cost alternative to increase seedlings quality (Lima et al., 2013).

Regarding different biochar uses, new studies are necessary in order to identify soil and biochar properties that help to maximize its application on agriculture production (Lima et al., 2016). To know more about biochar characteristics is important to avoid unwanted effects on soil fertility (Novak et al., 2014), such as excessive pH increase and nutrient unavailability.

Due to the Moringa importance and necessity of deeply studies related to biochar for forest seedling production, this work aims to evaluate the influence of three biochar types, in two concentrations, for production of *M. oleifera* seedlings.

2. Method

2.1 Study Area

The study was carried out at Universidade Federal de Sergipe, located in São Cristóvão, Sergipe, Brazil. The production of *M. oleifera* seedlings was conducted in an air circulation oven with temperature control, in which the cooling system was activated when the internal temperature reached 28 °C.

The soil used in this study was obtained from a surface horizon, classified as Red Yellow Argissolo, according to the Water Resources Digital Atlas of Sergipe State (2013). After collected, the soil was air dried, dewormed and sieved in a 2 mm mesh. Samples of this material were submitted to physical analysis, density and grain size composition, according to Donagema et al. (2011), and chemical analysis, potassium (P), calcium (Ca), magnesium (Mg), aluminum (Al), organic matter (OM), pH, electrical conductivity (EC), effective cation exchange capacity ($CEC_{effective}$), following methodology proposed by Silva (2009) (Table 1).

Table 1. Physical and chemical properties of a surface horizon soil, classified as red yellow argissolo used in this study

Element	Unit	Result
pH	-	4.64
EC	mS cm^{-1} at 25 °C	0.063
OM	g dm^{-3}	11.06
C		6.36
P Melich	mg dm^{-3}	1.82
K		25.40
Al	cmol dm^{-3}	0.45
K		0.06
Ca		0.72
Mg		0.65
SB		1.43
$CEC_{effective}$		1.88
Base Saturation	%	76.13
Density	g cm^{-3}	1.39
Sand	%	71.57
Silt		13.43
Clay		15.00
Textural Classification	--	Franco Sand

2.2 Biochar Production

Biochars were produced in a furnace adapted using a model developed by the International Biocarbon Initiative (IBI). The Top LidUp Draft (TLUD) model uses chimney as a second phase of burning to eliminate volatile products produced by pyrolysis. The pyrolysis process was conducted during 2 hours for sweage sludge biochar and 1 hour for dry coconut shells and orange bagasse. The temperature used was around 400-500 °C. Both vapors and non-condensable gases were burned to provide energy and to continue the carbonization process.

Biochars used for composing the substrates were submitted to laboratory analysis, in which the moisture content was determined on an oven-dry mass basis. Volatile matter, ash and fixed carbon were carried out in a muffle oven, following a methodology described by ABNT (1986) (Table 2). Determination of P and K available concentrations, pH and electrical conductivity was according to methodology used in the soil fertility determination. For Total Nitrogen (Nt) analysis was used a methodology to determine the plant tissue composition (Silva, 2009).

Table 2. Characteristics of biochars obtained with coconut shell, orange bagasse and sewage sludge

Element	Unit	Coconut Shell	Orange Bagasse	Sewage Sludge
pH	-	9.88	10.33	7.28
EC	mS cm^{-1} at °25 °C	2.19	2.75	10.39
Total Carbon (Dichromate)	%	62.40	62.30	34.04
N_{Total}		0.45	1.18	1.60
Moisture Content		8.20	7.55	3.95
Ash		8.97	9.75	48.13
Volatile matter		36.99	41.63	25.97
Fixed Carbon		54.04	48.51	25.40
C/N Ratio	-	0.72	52.78	21.28
$P_{available}$	g kg^{-1}	0.65	5.20	10.60
$K_{available}$		1.43	36.70	2.90

2.3 Substrates Formulation

Three types of substrates were formulated using a mixture of soil and biochar, produced from dry coconut shell, orange bagasse and sewage sludge. This biochar was obtained by means of production residues collected at rural property, waste generated by snack bars of the university and waste generated by sewage treatment provided by Sergipe water supply company, respectively.

After substrates being prepared, those residues samples were collected and sent to the Soil Analysis Laboratory of Federal University of Sergipe to perform the chemical and physical analysis in order to determinate available nutrient contents (Silva, 2009; MAPA, 2007), since most of the substrate is composed of soil. Then, it was evaluated the total porosity (P_{Total}), macroporosity ($Macro_P$), microporosity ($Micro_P$), field capacity at 33 kPa (FC), permanent wilt point at 1500 kPa (PWP) and total soil water availability (WA_{Total}).

2.4 Trial Design

The experiment was carried out in a completely randomized design with seven treatments: dry coconut shell biochar, sewage sludge biochar, orange bagasse biochar at 1 and 2 % and the control (soil without biochar). Four replicates were used for each treatment with a total of 28 seedlings.

Four seeds were sown in pots with a 1.5 L capacity, containing 2 kg of soil plus the biochar. After that, the roughing was done in order to leave the most vigorous plant. The filled pots with soil and biochar were irrigated and kept wet until the sowing day. This practice is necessary to induce bio-coal reactions with the soil to promote a balance of a system. Soil moisture was maintained close to the field capacity to minimize possible water stress.

One month after sowing, seedlings were evaluated biweekly for 60 days by measuring steam base diameter, shoot height and number of leaves. To measure seedlings steam diameter, a digital caliper with millimetric precision was used. Seedlings height were measured by means of a ruler from soil surface to the apical plant bud. After measure those growth parameters, seedlings were separated into shoot and root, washed and placed in paper bags for drying in an air circulating oven at 65 °C until reaching a constant mass. After that, the roots and shoots dry matter were weighed. The number of was given manually, starting with basal leaves until the last one totally opened (Carneiro, 1995).

At the end of the experiment, root dry matter (RDM), shoot dry matter (SDM), total seedling dry matter (TDM), ratio RDM/SDM and Dickson Quality Index (DQI) were obtained by the equation below (DICKSON et al., 1960):

$$DQI = TDM/(SH/SBD) + (SDM/RDM)$$

where, $TDM_{(g)}$ is the Total Dry Matter, $SH_{(cm)}$ is the Shoot Height, $SBD_{(mm)}$ is the Stem Base Diameter, $SDM_{(g)}$ is the Shoot Dry Matter and $RDM_{(g)}$ is the Root Dry Matter.

2.5 Statistical Analysis

The data were submitted to analysis of variance (ANOVA) and Tukey's multiple range tests ($P < 0.05$), using software R 3.2.2 (R Development Core Team. 2015). Variability in the biochar types treatment means was also expressed as the standard deviation of four replicates.

3. Results and Discussion

3.1 Effects of Biochar on Chemical Soil Properties

The addition of different biochar types in the soil had a significant impact on chemical soil properties of the *M. oleifera* seedlings substrates (Table 3). In general, all the biochars improved chemical characteristics compared to the control (soil without biochar), mainly pH, EC, CEC, Sum of Base (SB) and Base Saturation (BS).

Table 3. Chemical porperties of *Moringa oleifera* substrates treated with biochar form coconut shell (CSB), orange bagasse (OBB) and sewage sludge (SSB), at 1 and 2%

Chemical Porperties		Biochar treatment						
		Control	CSB 1%	CSB 2%	SSB 1%	SSB 2%	OBB 1%	OBB 2%
pH	-	4.9^{e}	5.4^{cd}	5.9^{b}	5.3^{d}	5.6^{c}	6.0^{b}	6.7^{a}
EC	$mS\ cm^{-1}$	549.4^{b}	818.8^{ab}	1024.8^{a}	847.4^{ab}	862.7^{ab}	$611.0a^{b}$	913.8^{ab}
P	$mg\ dm^{-3}$	7.8^{d}	8.3^{d}	9.5^{d}	28.3^{b}	44.9^{a}	16.9^{c}	27.0^{b}
K		31.5^{c}	199.8^{c}	428.0^{b}	40.0^{c}	47.5^{c}	427.0^{b}	875.3^{a}
Na		98.4^{d}	410.4^{a}	297.1^{ab}	215.4^{bcd}	242.9^{bc}	107.6^{cd}	155.9^{cd}
Ca	$cmol\ dm^{-3}$	1.9^{c}	1.6^{d}	1.4^{d}	2.6^{b}	2.9^{a}	2.0^{c}	1.9^{c}
Mg		0.8^{b}	0.8^{b}	0.8^{b}	1.1^{a}	1.2^{a}	1.0^{ab}	1.0^{ab}
Al		0.08^{ns}	0.09^{ns}	0.02^{ns}	0.02^{ns}	0.00^{ns}	0.02^{ns}	0.00^{ns}
H+Al		1.43^{a}	1.09^{ab}	0.80^{b}	1.43^{a}	1.42^{a}	1.21^{ab}	0.97^{ab}
CEC		4.66^{c}	5.30^{bc}	5.84^{abc}	6.20^{ab}	6.72^{a}	5.77^{abc}	6.84^{a}
SB		3.23^{c}	4.22^{bc}	5.05^{ab}	4.77^{ab}	5.30^{ab}	4.56^{b}	5.87^{a}
C	$g\ dm^{-3}$	0.55^{c}	1.00^{b}	1.85^{a}	1.67^{a}	1.70^{a}	1.70^{a}	1.97^{a}

Note. Means followed by the same letter within a row are not statistically different, according to the Tukey test at 5% probability.

All biochars applied to the substrates raised soil pH by 0.4-1.8 units. However, only CSB 2%, SSB 2% and OBB 1% reached pH values between 5.5 and 6.5, considered appropriated to forest seedlings production (Gonçalves & Poggiani, 1996). Dai et al. (2013) reported that pineapple peel biochar at 1 and 3% increased soil pH by 1.13 and 2.16 units compared with the control biochar treatment. Similarly, Zhang et al., (2015) indicated that soil pH had increased after application of peanut hull, rice and rape straw biochar by 0.70, 0.92 and 0.63 units, respectively. Raising soil pH could alter the form that nutrients are available for plants and make some element root absorption easier (Ding et al., 2016). However, the effects of biochar in the soil pH depends on pyrolysis process and biochar type (Ok et al., 2016).

The biochar application to the soil provided a great difference between BC types according to EC parameter. The CSB 2% and OBB 2% substrates showed the higest EC in the soil. A higher EC value is associated with a higher amount of leached salts in the solution. So that, salt excess in the soil may interfere in a germination process and seedling growth due to the necessity of a greater energy to absorve water, leading to damages in metabolic process (Tomé Júnior, 1997).

Applying biochar to the soil increased significantly some plant elemental concentracions, such as P, K, Na, Ca and Mg. The increase of P avaliable in the soil could be explained by a higher P content in the biochar applied or due to an increase of CEC, leading to a lower Al content in acidic soils (Ok et al., 2016). The CEC was significantly affected by the addition of OBB 2%, SSB 1 and 2% tratments. The beneficial effects of the biochar application on CEC have been widely reported (*e.g.* Vaccari et al., 2012; Kloss et al., 2014; Abujabhah et al., 2015; Hasen et al., 2016).

3.2 Effects of Biochar on Physical Soil Properties

The biochar influenced all soil physical properties analyzed of formulated substrates, except for macroporosity (Table 4). The CSB biochar type at both concentration and OBB at 2% improved total porosity when compared with the control. Only biochar using dry coconut shells increased significantly the total porosity and microporosity at the same time, but had no significant effect on macroporosity. These results disagreed with those of Pratiwi and Shinogi (2016) who reported that biochar amendment using rice husk at 4% raised significantly soil macropores, but had no significant effect on micropores. Although, these authors indicate that macropores had considerably decreased during the growing period while increased micropores.

Table 4. Physical porperties of *Moringa oleifera* substrates treated with biochar form coconut shell (CSB), orange bagasse (OBB) and sewage sludge (SSB), at 1 and 2%

Soil porperties		Biochar treatment						
		Control	CSB 1%	CSB 2%	SSB 1%	SSB 2%	OBB 1%	OBB 2%
P_{Total}	%	28.95^{c}	32.88^{b}	39.34^{a}	28.98^{c}	30.29^{bc}	30.09^{bc}	32.2^{b}
$Macro_P$	%	9.57^{a}	10.04^{a}	13.67^{a}	12.00^{a}	12.19^{a}	11.44^{a}	13.92^{a}
$Micro_P$	%	19.37^{c}	22.85^{b}	25.68^{a}	16.99^{c}	18.10^{c}	18.65^{c}	18.33^{c}
FC	%	8.02^{c}	9.04^{c}	13.18^{a}	8.71^{c}	9.21^{c}	8.18^{c}	11.21^{b}
PWP	%	5.22^{de}	7.61^{c}	11.40^{a}	4.12^{e}	5.01^{de}	5.80^{d}	9.55^{b}
WA_{Total}	mg H_2O g^{-1}	28.01^{abc}	14.31^{c}	17.89^{c}	45.92^{a}	42.02^{ab}	23.94^{bc}	16.59^{c}

Note. Means followed by the same letter within a row are not statistically different, according to the Tukey test at 5% probability. Total porosity (P_{Total}), macroporosity ($Macro_P$), microporosity ($Micro_P$), field capacity at 33 kPa (FC), permanent wilt point at 1500 kPa (PWP) and total soil water availability (WA_{Total}).

The total porosity was affected by biochar rates, in which higher the biochar rates are, higher is the total porosity. Similar effects were reported by Glab et al. (2016), using biochar formulated with straw of two species, miscanthus and winter wheat. Hardie et al. (2014) suggested that biochar may induce to changes in soil porosity and water retention due to three mechanisms: direct interference of biochar pores, creation of accomodation pores between biochar particles and soil aggregates and a higher persistence of soil pores by promoting agreggate stability.

The CSB at 2% had the highest values for FC and PWP, with a increase of 5.16 and 6.18 % compared to the control. The water hold capacity in the soil is related to its porosity. The effects of biochar on FC, PWP and WA_{Total} were variable for different biochar types, similar was verified by Sun and Lu (2014). However, Biochar application had no significant increase on water avalability, compared to the control. This fact may be related with the increase of a small pores, reflecting in $Micro_P$ values that raised, especially in CSB at 1 and 2%.

3.3 Effects of Biochar on Seedling Growth Parameters

The effects of biochar on seedling growth parameters along the time, after 30, 45, 60 and 75 days after sowing is represented in Figure 1. Evaluating biochar effects on shoot height 30 days after sowing had significant differences among the treatments. The control had the highest values for this parameter, however from 45 to 75 days after sowing all biochar treatments had no diferences. In the last evaluation, SSB at 2% increased 10.5 cm of shoot height, compared to the control. From 60 days after sowing, SSB at 2% also stood out for having the highest values of stem base diameter. Number of leaves had a siginificant decrease with CSB at 2% application and OBB at 2% 45 and 75 days after sowing, respectively.

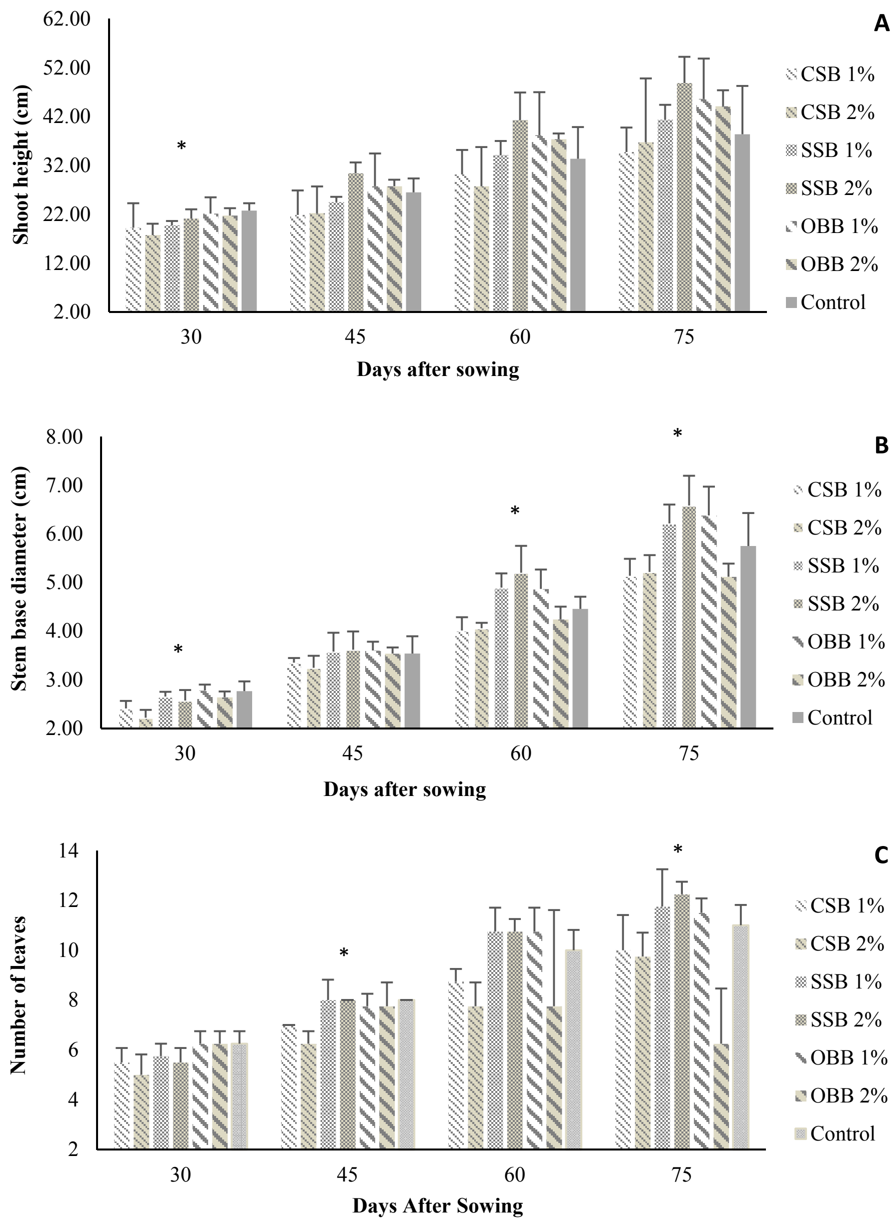

Figure 1. Shoot height (A), Stem base Diamter (B) and Number of leaves (C) of *Moringa oleifera* substrates treated with biochar form coconut shell (CSB), orange bagasse (OBB) and sewage sludge (SSB), at 1 and 2% after 30, 45, 60 and 75 days after sowing. Note error bars indicate ± standard deviation. The asterisk indicates the significant difference ($p < 0.05$) according Tukey test among treatments

Biochar amendment signficantly increased steam base diameter (SBD) and shoot dry matter (SDM) (Table 5). The biochar type SSB at 1 and 2% and OBB at 1% had the highest means of SBD, with an increase of 0.46, 0.83 and 0.62 mm compared to the control, respectively. However, only SSB at 2 % had the highest value of SDM, compared to the control. Raising biochar concentration at 2% of type OBB, there is a reduction of these parameters expression, showing statistically similar results compared to the contol. Same behavior was observed for the TDM, in which increasing OBB concentration from 1 to 2% caused reduction of 1.7 g.plant^{-1}. Stem base diameter is considered a great parameter to estimate forest seedling survival at field conditions, mainly because it is easy to measure and is non-destructive. The larger stem base diameter, the better is shoot growth balance (Gomes & Paiva 2013).

Table 5. Growth parameters of *M. oleifera* substrates treated with biochar form coconut shell (CSB), orange bagasse (OBB) and sewage sludge (SSB), at 1 and 2%

Growth Parameters	Biochar Treatment						
	Control	CSB 1%	CSB 2%	SSB 1%	SSB 2%	OBB 1%	OBB 2%
SBD (mm)	5.75[b]	5.13[b]	5.21[b]	6.21[a]	6.58[a]	6.37[a]	5.11[b]
SH (mm)	383.7[a]	347.5[a]	367.5[a]	413.7[a]	488.7[a]	456.2[a]	441.2[a]
NF	11.00[a]	10.00[a]	9.75[a]	11.75[a]	12.25[a]	11.50[a]	6.25[b]
SDM (g. planta^{-1})	2.27[b]	1.81[c]	1.72[c]	2.47[b]	3.15[a]	2.52[b]	1.70[c]
RDM (g planta^{-1})	2.68[a]	2.17[a]	2.26[a]	3.37[a]	3.20[a]	2.96[a]	2.07[a]
TDM (g planta^{-1})	4.95[a]	3.98[b]	3.98[b]	5.84[a]	6.35[a]	5.48[a]	3.78[b]

Note. Means followed by the same letter within a row are not statistically different, according to the Tukey test at 5% probability. Strem base diameter (SBD), Shoot height (SH), Number of leaves (NF), Shoot dry matter (SDM), Root dry matter (RDM), Total dry matter (TDM).

The SSB at 1% improved SDM in 0.88 g.plant^{-1}, compared to the control. A number of researchers have suggested that biochar amendment had positive effects on forest seedlings growth (Petter et al., 2012; Rezende et al., 2016; Souchie et al., 2011). Shouchie et al. (2011) studied the biochar application produced with *Eucalyptus sp.* in forest seedlings substrates showed an increase in seedling height, diameter and dry matter. However, this effect was observed just starting with biochar concentration of 12.5%.

There was no statistically differences among the treatments for the variables SH and RDM. Theses results are in accordance with Neves et al. (2010) findings, in which organic materials for *M. oleifera* seedlings subtrates had no different effects among the treatments using coconut and sewage sludge. Similar findings were reported by Lima et al. (2016) using *Eucalyptus* sp. wood for beet production. The SSB biochar type at 2% had the highest means for all seedling growth variables, being recommend by Gonzaga et al. 2018 as a soil condiotioner for improving *Eucalyptus* seedling growth.

The material type used in substrates composition for seedlings production directly influences on compound physical characteristics. Normally, improving these physical characteristics leads to a better seedling growth. It may be noticed that the sewage sludge substrate using a dose of 2% provided a significant increase in the amount of water available in the soil (Table 4). Probably, it provoked a greater stem base diameter and leaves number increase, 45 days after sowing (Figure 1 and Table 5). Although the seedlings development is directly related to the substrates physical characteristics, improving substrate chemical characteristics may also lead to a better plants development. Table 3 shows that the sewage sludge biochar at a dose of 2% presented a higher phosphorus concentration, which may have contributed to the increase seedlings performance under this treatment (Figure 1 and Table 5). In addition, all biochars caused, in general, an increase of carbon concentration relative to the control (Table 3). This fact may also have influenced the greater seedling growth in the sewage sludge biochar.

The seedlings quality indexes are showed in Table 6. It is noted that the quality indexes had no significant differences among biochar treatments. So that, the biochar types used did not influence in the quality of *M. Oleifera* seedlings. These findings corroborate with results observed by Lima et al. (2016). *M. oleifera* is a rustic plant and this fact could be limited the biochar expression in the seedlings.

Table 6. Quality Indexes of *Moringa oleifera* substrates treated with biochar form coconut shell (CSB), orange bagasse (OBB) and sewage sludge (SSB), at 1 and 2%

Quality Indexes	Biochar treatment						
	Control	CSB 1%	CSB 2%	SSB 1%	SSB 2%	OBB 1%	OBB 2%
SH/SBD	6.73[a]	6.94[a]	6.68[a]	7.43[a]	7.26[a]	8.66[a]	6.59[a]
RDM/SDM	0.91[a]	0.78[a]	0.77[a]	1.12[a]	0.91[a]	0.85[a]	0.85[a]
DQI	0.54[a]	0.56[a]	0.79[a]	0.74[a]	0.69[a]	0.40[a]	0.69[a]

Note. Means followed by the same letter within a row are not statistically different, according to the Tukey test at 5% probability. Shoot height/Stem base diameter (SH/SBD), Root dry matter/Shoot dry matter (RDM/SDM), Dickson quality index (DQI).

The DQI varied from 0.40 to 0.79, being considered as the good quality seedlings for field conditions. According to Gomes and Paiva (2004), the higher DQI value is, higher is seedlings quality and values below 0.2 are not considered good for taking in account field contions. The DQI represents a promising weighted morphological measure, since it consideres not only the seedling vigor, but also plant biomass distribution (Merlo et al., 2008). As biochar concentrtation increased, SH/SBD and RDM/SDM indexes also increased. The RDM/SDM index is an important parameter to evaluate when the seedlings are ready to be submitted to field conditions. Plant shoot should not be much higher to roots due to occurance problems in water absorption for the stem (Caldeira et al., 2008).

5. Conclusions

The application of biochar improved physical and chemical soil properties, although the effect is not always siginificant. The different biochar types affected positively growth parameter of *M. oleifera* seedlings. In general, the sewage sludge application is highlighted for having the greatest values for most of the analyzed parameters.

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