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SEWAGE SLUDGE DERIVED BIOCHAR AND ITS EFFECT ON THE GROWTH AND MORPHOLOGICAL TRAITS OF *Eucalyptus grandis* W.Hill ex Maiden SEEDLINGS

BIOCARVÃO DE LODO DE ESGOTO E SEU EFEITO NO CRESCIMENTO E NAS CARACTERÍSTICAS MORFOLÓGICAS DE MUDAS DE EUCALIPTO (*Eucalyptus grandis* W.Hill ex Maiden)

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ABSTRACT

The use of sewage sludge (biosolids) biochar in the forest seedlings growing media can become an environmentally interesting strategy to improve seedlings quality. Therefore, the aim of this study was to evaluate different rates of application (0, 20, 40, 80 and 100 t ha^{-1}) of biosolid biochars on the growth and morphological traits of eucalyptus (*Eucalyptus grandis* W. Hill ex Maiden) seedlings. The treatments were arranged in a completely randomized design with four replications. Ten-week old eucalyptus seedlings were transferred to the pots and allowed to grow for eight weeks. Chlorophyll content, plant height and stem diameter were measured at 0 (time 1), 30 (time 2) and 60 (time 3) days after transplant. Shoot and root biomass were measured after plant harvest. Biochar was effective in improving the seedlings quality. Increasing rate of application of biochar did not significantly influence chlorophyll content. At 60 days after planting, plant height increased and stem diameter decreased when high doses of biochar were applied. The use of sewage sludge biochar improved the growth and the morphological traits of the eucalyptus seedlings, therefore its application can be recommended as soil amendment and soil conditioner. According to the Dickson Quality Index, the best eucalyptus seedlings were obtained with 20 and 40 t ha^{-1} of biochar.
Keywords: Biosolids; pyrogenic carbon; forest plant species.

RESUMO

O uso de biocarvão de lodo de esgoto (biossólido) no substrato para crescimento de mudas de espécies florestais pode ser uma estratégia ambientalmente interessante para a melhoria da qualidade das mudas. O objetivo do estudo foi avaliar o efeito de diferentes doses (0, 20, 40, 80 e 100 t ha^{-1}) de biocarvão de lodo de esgoto no crescimento e características morfológicas de mudas de eucalipto (*Eucalyptus grandis* W. Hill ex Maiden). Os tratamentos foram dispostos em delineamento inteiramente casualizado, com quatro repetições. Mudas de eucalipto com 10 semanas foram transferidas para potes e cultivadas por oito semanas. O teor de clorofila, a altura da planta e o diâmetro do coleto foram avaliados aos 0 (tempo 1), 30 (tempo 2) e 60 (tempo 3) dias após o transplante. A biomassa da parte aérea e das raízes foi avaliada após a colheita. O biocarvão melhorou a qualidade das mudas e não influenciou o teor de clorofila. A altura das plantas aumentou enquanto o diâmetro do coleto diminuiu com o aumento das doses de biocarvão, aos 60 dias do transplante. O uso de biocarvão de lodo de esgoto melhorou o crescimento e as características morfológicas das mudas de eucalipto, sendo assim, sua aplicação pode ser recomendada como insumo e condicionador de solo. De acordo com o Índice de Qualidade de Dickson, as melhores mudas foram obtidas com o uso de

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20 e 40 t ha^{-1} de biocarvão.
Palavras-chave: Biossólido; carbono pirogênico; espécies florestais.

INTRODUCTION

Sewage sludge or biosolids is an enriched organic matter waste that is produced in the Water (WTP) and Waste Water Treatment Plants (WWTP). Its disposal has become a major challenge because the amount that is produced increases proportionally as the population grows (LOBO; GASSI FILHO; KUMMER, 2014).

A national survey carried in 2008 reported that, from a total of 5564 municipalities, 37.7% had an active WTP and only 27.2% had a WWTP. However, those numbers are about to increase according to the new environmental regulations in Brazil. Currently, the sewage sludge annual production ranges from 150 to 220 thousand tons, making its proper disposal a difficult task due to the high costs.

Several options are available for the disposal of biosolids, such as incineration, ocean dumping, landfills and land application. Land application of biosolids is a promising strategy due its high concentration of plant nutrients and organic matter. However, more research is needed to establish an application criterion since biosolids also contains pathogenic organisms and high concentration of heavy metals that could cause environmental contamination (GUEDES; ANDRADE; POGGIANI, 2006).

The thermal degradation of biosolids through pyrolysis produces biosolid biochar and reduces the amount of pathogenic agents as well as contaminant availability, which improves the quality of the organic residue and makes possible its application in soil both as soil amendment and soil conditioner (LIU; LIU; ZHANG, 2014). Biochar can be used as a soil conditioner because it is a good quality carbon rich material that stays in soil for hundreds of years (LEHMANN, 2003). It also can be used as a soil amendment due its high concentration of nutrients (MÉNDEZ et al., 2012). However, only few studies have been reported on the use of biosolid biochar in soil. Considering that the components used in the production of substrates influence directly the growth cycle, quality and consequently the cost of production of seedlings (SIMÕES; SILVA; SILVA, 2012), the use of biochar in tree seedlings growing media, although still very incipient, could become an economically and environmentally friendly practice. Solla-Gullon, Santalla and Rodriguez-Soalleiro (2006) observed an 18% increase in plant height of a five-year old tree stand after application of 20 t ha^{-1} biochar. Omil, Piñeiro and Merino (2013), found good results in a forest field when applied 4.5 t ha^{-1} biochar mixed with ash. Therefore, the use of biosolid biochar in forest plants can be a wise strategy to cope with the problem of sewage sludge disposal while improving plant productivity. Therefore, the objective of the study was to evaluate the effect of increasing rate of application of biosolid biochar in the growth and morphological traits of eucalyptus seedlings under greenhouse environment.

MATERIAL AND METHODS

Collection of sewage sludge and production of biochar

Sewage sludge was taken from a drying bed, at a municipal wastewater treatment facility (WWTF), located in Tallahassee, Florida, U.S.A, where wastewater is treated through an activated sludge treatment process. Sewage sludge is sampled quarterly by the facility staff and presented the following mean ± std composition prior drying: 91±2% moisture, 6.8±0.3 pH units. It also contained the following total elemental composition:5.7±0.9%. N, 1.3±0.5% P, 0.2±0.1% K, 940±213 mg kg^{-1}Cu, 888±210 mg kg^{-1}Zn, 18±3 mg kg^{-1}Mo, 4.0±0.8 mg kg^{-1}As, 0.80±0.26 mg kg^{-1}Cd, 20±9 mg kg^{-1}Pb and 3.6±0.6 mg kg^{-1}Ni.

Biochar was produced in a Top-Lit Updraft retort unit, which is a micro-kiln that uses a reburner to eliminate volatile by-products of pyrolization, developed by the International Biochar Initiative (IBI) (NSAMBA, 2015).

Ground samples of the biochar were sent to a commercial laboratory (Huffman Labs, Boulder, CO, USA) for the proximate analysis (ash content, volatile matter and fixed carbon). The laboratory also conducted ultimate analysis (elemental C, N, H and S), using an elemental analyzer via flush combustion at 1,020^{o}C.

Oxygen was estimated as follows (eq. 1):

$$O\ (\text{mass }\%) = 100 - [(\frac{C+H+N+S}{\text{Total}})] \qquad \text{eq. 1}$$

Biochar pH was determined in a 1:5 (w/w) biochar:water ratio, after 1.5 h shaking in a reciprocating shaker and 1h equilibration (GASKING; STEINER; HARRIS, 2008). Electric conductivity (EC) was then determined in the same extract. Biochar characteristics are presented in Table 1.

Soil collection and experimental set up

The soil used in this experiment was taken from a fallow field at North Florida Research and Education Center (NFREC), Quincy, Florida. The soil was air-dried and sieved to pass through a 2-mm screen. The soil is classified as a Loamy, kaolinitic, thermic Grossarenic Kandiudults (SOIL SURVEY STAFF, 2007), with pH (ratio of 1:5 w/v) of 5.8.

The experiment was conducted as a completely randomized design with five treatments (rates of application of biochar: 0, 20, 40, 80 and 100 t ha^{-1}), with 4 replications. The plants were grown in a greenhouse with an average night/day temperature of 14/30^{o}C and an average photosynthetically active radiation flux of 825 umol m^{-2} s^{-1}. All treatments received NPK fertilization as recommended for eucalyptus seedlings, which was done by applying 0.7g per pot of osmocote (14-14-14), a controlled release fertilizer, as source of NPK.

TABLE 1: Sewage sludge biochar characteristics.
TABELA 1: Características do biocarvão de lodo de esgoto.

Variable	Proximate analysis			Ultimate analysis					pH	EC
	Ash	VM	Fixed C	C	N	H	S	O		
	%			%						dS m^{-1}
	25.6	54.2	20.2	45.5	7.43	4.85	1.14	15.5	7.5	7.29

Em que: VM = volatile matter; EC = electrical conductivity

A known weight (3.0 kg) of air-dried and sieved (2 mm) soil was put into plastic bags and thoroughly mixed with the appropriate rate of biosolid biochars, then transferred into 5 L plastic pot. After a one-week incubation period at field capacity, a health 10-week eucalyptus seedling (from seeds), with 38 cm height and 4 mm stem diameter, was transferred to each pot and maintained at 80% of field capacity by weighing the pots daily and adding the equivalent volume of water during the plant growth period. The seedlings were donated by Arborgen. At 0 (T1), 30 (T2) and 60 (T3) days after transplant, chlorophyll content, plant height and colon diameter were measured. Chlorophyll content of the mid-section, fully expanded leaves was measured using a hand-held chlorophyll meter (SPADmeter). Stem diameter was measured 2 cm above soil level using a digital caliper. The plants were allowed to growth for 60 days. Plants were then harvested and separated into roots and shoots. Plant tissues were washed thoroughly with tap water, then rinsed with deionized water. The shoots and roots were oven-dried for 3 days at 65°C and weighed.

Dickson Quality Index (DQI) was calculated to evaluate seedling quality as a function of total dry matter (TDM), shoot height (SH), stem base diameter (SBD), shoot dry matter (SDM) and root dry matter (RDM), and is given as follows (eq. 2) (DICKSON et al., 1960):

$$IQD = \frac{TDM(g)}{\frac{SH(cm)}{SBD(cm)} + \frac{SDM(g)}{RDM(g)}} \qquad \text{eq. 2}$$

Statistical analyses

Reported results are the means of the four replicates. One and two-way analyses of variance (ANOVA) were conducted to test the significance of the treatments using SISVAR software package (FERREIRA, 2011). The Tukey mean separation test was applied to treatment means at $P < 0.05$ probability level.

RESULTS AND DISCUSSION

The application of sewage sludge biochar had significant impact ($P < 0.001$) on the chlorophyll content, plant height and colon diameter of the eucalyptus seedlings (Table 2).

TABLE 2: ANOVA mean square for chlorophyll content, plant height and stem diameter of eucalyptus seedlings treated with sewage sludge biochar. Quincy, Florida.
TABELA 2: Quadrados médios para os teores de clorofila, altura de planta e diâmetro do caule das mudas de eucalipto tratadas com biocarvão de lodo de esgoto. Quincy, Flórida.

Factor of variation	GL	Chlorophyll	Plant height	Stem diameter
Rate of application (RA)	4	144,0**	100,0	1,7
Time (T)	2	857,0	6669,0	74,7
(RA) x (T)	8	51,7	82,5	2,1
C. V. (%)		9,0	8,3	9,7

Em que: **Significant at 1% probability.

The chlorophyll content is related to the N concentration in plant leaves. It is interesting to note that, according to the Ultimate analysis (Table 1), N concentration is higher in the sewage sludge biochar than in the uncharred sewage sludge (5.7%), which is a desired characteristic if sewage sludge biochar is to be considered as soil amendment. However, N in biochar does not seem to be in available form based on the results of the chlorophyll content, which are similar to the treatment without biochar, in the two lowest rates of application. Without biochar, time of measurement did not have any effect on the chlorophyll content of the eucalyptus seedlings. However, the presence of biochar caused an increase in the chlorophyll content with time, regardless of the rate of application, even though the results were more pronounced at T2, probably due to a higher availability of N in the first month (FIGUEREDO; SILVA; DIAS, 2014).

Chlorophyll content varied from 40.8 to 54.5 SPAD and from 35.2 to 46.8 SPAD in T2 and T3, respectively (Figure 1). Comparing with the control, the chlorophyll content was higher in the two highest rates of biochar application, at T2 and T3 (Figure 1).

However, there was no significant difference among the organic treatments. Santiago et al. (2009) found mean values around 32.6 SPAD for the chlorophyll content of different species of eucalyptus at 5-9 month-age. Yet, Oliveira Júnior, Cairo and Novaes (2011) observed lower values (21.0 to 29.78 SPAD) for the chlorophyll content of *Eucalyptus urophylla* after growing for 100 days in different growing media. Based on these findings, the presence of biochar in the growing media, in our study, did not have any negative effect on the chlorophyll content of the eucalyptus seedlings.

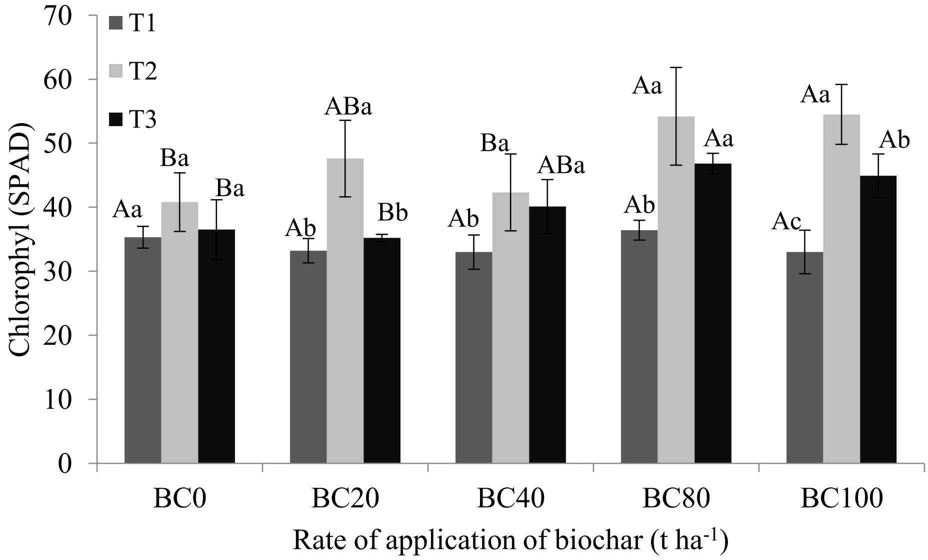

FIGURE 1: Chlorophyll content in the leaves of eucalyptus seedlings treated with sewage sludge biochar (BC) at different rates of application (0, 20, 40, 80 and 100 t ha^{-1}) and at time 1 (T1: day of planting), time 2 (T2: 30 days after planting) and time 3 (T3: 60 days after planting), under greenhouse condition. Each bar represents the mean and SD (n = 4). Different uppercase letters indicate significant differences ($p < 0.05$) between treatment means within the same time of evaluation. Different lowercase letters indicate significant differences ($p < 0.05$) between treatment means within the same rate of application.

FIGURA 1: Teor de clorofila nas folhas de mudas de eucalipto tratadas com diferentes taxas de aplicação (0, 20, 40, 80 e 100 t ha^{-1}) de biocarvão de lodo de esgoto aos 0 (T1), 30 (T2) e 60 (T3) dias do plantio, em casa de vegetação. Barras representam desvio padrão (n = 4). Diferentes letras maiúsculas indicam diferença significativa entre as médias dos tratamentos dentro de um mesmo tempo de avaliação. Diferentes letras minúsculas indicam diferença significativa entre as médias dos tratamentos dentro de uma mesma dose de biocarvão.

Thirty days after the plant transfer, no significant difference was observed in plant height (Figure 2). However, at T2, there was a significant reduction in stem diameter when biochar was applied at 80 and 100 t ha^{-1} (Figure 3). At T3, plant height significantly increased (16.8, 23.1, 32, 35%) with increase rate of biochar application, whereas stem diameter increased by 31.3, 23.6, 12.2 and 9.7% with increasing dose of biochar when compared with the control. Plant height and colon diameter significantly increased with time for all treatments, except colon diameter in the control. Freitas, Cardoso e Souza (2015) did not find significant differences in plant height and stem diameter of *Dipteryx odorata* seedlings when biochar was added to the growing medium. In our study, application of 80 t ha^{-1} biochar increased plant height by 35%. Even though, it was observed an increase in plant growth with increasing rate of application of biochar, at T2, that was also probably due to light competition, which forced the plant to allocate more energy and photosynthates toward the height growth than the stem, which could be observed in Figure 2. At the rate of application of 20 t ha^{-1}, there was an increase in stem diameter and no increase in plant height.

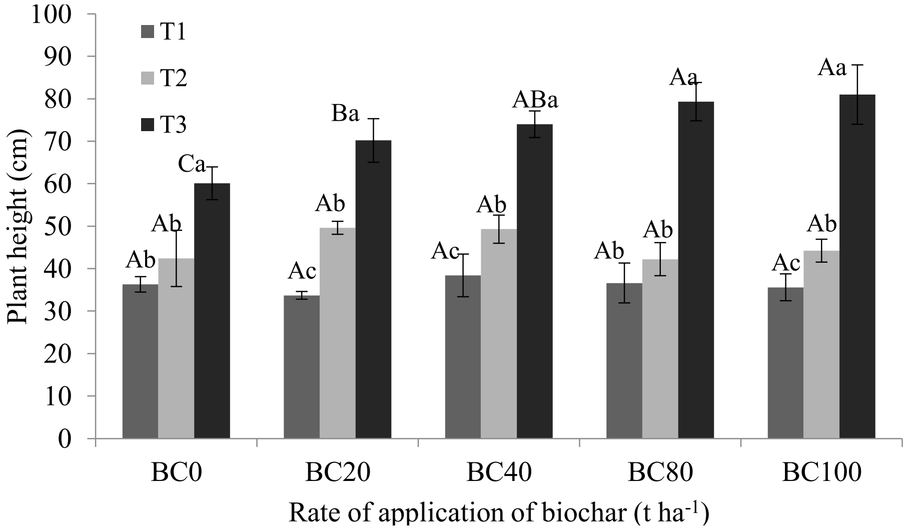

FIGURE 2: Plant height of eucalyptus seedlings treated with sewage sludge biochar (BC) at different rates of application (0, 20, 40, 80 and 100 t ha^{-1}) and at time 1 (T1: day of planting), time 2 (T2: 30 days after planting) and time 3 (T3: 60 days after planting), under greenhouse condition. Each bar represents the mean and SD (n = 4). Different uppercase letters indicate significant differences ($p < 0.05$) between treatment means within the same time of evaluation. Different lowercase letters indicate significant differences ($p < 0.05$) between treatment means within the same rate of application.

FIGURA 2: Altura de mudas de eucalipto tratadas com diferentes taxas de aplicação (0, 20, 40, 80 e 100 t ha^{-1}) de biocarvão de lodo de esgoto aos 0 (T1), 30 (T2) e 60 (T3) dias do plantio, em casa de vegetação. Barras representam o desvio padrão (n = 4). Diferentes letras maiúsculas indicam diferença significativa entre as médias dos tratamentos dentro de um mesmo tempo de avaliação. Diferentes letras minúsculas indicam diferença significativa entre as médias dos tratamentos dentro de uma mesma dose de biocarvão.

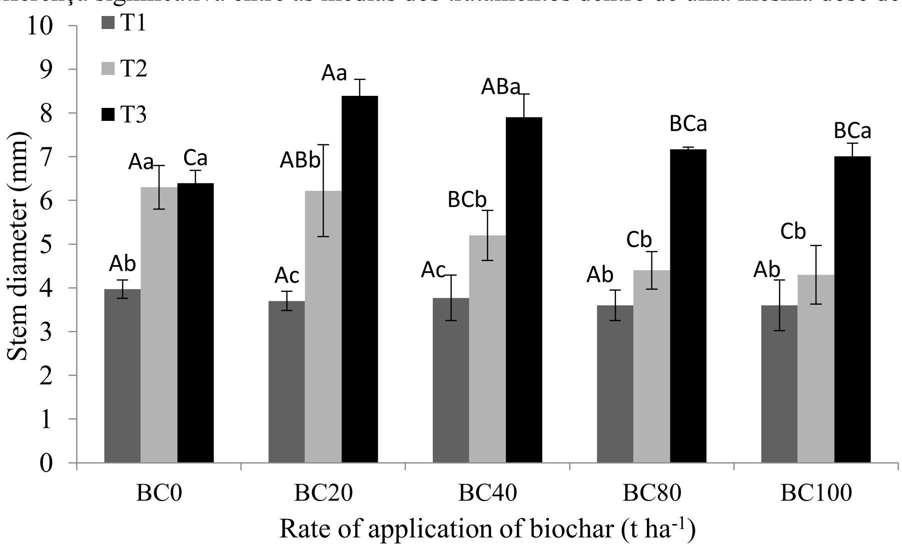

FIGURE 3: Stem diameter of eucalyptus seedlings treated with sewage sludge biochar (BC) at different rates of application (0, 20, 40, 80 and 100 t ha^{-1}) and time 1 (T1: day of planting), time 2 (T2: 30 days after planting) and time 3 (T3: 60 days after planting) under greenhouse condition. Each bar represents the mean and SD (n = 4). Different uppercase letters indicate significant differences ($p < 0.05$) between treatment means within the same time of evaluation. Different lowercase letters indicate significant differences ($p < 0.05$) between treatment means within the same rate of application.

FIGURA 3: Diâmetro do caule de mudas de eucalipto tratadas com diferentes taxas de aplicação (0, 20, 40, 80 e 100 t ha^{-1}) de biocarvão de lodo de esgoto aos 0 (T1), 30 (T2) e 60 (T3) dias do plantio, em casa de vegetação. Barras representam desvio padrão (n = 4). Diferentes letras maiúsculas indicam diferença significativa entre as médias dos tratamentos dentro de um mesmo tempo de avaliação. Diferentes letras minúsculas indicam diferença significativa entre as médias dos tratamentos dentro de uma mesma dose de biocarvão.

The presence as well as the amount of sewage sludge biochar in the growing media had a significant influence on shoot (Figure 4) and root biomass (Figure 5) of the eucalyptus seedlings. Biochar significantly improved shoot biomass by 97.8% (20 t ha^{-1}), 65.0% (40 t ha^{-1}), 31.0% (80 t ha^{-1}) and 19.5% (100 t ha^{-1}) as compared with the control. However, increasing rate of application of biochar from 20 t ha^{-1} to 100 t ha^{-1} significantly reduced shoot biomass (Figure 4). The lowest doses of biochar (20 and 40 t ha^{-1}) significantly increased root biomass by 43% and 28% as compared with the control; whereas the highest doses (80 and 100 t ha^{-1}) significantly reduced root biomass by 12% and 16%, respectively. Plant grew four times more shoot than root in the biochar treated pots and only three times more shoot than root in the control. The lack of positive response in both shoots and root growth as a result of high rates of biochar application is probably related to an increase in soluble substances in soil solution that could have harmed the plants.

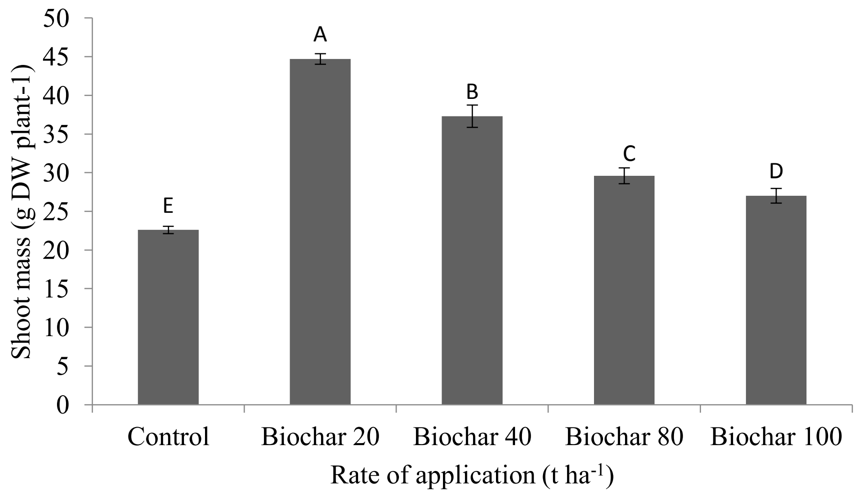

FIGURE 4: Shoot dry mass of eucalyptus seedlings treated with sewage sludge biochar at different rates of application (0, 20, 40, 80 and 100 t ha^{-1}), under greenhouse condition. Each bar represents the mean and SD (n = 4). Different letters indicate significant differences ($p < 0.05$) between treatment means.

FIGURA 4: Massa seca da parte aérea de mudas de eucalipto tratadas com diferentes taxas de aplicação (0, 20, 40, 80 e 100 t ha^{-1}) de biocarvão de lodo de esgoto, em casa de vegetação. Barras representam o desvio padrão (n = 4). Letras diferentes indicam diferença significativa ($p < 0.05$) entre os tratamentos.

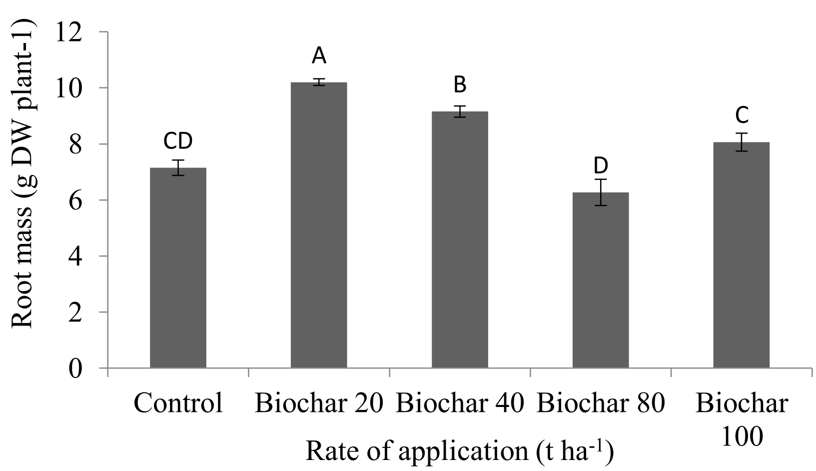

FIGURE 5: Root dry mass of eucalyptus seedlings treated with sewage sludge biochar at different rates of application (0, 20, 40, 80 and 100 t ha^{-1}), under greenhouse condition. Each bar represents the mean and SD (n = 4). Different letters indicate significant differences ($p < 0.05$) between treatment means.

FIGURA 5: Massa seca das raízes de mudas de eucalipto tratadas com diferentes taxas de aplicação (0, 20, 40, 80 e 100 t ha^{-1}) de biocarvão de lodo de esgoto, em casa de vegetação. Barras representam o desvio padrão (n = 4). Letras diferentes indicam diferença significativa ($p < 0.05$) entre os tratamentos.

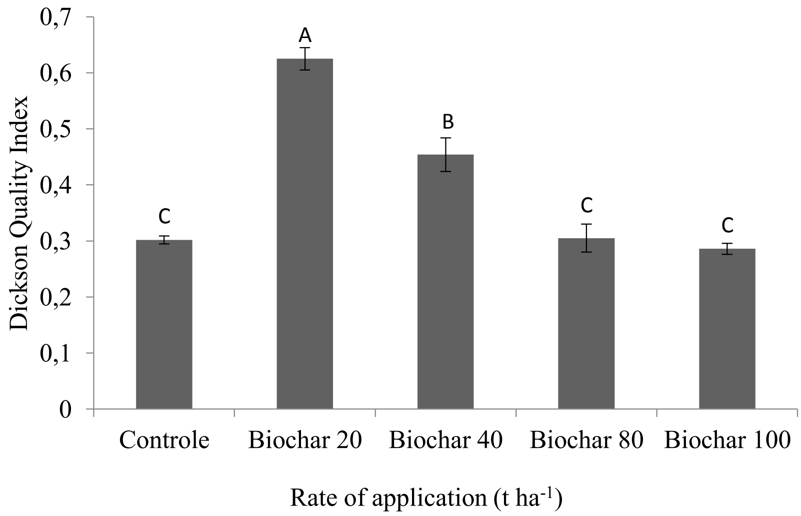

FIGURE 6: Dickson Quality Index (DQI) of eucalyptus seedlings treated with sewage sludge biochar at different rates of application (0, 20, 40, 80 and 100 t ha^{-1}), under greenhouse condition. Each bar represents the mean and SD (n = 4). Different letters indicate significant differences ($p < 0.05$) between treatment means.

FIGURA 6: Índice de Qualidade de Dickson de mudas de eucalipto tratadas com diferentes taxas de aplicação (0, 20, 40, 80 and 100 t ha^{-1}) de biocarvão de lodo de esgoto, em casa de vegetação. Barras representam o desvio padrão (n=4). Letras diferentes indicam diferença significativa ($p < 0.05$) entre os tratamentos.

According to Bernardino, Paiva and Neves (2005), high values of the DQI suggest high quality seedlings. Therefore, as it is shown in our study, sewage sludge biochar has the potential to be used as soil amendment for improving the quality of eucalyptus plants, with results even better than those observed by Caldeira (2013) when applied sewage sludge in the growing medium for the growth of eucalyptus seedlings. The authors found values of the DQI that varied from 0.09 (100% sewage sludge) to 0.24 (80% sewage sludge and 20% vermiculite). In the present study, the DQI of 0.23 was observed in the two highest doses of biochar, which suggests the beneficial effect of transforming sewage sludge into biochar. Rezende et al. (2016) used biochar, from fresh sawdust of tropical native forest, in the commercial substrate for the growth of *Tectona grandis* seedlings and observed that biochar did not improve quality parameters of seedlings. Their results were probably related to the poor nutrient content of the sawdust biochar.

CONCLUSION

The use of sewage sludge biochar improved the growth and the morphological traits of the eucalyptus seedlings, therefore its application can be recommended as soil amendment and soil conditioner. According to the DQI, the best eucalyptus seedlings were obtained with 20 and 40 t ha^{-1} of biochar.

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