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BIOSOLID AND COFFEE STRAW IN THE SUBSTRATE COMPOSITION OF ‘Cravo’ LEMON TREE SEEDLING

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ABSTRACT - The selection of an appropriate substrate promotes optimal growth and initial development of plants. Increasingly, organic residues are incorporated into substrate compositions due to their physicochemical characteristics, including biosolids and coffee husks. This study hypothesizes that biosolids and coffee husks can serve as alternative substrates in the initial production of ‘Cravo’ lime rootstock seedlings. The experiment was conducted in two phases using a randomized block design, consisting of seven treatments and four blocks, each block comprising ten plants. The treatments included mixtures of commercial substrate (CS), biosolids (BIO), and coffee husks (CH) in the following proportions (v/v/v): 50:50:0 (T1), 50:40:10 (T2), 50:30:20 (T3), 50:20:30 (T4), 50:10:40 (T5), 50:0:50 (T6), and 100:0:0 (T7). In Experiment 1, the seven treatments were evaluated up to 140 days after planting, while in Experiment 2, treatments T1, T3, T6, and T7 were evaluated up to 260 days. The variables measured included plant height (cm), stem diameter (mm), number of leaves, leaf area (cm²), root length (cm), dry biomass (g), growth ratios (height/diameter and shoot dry mass/root dry mass), and the Dickson Quality Index (DQI). The use of residues such as coffee husks and biosolids represents a viable alternative for producing ‘Cravo’ lime rootstock seedlings. Coffee husks can be used in proportions of up to 50% of the substrate volume. Biosolids can also be utilized at a proportion of 50% of the commercial substrate. Furthermore, a substrate formulation containing both residues comprising 50% commercial substrate, 30% biosolids, and 20% coffee husks can be developed without compromising the growth of ‘Cravo’ lime seedlings.
Keywords: *Citrus limonia* Osbeck, sewage sludge, organic waste.

BIOSSÓLIDO E PALHA DE CAFÉ NA COMPOSIÇÃO DE SUBSTRATO DE MUDAS DE LIMOEIRO CRAVO

RESUMO - A escolha do substrato adequado proporciona um bom crescimento e desenvolvimento inicial das plantas. Cada vez mais se utiliza resíduos orgânicos na sua composição devido às suas características físico-químicas, dentre eles o biossólido e palha de café. O trabalho tem como hipótese o uso do biossólido e palha de café como substratos alternativos na produção inicial de mudas do porta-enxerto de limoeiro cravo. O experimento contou com duas fases, montados em bloco casualizados, composto por sete tratamentos e quatro blocos, sendo cada bloco composto por dez plantas. Os tratamentos consistem na mistura de substrato comercial (SC), biossólido (BIO) e palha de café (PC), na proporção 50:50:0 (v/v/v) = T1, 50:40:10 (v/v/v) = T2, 50:30:20 (v/v/v) = T3, 50:20:30 (v/v/v) = T4, 50:10:40 (v/v/v) = T5, 50:0:50 (v/v/v) = T6 e 100:0:0 (v/v/v) = T7. No experimento 1, os sete tratamentos foram avaliados até 140 dias após o plantio, enquanto no experimento 2 os tratamentos T1, T3, T6 e T7, avaliados até 260 dias, sendo mensurados: altura das plantas (cm), diâmetro de caule (mm), número de folhas, área foliar (cm²), comprimento radicular (cm), biomassa seca (g), relações de crescimento (altura/diâmetro e massa seca da parte área/massa seca da raiz) e IQD (Índice de Qualidade de Dickson). O uso de resíduos como palha de café e biossólido são uma boa alternativa na produção de mudas para porta-enxertos de limoeiro ‘Cravo’. A palha de café pode ser utilizada na proporção de 50% do volume do substrato. O biossólido também pode ser utilizado na proporção de 50% do substrato comercial. Além disso, pode ser formulado um substrato contendo ambos os resíduos, na formulação volumétrica de 50% de substrato comercial, 30% biossólido e 20% palha de café, sem prejuízo ao desenvolvimento de mudas de limoeiro ‘Cravo’.
Palavras-chave: *Citrus limonia* Osbeck, lodo de esgoto, resíduo orgânico.

INTRODUCTION

In the process of producing high-quality seedlings, the substrate plays a fundamental role in supporting and providing suitable conditions for the plant, thereby contributing to its initial growth, greater survival in the field, and resistance to environmental stress (SILVA et al., 2020). To achieve this, the substrate's chemical and physical characteristics must be carefully considered, as they directly influence nutrient availability and water retention, adequately meeting the seedlings' requirements (SILVA et al., 2022a).

For optimal seedling growth, the recommended approach is to formulate the substrate using diverse materials to create a high-quality medium capable of providing the necessary conditions for plant development (SILVA et al., 2022a). Organic materials, in particular, are essential as they contribute to moisture retention, thermal amplitude control, and partial nutrient supply (BERILLI et

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al., 2017). Furthermore, the use of organic residues- especially those readily available in the cultivation area- can reduce environmental contamination risks associated with improper disposal and lower production costs (MONACO et al., 2020).

Currently, the use of alternative substrates in seedling production and plant cultivation has been extensively studied as a strategy to utilize organic residues and promote sustainability. Among these, biosolids and coffee husks have garnered interest as agricultural substrates due to their potential benefits.

The use of biosolids in substrate formulation is considered a promising alternative due to their chemical and physical properties, providing a valuable source of organic matter and essential nutrients for plants (SILVA et al., 2022a; CARVALHO et al., 2022). However, despite their favorable chemical attributes, biosolids must be combined with other components to balance nutrient supply and physical properties (CALDEIRA et al., 2018). The reuse of sewage sludge as biosolids represents a viable alternative in substrate composition, reducing soil use for seedling production and facilitating management through lighter trays (SILVA et al., 2022b).

Coffee husks, a renewable residue derived from coffee bean processing, are abundant and cost-effective in major coffee-producing regions, such as Minas Gerais, Espírito Santo, and São Paulo (FARIA et al., 2020). Given their high content of organic matter, nutrients, and secondary compounds, coffee husks can be recycled or reused in nature (HOSEINI et al., 2021). Thus, they are suggested as an alternative component in substrate formulations, offering benefits such as waste volume reduction and cost amortization for producers during seedling production (SILVA et al., 2020).

The selection of an appropriate substrate is therefore crucial for the healthy and vigorous growth of seedlings, including those for citrus production. Citrus cultivation is a globally significant agricultural activity, with Brazil standing out among fruit-producing nations due to its high production volume and value, both for the domestic fresh fruit market and juice exportation (CARVALHO et al., 2019). However, studies examining the combined use of biosolids and coffee husks in substrate formulations for seedling production remain scarce in the literature.

This study hypothesizes that biosolids and coffee husks can serve as alternative substrates for the initial production of ‘Cravo’ lime rootstock seedlings.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse located at the Experimental Farm of the Centro de Ciências Agrárias e Engenharias at Universidade Federal do Espírito Santo (UFES), in the municipality of Alegre, Espírito Santo, Brazil, during the year 2022. A randomized block design was employed, comprising seven treatments and four blocks, with each block consisting of ten plants.

The treatments corresponded to different proportions of a mixture containing biosolids, commercial substrate, and coffee husks, as detailed in Table 1. The biosolids were produced from sewage sludge sourced from the Effluent Treatment Plant of the Autonomous Water and Sewage Service (SAAE) in the city of Jerônimo Monteiro, Espírito Santo. The biosolids underwent a solarization process for stabilization and sanitization.

TABLE 1 - Proportion of commercial substrate (Provaso®), biosolid, and coffee husks in the different treatments studied.

Treatments	Commercial substrate	Biosolid	Coffee husks
		%	
T1	50	50	0
T2	50	40	10
T3	50	30	20
T4	50	20	30
T5	50	10	40
T6	50	0	50
T7	100	0	0

The commercial substrate (Provaso®) used in the experiment was a bio-stabilized compost made from sugarcane bagasse, peat, limestone, agro-industrial organic waste (class A), manures and poultry bedding, ash, and plant residues. The coffee husks were obtained after the processing of Arabica coffee fruits from local coffee producers and were sun-dried before use.

Initially, the seedlings were monitored every two days and evaluated for germination percentage (GP) and germination speed index (GSI), calculated using Equation 1 and Equation 2, respectively, where N (number of germinated seed), A (total number of seeds in the sample), ni (number of seeds germinated at time “i”) and ti (time (days) after the test setup).

$$GP = \frac{N}{A} * 100 \quad \text{(Equation 1)}$$

$$GSI = \Sigma \frac{ni}{ti} \quad \text{(Equation 2)}$$

The mean germination time (MGT) and mean germination speed (MGS) were also calculated using Equation 3 and Equation 4, respectively, as described below, where t is the average germination time (CETNARSKI FILHO; CARVALHO, 2009).

$$MGT = (\sum niti)/\sum ni \quad \text{(Equation 3)}$$

$$MGS = \frac{1}{t} \quad \text{(Equation 4)}$$

When the plants developed two pairs of leaves, thinning was carried out, leaving only one plant per container. The first growth analysis was conducted when the seedlings were 140 days after emergence, analyzing four plants from each repetition. The non-destructive variables evaluated were plant height (measured from the substrate level to the apical bud using a measuring tape, cm); stem diameter at the base (measured at the substrate level using a digital caliper, mm) and number of leaves. Afterward, the plants were removed from the container and washed under running water for destructive evaluation. Root length (cm) and leaf area (cm²) and root area (cm²) were measured through digital scanning and analysis using ImageJ software (ABRAMOFF et al., 2004).

Subsequently, the dry biomass of the roots (DBR, g) and dry biomass of the shoot (DBS, g) were dried and weighed. The plants were dried in an oven with forced air circulation at 60°C for 48 hours and then weighed on a precision electronic balance, with the total dry biomass (TDB, g) calculated. The height/diameter ratio (H/D) and DBS/DBR were also calculated, and the seedling quality was assessed using the Dickson Quality Index (DQI) (Equation 5).

DQI = [DBR/ (H/D + DBS/DBR)] $IQD = \frac{BST}{\frac{H}{D}+\frac{DBS}{DBR}}$

(Equation 5)

The seedlings that were not analyzed in the experiment were transferred to 400 cm³ plastic bags to promote further plant growth. At this stage, the number of treatments was reduced to four, based on the data obtained in the first analysis. Thus, treatments T1 (50:50:0), T3 (50:30:20), T6 (50:0:50), and T7 (100:0:0) were maintained. The analysis of the plants was carried out 120 days after transferring the seedlings to plastic bags, totaling 260 days after emergence. The same analyses described in the first part of the experiment were performed.

The analysis of variance (ANOVA) was performed with a 5% probability of error, and when significant, the means were subjected to the Scott-Knott test. The data were analyzed using R Studio® software, version 1.3.1073 (TEAM, 2022).

RESULTS AND DISCUSSION

The analysis of variance related to the seedling emergence variables is presented in Table 2. The germination percentage was not significant, considering p<0.05, while the germination speed index, average germination time, and mean germination rate were significant.

Based on the data in Table 3, it was observed that the GSI was higher in treatments T1 (50:50:0) and T7 (100:0:0). Since GSI is inversely related to MGT and MGR, these treatments showed lower indexes for these variables. Thus, the higher the GSI, the lower the mean germination time and mean germination rate, with substrates composed solely of commercial substrate and with 50% biosolid in their composition resulting in faster and more uniform germination, reducing the seedling production time.

TABLE 2 - ANOVA of variables: germination percentage (GP), germination speed index (GSI), mean germination time (MGT), and mean germination rate (MGR) of 'Cravo' lemon seeds in different substrates.

Variables	F	P<0,05	VC(%)
GP	2.605	0.053^{ns}	18.96
GSI	4.389	0.006**	20.31
MGT	3.970	0.010**	8.34
MSR	4.816	0.004**	7.72

**Significant by F Test at 5% error probability. ns = not significant. F = F-test, P = p-value of significance, CV = coefficient of variation.

TABLE 3 - Mean germination percentage (GP), germination speed index (GSI), mean germination time (MGT), and mean germination rate (MGR) of 'Cravo' lemon seeds in different mixtures containing commercial substrate (CS), biosolid (BIO), and coffee husk (CH).

Treatment	GP	GSI	MGT	MGR
(CS:BIO:CH)	%	-	dias	dias
T1 (50:50:0 v/v/v)	70.0	19.7 a*	36.7 b	0.027 a
T2 (50:40:10 v/v/v)	70.0	18.7 b	40.2 a	0.025 b
T3 (50:30:20 v/v/v)	70.0	16.7 b	43.4 a	0.023 b
T4 (50:20:30 v/v/v/)	67.5	15.6 b	45.3 a	0.022 b
T5 (50:10:40 v/v/v/)	52.5	13.0 b	42.6 a	0.025 b
T6 (50:0:50 v/v/v/)	62.5	15.9 b	41.5 a	0.024 b
T7 (100:0:0 v/v/v/)	87.5	24.8 a	36.2 b	0.028 a

*Means followed by the same letter in the column do not differ from each other by the Scott-Knott test at 5% error probability.

According to the results obtained by Elloumi et al. (2016), it was demonstrated that different levels of biosolids increased both seed germination and seedling growth, which may be the result of increases in plant nutrients provided by the waste. However, in studies by Braga and Pasin (2020), coffee husk inhibited the germination and initial development of some plant species (bidens, yellow bean, and pumpkin) due to its allelopathic effect, causing the appearance of abnormal seedlings, with root necrosis being one of the most common symptoms of allelopathy.

According to the analysis of variance described in Table 4, the results obtained after 140 days of planting indicated significance only for the variables number of leaves, root dry mass, DBS/DBR ratio, and DQI of 'Cravo' lemon seedlings. The morphological parameters most commonly used to characterize seedling quality are height, diameter, height/diameter ratio, shoot biomass, root biomass, and total biomass (GOMES et al. 2019).

TABLE 4 - Summary of the analysis of variance of ‘Cravo’ lemon seedlings, 140 days after planting, grown in different substrates.

Variables	F	P<0,05	CV(%)
Height (cm)	1.845	0.140^{ns}	25.73
Diameter (mm)	1.444	0.252^{ns}	11.63
Number of leaves	3.100	0.028**	8.40
Leaf area (cm²)	0.988	0.461^{ns}	22.78
Shoot dry biomass (DBS) (g)	1.393	0.270^{ns}	23.95
Root dry biomass (DBR) (g)	3.636	0.015**	28.94
Total dry biomass (TDB) (g)	2.318	0.077^{ns}	25.56
Root length (cm)	1.354	0.281^{ns}	9.40
Height/Diameter	1.517	0.228^{ns}	23.51
DBS/DBR	5.558	0.002**	13.53
Dickson Quality Index (DQI)	1.498	0.235**	31.78

**Significant by F Test at 5% error probability. ns = not significant. F = F-test, P = p-value of significance, CV = coefficient of variation.

Table 5 presents the mean values of the variables for the shoot part of ‘Cravo’ lemon seedlings. It can be observed that the number of leaves in the treatments 50:50:0, 50:20:30, 50:0:50, and 100:0:0 (commercial substrate: biosolid: coffee husk, v/v/v) obtained a higher mean compared to the other treatments. According to Leila et al. (2017), an increase in the number of leaves is a good indicator of adequate water and nutrient supply for plants, as *E. camaldulensis* (red eucalyptus) seedlings fertilized with sludge showed a 40% increase in the number of leaves.

TABLE 5 – Height, diameter, number of leaves, leaf area, and shoot dry biomass of ‘Cravo’ lemon seedlings, 140 days after planting.

Treatments (CS:BIO:CH)	Height (cm)	Diameter (mm)	Number of leaves	Leaf area (cm²)	Shoot dry biomass(g)
T1 (50:50:0)	9.22*	1.82	8.50 a	33.32	0.30
T2 (50:40:10)	7.98	1.66	7.93 b	34.77	0.28
T3 (50:30:20)	7.45	1.66	7.81 b	29.91	0.23
T4 (50:20:30)	7.95	1.79	8.75 a	34.62	0.80
T5 (50:10:40)	7.86	1.66	7.81 b	31.24	0.26
T6 (50:0:50)	11.86	1.84	9.18 a	33.89	0.30
T7 (100:0:0)	9.65	2.00	9.25 a	41.36	0.36

*Means followed by the same letter in the column do not differ from each other by the Scott-Knott test at 5% error probability.

Oliveira et al. (2019) observed that treatments with 15%, 30%, and 45% coffee husk showed higher means of leaf area, plant height, and stem diameter of papaya seedlings; however, the number of leaves was not significant among treatments. According to Silva et al. (2020), coffee husk positively contributed to diameter growth, possibly due to improvements in the physical-water conditions of the substrate. Root dry biomass had a higher mean in treatments 50:50:0, 50:0:50, and 100:0:0, while the DBS/DBR ratio was lower in the compositions 50:50:0 and 100:0:0 (Table 6). Although DQI showed significance in the analysis of variance, this was not the case for the means test.

Various results regarding the effect of biosolids on plant growth have been observed in the literature. Souza et al. (2022b) found that biosolids in the substrate composition had a significant effect on almost all evaluated morphological and quality characteristics of zinnia (*Zinnia elegans*) seedlings, except for the shoot height/stem diameter ratio. In the production of watercress seedlings (*L. sativum*), smaller doses of sludge were associated with greater promotion of root length (URBANIAK et al., 2017), while Oliveira et al. (2019) did not observe significant differences in the root system length of papaya plants.

For shoot biomass and root system characteristics of papaya seedlings, treatments with 100% commercial substrate and 15%, 40%, and 45% coffee husk showed superior values compared to other treatments for both characteristics. The use of low-density and coarser granulometry materials for substrate composition, such as raw coffee husk, provided greater aeration space, reduced bulk density, and available water (CALDEIRA et al. 2014). The relationship between shoot and root dry biomass must be observed so that the upper part of the seedlings is not significantly superior to the root part, making it difficult to absorb and transfer water to the shoot, especially under field conditions (GOMES et al. 2019). In this case, it can be observed that treatments containing the addition of coffee husk to the substrate increased the DBS/DBR ratio.

TABLE 6 - Root dry biomass (DBR), total dry biomass (TDB), root length, height/diameter ratio, DBR/TDB ratio, and Dickson Quality Index (DQI) of 'Cravo' lemon seedlings, 140 days after planting.

Treatments (CS:BIO:CH)	Root dry biomass	Total dry biomass (g)	Root length (cm)	Height/diameter ratio	DBR/TDB ratio	DQI
T1 (50:50:0)	0.23 a*	0.54	16.87	5.04	1.31 b	0.085
T2 (50:40:10)	0.18 b	0.47	15.61	4.79	1.60 a	0.073
T3 (50:30:20)	0.14 b	0.37	14.45	4.49	1.66 a	0.060
T4 (50:20:30)	0.18 b	0.47	14.99	4.42	1.55 a	0.0790
T5 (50:10:40)	0.17 b	0.44	14.53	4.73	1.62 a	0.0696
T6 (50:0:50)	0.28 a	0.58	14.56	6.54	1.61 a	0.0891
T7 (100:0:0)	0.29 a	0.65	15.78	4.81	1.28 b	0.109

*Means followed by the same letter in the column do not differ from each other by the Scott-Knott test at 5% error probability.

TABLE 7 - Summary of variance analysis of 'Cravo' lime seedlings, 260 days after planting.

Variables	F	P<0,05	CV(%)
Height (cm)	0.621	0.618^{ns}	10.83
Diameter (mm)	0.779	0.534^{ns}	11.51
Number of leaves	4.523	0.034**	13.41
Leaf area (cm²)	2.452	0.130^{ns}	21.46
Shoot dry biomass (DBS) (g)	2.146	0.164^{ns}	23.98
Root dry biomass (RDB) (g)	1.659	0.244^{ns}	27.79
Total dry biomass (g)	2.037	0.179^{ns}	24.64
Root length (cm)	4.184	0.041**	14.00
Height/Diameter	0.615	0.622^{ns}	12.21
DBS/RDB	0.692	0.597^{ns}	10.26
Dickson Quality Index	1.945	0.193^{ns}	28.83

**Significant by F Test at 5% error probability. ns = not significant. F = F-test, P = p-value of significance, CV = coefficient of variation.

TABLE 8 - Height, diameter, number of leaves, leaf area, biomass dry of the aerial part (BDAP), biomass dry of the root, total biomass dry, root length, height/diameter ratio, biomass dry of the aerial part /total biomass dry ratio (BDAP/TBD) and Dickson Quality Index (DQI) of 'Cravo' lime seedlings, 260 days after planting.

Treatment (CS:BIO:CH)	Height (cm)	Diameter (mm)	Number of leaves	leaf area (cm^2)	BSAP (g)	
T1 (50:50:0)	39.75*	4.65	31.75 a	341.99	4.27	
T3 (50:30:20)	38.95	4.88	29.87 a	286.16	4.20	
T6 (50:0:50)	36.04	4.39	26.08 a	303.04	3.57	
T7 (100:0:0)	37.62	4.90	36.75 a	413.21	5.41	
Treatment (CS:BIO:CH)	BDR (g)	TBD (g)	Root length (cm)	Height/diameter ratio	BDAP/TBD ratio	DQI
T1 (50:50:0)	1.46	5.73	18.08 b	8.61	2.91	0.50
T3 (50:30:20)	1.32	5.52	17.33 b	8.04	3.23	0.50
T6 (50:0:50)	1.19	4.76	16.87 b	8.23	3.05	0.42
T7 (100:0:0)	1.79	7.20	22.70 a	7.68	3.05	0.67

*Means followed by the same letter in the column do not differ from each other by the Scott-Knott test at 5% error probability.

The Dickson Quality Index (DQI) is widely used in seedling quality assessment as it considers the vigor and balance of biomass distribution in the seedling, along with parameters such as height, diameter, shoot dry biomass, root dry biomass, and their ratios (GOMES et al., 2019). The higher the index value, the better the seedling quality. Although the DQI was not statistically different among the treatments, it was observed that the highest value was in 'Cravo' lemon seedlings produced in commercial substrate. This result is similar to that found by Alonso et al. (2021), where the DQI reached the highest values in the control treatment with commercial substrate.

Although most of the analyzed variables did not show significant differences, this means that the performance of 'Cravo' lemon seedlings in substrates containing biosolids and coffee husks was similar to those containing only commercial substrate. Thus, the good performance of seedlings produced in substrates with coffee husk and biosolids is due to the physico-chemical characteristics of these materials.

The commercial substrate, despite receiving base fertilization in its formulation, may have low total nutrient levels, with available values tending to leach after the first few weeks of irrigation, while biosolids have considerable total nutrient levels that are gradually released to the plants over time (ALONSO et al., 2022).

Biosolids positively influence the fertility attributes of the substrate, especially in terms of N and micronutrient levels, such as Zn, Cu, Fe, and Mn (COSTA et al., 2023). They also increase density, microporosity, and water retention capacity (ALONSO et al., 2022). Coffee husk is rich in N, P, and K and lignocellulosic materials, making it an ideal substrate for microbial processes (HOSEINI et al., 2021).

The best proportion of biosolids and coffee husks to be incorporated into the substrate will depend on the species being studied. When using biosolids in seedling production, a proportion of 50% is recommended for aroeira (CARVALHO et al., 2022), 40 to 60% for pata-de-vaca (*Bauhinia forficata* Link) (VINCIGUERRA et al., 2023), and *Syagrus romanzoffiana* (known as jerivá) (SOUZA et al., 2022). Adding up to 30% coffee husk to the commercial substrate proved to be a viable measure for producing quality Hawaiian papaya seedlings (OLIVEIRA et al., 2019). For clonal eucalyptus seedlings *(E. urophylla* × *E. grandis*), coffee husk can be used in the alternative substrate composition at a proportion of up to 28% (MONACO et al., 2020), while for *E. grandis*, the most recommended composition is 80% sewage sludge + 20% raw coffee husk (CALDEIRA et al., 2014).

CONCLUSIONS

The use of residues such as coffee husk and biosolids appears as a good alternative in the production of seedlings for 'Cravo' lemon rootstocks. Coffee husk can be used at a proportion of 50% of the substrate volume. Biosolids can also be used at a proportion of 50% of the commercial substrate. Furthermore, a substrate containing both residues can be formulated, with a volumetric composition of 50% commercial substrate, 30% biosolids, and 20% coffee husk, without hindering the development of 'Cravo' lemon seedlings.

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