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Charcoal fine residues used as biochar in heavy clayey soil improve carrot production

O fino de carvão usado como biochar em solo muito argiloso incrementa a produção de cenoura

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ABSTRACT

Carrot plants do not develop well in clayey soils. In its turn, the charcoal fine (CF), which is a residue composed of porous particles, has the potential to be mixed in the soil to reduce its density. However, there is no evidence that the application of CF improves carrot production in clayey soil. Thus, an experiment in pots was designed, consisting of 16 treatments composed of the combination of 5 sizes of CF particles (1-2 mm, 2-4 mm, 4-8 mm, 8-16 mm, 16-32 mm) with 3 volumetric rates of CF (25%, 50% and 75%, plus a control (soil alone), aiming to verify which situation promotes the better plant growth and production. It was observed that mixing CF into the soil decreases substrate density and increases substrate water retention. The results of this research also revealed that the highest yield (fresh and dry weight) of carrots was achieved with CF mixed with the soil at a rate of 50%, using a CF particle size of 2-4 mm. The greatest length and diameter of carrots were obtained with a dose of CF of around 45%. CF-containing substrates delayed initial plant growth up to 45 DAE, but increased plant development after 75 DAE and improved plant performance and carrot yield measured at the harvest time (90 DAE).

Index terms: *Daucus carota*; fine coal residue; particle size; soil moisture.

RESUMO

As plantas de cenoura não se desenvolvem bem em solos muito argilosos. Por sua vez, o fino de carvão vegetal (CF) que é um resíduo composto por partículas porosas, tem potencial para ser misturado no solo visando diminuir a densidade do mesmo. Entretanto, não existe evidência de que a aplicação de CF melhora a produção de cenoura em solo argiloso. Assim, foi idealizado um experimento em vasos, composto por 16 tratamentos compostos pela combinação de 5 tamanhos de partículas de CF (1-2 mm, 2-4 mm, 4-8 mm, 8-16 mm, 16-32 mm) com 3 doses (proporções volumétricas) de CF (25%, 50% e 75%), mais um controle (somente solo), visando verificar qual a situação que promove o melhor crescimento e produção das plantas. Foi observado que a mistura de CF no solo diminui a densidade do mesmo e aumenta a retenção de água. Os resultados desta pesquisa também revelaram que a maior produção (peso fresco e seco) de cenouras foi alcançada com CF misturado no solo na proporção de 50%), usando tamanho de partículas de CF de 2-4 mm. O maior comprimento e diâmetro da cenoura foi obtido com dose de CF em torno de 45%. Substratos contendo CF retardaram o crescimento inicial das plantas até 45 DAE, mas aumentaram o desenvolvimento das plantas após 75 DAE e melhoraram o desempenho das plantas e a produção de cenoura, quando avaliados na época da colheita (90 DAE).

Termos para indexação: *Daucus carota*; fino de carvão; tamanho da partícula; umidade do solo.

INTRODUCTION

Carrot plants require soil with low bulk density, high aeration, and good drainage to produce roots of commercial quality (Lima Junior et al., 2012). Because of these requirements, carrots production with plants grown on heavy clayey soils is difficult. On the other hand, charcoal fine (CF) is a material with low density due to being composed of highly porous particles. Then, we hypothesize that mixing CF to the soil in a suitable proportion and particle size, should decrease the soil density besides improving other soil quality, which may lead to better carrot plant growth and production.

The mentioned CF are particles of charcoal coming from the charcoal production chain, originating mainly from abrasion and mechanical chocks during charcoal manipulation. It is a solid waste considered a serious environmental pollutant, but it could be used as a soil amendment (Ronix et al., 2021) if well managed.

Charcoal is a material obtained subjecting wood or other organic materials to pyrolysis (heating to a high temperature associated with low oxygen availability). When the production of this material aims at its use as fuel, it is commonly named charcoal, but when the objective is to use it as a soil conditioner, it is more appropriate to refer to it as biochar (Tenenbaum, 2009; Novotny et al., 2015).

Several reports showed that biochar, and therefore CF, mixed in the soil reduces soil bulk density and increases soil water holding capacity (Basso et al., 2013; Adekiya et al., 2019, Agbede, 2021). Other studies suggested that the application of charcoal alone or associated with other fertilizers also improves soil conditions by factors like increasing its carbon stock (Novotny et al., 2009); protecting organic matter against mineralization (Zheng et al., 2021); improving soil fertility traits (Gaskin et al., 2010; Agegnehu et al., 2015, Ronix et al., 2021); increasing nutrient use efficiency (Alburquerque et al., 2013); enhancing chemical and biological processes in the soil (Wang et al., 2012; Zhu et al., 2018); reducing nutrient losses by leaching (Zhang et al., 2013); and reducing the bioavailability and phytotoxicity of heavy metals (Park et al., 2011). Altogether these factors would justify the improvement in plant growth (Góes et al., 2010), besides reducing greenhouse gas emissions (Wang et al., 2012). Enhancement of the morphology and vitality of roots from plants grown on soil amended with biochar was also reported by Zhu et al. (2018). However, it is logical to believe that plant response should depend on biochar, particle size, and the rate applied to the soil (Trifunovic et al., 2018). Despite the apparent potential of biochar to improve soil characteristics and agricultural productivity, several contradictory results lead to concern that may have risks of unfavorable responses to crop productivity when biochar is inappropriately used (Mukherjee; Law, 2014; Brtnicky et al., 2021). In the same way, such concerns with biochar should be valid also for CF residue, which justifies the development of this study.

The uncertainties related to the use of biochar, and therefore of CF, in agriculture are because scientific literature on the effect of biochar application in cropland is still incipient (Singh et al., 2014). For instance, it is currently unknown whether the results of a given biochar application would be the same in different types of soils and crops (Pereira et al., 2015). Likewise, there is still not enough information on the effect of rates and particle size required by specific crops (Zanetti et al., 2003; Souchie et al., 2011; Petter; Madari, 2012). It is also unclear whether the size and rate of CF particles have independent effects on soil and plant or if they interact somehow to affect soil quality and plant growth.

Thus, it is very likely that the CF residue effects resulting from the rate of application and particle size will also be dependent on the crop and soil type. In this context, we hypothesized that mixing an appropriate rate of CF residue with a suitable particle size would be a feasible strategy to improve soil bulk density, aeration, water retention, and drainage of heavy clayey soils to make carrot production enhanced under this condition. In this sense, this study aims to determine the effects of CF rates and particle size when applied in clayey soil to verify what is the better proportion (rate), and suitable particle size of CF to be mixed with a clayey soil to enhance the growth and production of carrot plants. If this work is successful, we will have contributed to enabling the production of carrots in heavy clayey soils and have contributed to guiding the environmentally correct disposal of CF residues.

MATERIAL AND METHODS

Site description and experimental design

The experiment was conducted from January 26 to May 12, 2020, in a greenhouse located in the municipality of Bebedouro, SP, Brazil (20° 53’20.0436’’S, 48° 27’39.8088’’W, altitude of 597 m). The climate in this region is Cwa (rainy tropical with a dry winter) according to Köppen’s classification system, and the mean temperature is 18 °C. The essay consisted of 16 treatments disposed of in a 5×3+1 factorial scheme (5 particle sizes × 3 rates of charcoal fine + 1 control without charcoal fine), with 4 replicates, totaling 64 experimental units, arranged in a completely randomized design. Each experimental unit was composed of a plastic pot, containing 8 dm^{-3} of corresponding treatment (soil alone or mixed with distinct rates, and particle size of CF). To prevent nutrients from leaching, each pot was lined with a plastic tray.

Soil physical and chemical properties

The soil used in this experiment was classified as a dark red oxisol with a clayey texture (EUTRUSTOX), Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA, 2013), and its physical-chemical characterization is presented in Table 1.

Charcoal fine residue (CF) physical and chemical properties

The charcoal fine residue (CF) used in the study was obtained from Eucalyptus citriodora wood, in sizes corresponding to the ranges of 1-2 mm, 2-4 mm, 4-8 mm, 8-16 mm, and 16-32 mm. The availability of nutrients, as well as pH and electrical conductivity (EC) of distinct CF fractions, were measured in an aqueous extract 1:5(v/v) prepared by stirring this volumetric mixture at 40 rotations per minute, at 25 °C, for 1 h (Ministério da Agricultura, Pecuária e Abastecimento - MAPA, 2007). The nutrient contents were determined in the obtained aqueous extract after being subjected to different digestion processes: sulfuric digestion (N), and nitro perchloric (P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn). After digestion, aqueous extracts were analyzed for the concentrations of K, Ca, Mg, Cu, Fe, Mn, and Zn, by using an atomic absorption spectrophotometer; S was determined by turbidimetry and P by molecular spectrophotometry. Total N was determined in the sulfuric digestion of the aqueous extract by distillation according to the Kjeldahl method (Carmo et al., 2000). Results are presented in Table 2.

Treatments preparation

The 16 treatments were composed of a combination of 5 particle sizes of CF (1-2 mm, 2-4 mm, 4-8 mm, 8-16 mm, 16-32 mm) with 3 rates of charcoal fine (25%, 50% and 75%, volumetric basis), plus one control treatment (soil alone). By using a volumetric container, corresponding volumes of soil and CF fractions were measured and mixed to compose each CF rate. Thus, for the CF rate of 25%, the content of one volumetric container of CF was mixed with the amount of three volumetric containers of soil; for the CF rate of 50%, an equal volume of CF and soil were mixed; and for the CF rate of 75%, one volumetric container of soil was mixed with three volumetric containers of CF.

The control treatment (soil alone) showed a bulk density of 3136 g dm^{-3} (Table 1), and the bulk density obtained for the other 15 treatments (combination of the 5 CF particle size with three rates) are presented in Table 2.

All 16 treatments were prepared in four replications, leading to 64 experimental units. Each experimental unit was composed of a plastic pot containing 8 dm^{-3} of the corresponding treatment. To prevent nutrients from leaching, each pot was lined with a plastic tray. Those 64 experimental units were placed in a greenhouse and disposed of in a complete randomized design.

Table 1: Chemical and physical characteristics of the soil used to prepare treatments (soil alone or mixed with distinct sizes and rates of charcoal fine).

Chemical variables													
pH	OM	P (res.)	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	CEC	V%	B	Cu	Fe	Mn	Zn
-	g dm^{-3}	mg dm^{-3}	---------------- mmol$_c$ dm^{-3}----------------					%	---------------- mg dm^{-3} --------------------				
5.7	19	5.1	3.4	3.6	1.3	0.0	73.4	71	0.32	4.2	11	24	1.6

Physical variables			
Bulk density	Sand	Silt	Clay
g dm^{-3}	---------------------------- g kg^{-1} ----------------------------		
3136	193	237	570

Table 2: Chemical characteristics of the aqueous extract (1:5v/v, sample: water) of charcoal fine fractions, and substrate (mixture of soil + charcoal fine) bulk density corresponding to volumetric rates of 25%, 50% and 75%.

Particle size (mm)	Chemical characteristics of charcoal fine												Rates (volumetric basis) of charcoal fine 25% 50% 75%		
	EC (mS cm^{-1})	pH	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	Bulk density		
			------------------------------ mg dm^{-3} --------------------------------------										---------- g dm^{-3} ----------		
1-2	543	6.68	0.5	29	4.16	1.28	1.84	6	0.01	0.30	nd	0.02	2532	2018	1286
2-4	546	6.78	0.5	32	3.46	1.34	2.12	141	0.01	1.86	nd	nd	2595	2257	1678
4-8	429	6.68	0.5	24	3.29	1.22	1.88	107	0.01	1.84	nd	nd	2639	2283	1804
8-16	328	6.60	0.5	19	2.04	0.98	1.4	88	0.01	2.26	nd	nd	2588	2236	1785
16 -32	192	6.64	0.5	3	1.18	0.92	0.6	78	0.01	0.84	nd	nd	1642	1632	1265

nd = non detected (< 0.01 mg dm^{-3}).

Water holding capacity of treatments

The water retention capacity of treatments (soil alone or mixed with CF) was determined by employing the weighting method. For this determination, another set of 64 experimental units (16 treatments with 4 replications), in pots containing 1 dm^{-3} of treatments, was prepared. Then, all those experimental units were weighed, and placed in a tank containing a water level 2 cm higher than the level of soil (or subtracts) surface in the pots. The water entered the pots through small holes done in the pot's bottom. After the contents of pots were completely flooded, pots were removed from the water and left to drain for 12 hours, and weighed again to determine the amount of water retained in each pot. The corresponding volume (cm^3) of water was calculated considering a water density of 1 g cm^{-3} and expressed as cm^3 of water retained per dm^3 of treatments (soil or soil-CF mixtures).

Fertilization, carrot sowing, determination of plant growth and yield variables

All experimental units were fertilized in the exact same way, considering the results of the soil analysis, and following the indications of Raij et al. (1996). Thus, before sowing time, rates equivalent to 20 kg ha^{-1} N, 240 kg ha^{-1} P_2O_5, and 60 kg ha^{-1} K_2O were applied, and after the emergence of plants more 80 kg ha^{-1} N, and 60 kg ha^{-1} K_2O were divided into three parts, and each part applied at 15, 30 and 52 DAE (days after emergence). The sources of nutrients used in the fertilization were NH_4NO_3, $NH_4H_2PO_4$, and KCl.

Carrot seeds (variety Mariana) were sown in four pits per pot, arranging two seeds every 3 cm deep pit, with a 10 cm spacing from each other. The experiment was carried out with a daily manual watering event.

Plants emergence was defined at 15 days after sowing (DAS) when 90% of the plants had emerged. Thinning was performed at 40 DAS, leaving four plants per pot. Subsequently, at 15, 30, 45, 60, 75, and 90 days after plant emergence (DAE) the plant's height was determined. This determination was accomplished by using a scale and measuring the distance from the soil surface to the highest plant top. Carrot plants were harvested at 90 DAE and both their shoot and root (carrots) were separated, so that the length (measured by using a scale, in cm), the largest diameter (measured by using a pachymeter, in mm), and fresh weight (by using a balance with a precision of 0.1 g) were determined. After this first evaluation stage, all samples were placed in an oven dryer with forced air circulation at 65-70 °C and dried until constant weight to determine the dry matter content, by using a balance with a precision of 0.1 g.

Statistical analysis

All data collected in this study were submitted to a variance analysis by the F test, considering a factorial scheme 5×3+1, corresponding to five particle sizes combined with three charcoal fine rates, and one control treatment (soil without CF). For statistical analysis, the initial value of each fraction of particle sizes was considered, namely 1 mm, 2 mm, 4 mm, 8 mm, and 16 mm, respectively for the ranges of 1-2 mm, 2-4 mm, 4-8 mm, 8-16 mm and 16-32 mm. These statistical evaluations were carried out by using the software AgroEstat (Barbosa; Maldonado Junior, 2015). Always the statistical analysis (analysis of variance and F test) indicated independent ($p<0.05$) effects of each factor and subsequent polynomial regressions analysis was performed. Otherwise, when interactions showed a significant ($p<0.05$) effect, statistics involving the surface response of data were used to better interpret results. Complementarily, aiming to observe the similarity among treatments, a multivariate exploratory analysis was made, using the software Statistics 7.0 (Statsoft Inc., 2004). Using the same software, a cluster analysis was carried out by the hierarchical method, to classify the treatments in dendrograms, excluding subgroups (Sneath; Sokal, 1973). The Euclidean distance was used as a similarity coefficient, and Ward's method was used as a clustering strategy. To differentiate treatment groups and their relationship with the studied variables, a multivariate non-hierarchical method (k-means clustering method) was also performed.

RESULTS AND DISCUSSION

The application of charcoal fine (CF) in the soil reduced the bulk density of substrates (mixtures of soil with CF) in comparison to soil alone (control treatment). Soil alone showed a bulk density of 3136 g dm^{-3}, while substrates varied from 2639 to 1265 g dm^{-3}. There was a clear trend of reducing bulk density as the CR rate increased. However, greater densities occurred for intermediate particle sizes within each rate (volumetric proportion) of CF applied to the soil (Tables 1 and 2). These results suggest that intermediate particle sizes (from 2 to 16 mm) have greater densities compared to CF powder (particles < 2 mm) and to gross particles (>16 mm), and/or better incorporation in the soil.

Soil and substrate water retention results indicated a significant ($p < 0.01$) effect of interaction between particle size and CF rate (Table 3). It is noteworthy that absorption of water by the soil without CF (control treatment, represented in the figures by the rate of 0% and particle size of 0 mm, and indicated an arrow) was always lower ($p>0.01$) than that of other treatments (Table 3, Figure 1A). This result indicates that CF addition always improved soil water retention.

Table 3: Analysis of variance (F values) of water holding capacity, and carrot plant shoots height at 30, 45, 60, 75, and 90 days after plants emergence, for treatments (substrates of soil containing distinct rate (R) and particle size (S) of charcoal fine).

Causes of variations	Degrees of freedom	Water holding capacity	Plant height Days after plant emergence (DAE)				
			30	45	60	75	90
Particle size (S)	4	634.99**	9.45**	9.03**	4.24**	7.00**	24.26**
Rate (R)	2	2098.40**	76.29**	107.00**	26.58**	32.08**	86.18**
Interaction (S × R)	8	1002.94**	1.29ns	1.81ns	2.34**	3.54**	6.80**
Factorial	14	1054.31**	14.33**	18.90**	6.34**	8.60**	23.12**
Control × Factorial	1	3003.34**	18.96**	15.60**	0.02ns	14.32**	42.93**
Treatments	15	1184.24**	14.64**	18.68**	5.92**	8.98**	24.45**
Residual variations	48	---------	--------	--------	--------	--------	--------
Total	63	---------	--------	--------	--------	--------	--------
CV (%)	-----	1.12	19.06	12.64	8.14	5.10	4.25

Significance of the F test: ** = p < 0.01, * = p < 0.05, ns = p > 0.05; CV (%) = coefficient of variation.

The water retention in substrates, determined just after substrates preparation, was dependent on the interaction rate × particle size (Table 3), with the highest values for substrates containing about 50% and CF particles from 2-4 mm to 8-16 mm (Figure 1A), but plants development do not always respond directly to this variable (Figure 1B-1F). Carrot plants at the initial development stage (height evaluated until 45 DAE) responded to both rate and particle size in an independent ($p>0.05$) way (Table 3) and did not follow the same trend of initial water retention data (Figure 1A-1C). However, from 60 DAE to 90 DAE (Figure 1D-1F) plant height tended to fit data observed for water retention (Figure 1A). It is known that CF particles do not have good contact with the soil body just after application, but increase their interaction with soil particles along the cultivation time (Souchie et al., 2011). This initial lack of close contact between soil and CF particles may explain why plant height showed no significant ($p>0.05$) effect of interaction size × rate until 45 DAE, but a significant ($p<0.01$) effect afterward (Table 3). Also, if there is a delay in suitable contact between CF and soil, all chemical effects assigned to biochar, like improving soil fertility traits (Deluca et al., 2006, Gaskin et al., 2010; Agegnehu et al., 2015, Ronix et al., 2021), increasing soil water holding capacity (Basso et al., 2013), reducing the bioavailability and phytotoxicity of heavy metals (Park et al., 2011), as well as the increasing the microbial activity in the soil (Deluca et al., 2006) among other effects, also would be delayed and, consequently, also on plant growth delay. This delay seems to be responsible for changes in plant height from 30 DAE to 90 DAE, in this research (Figures 1B to 1F).

Both studied factors (rates and particle size of CF) affected carrot plants height ($p<0.01$) at 30, 45, 60, 75, and 90 DAE (Table 3). However, the interaction between these factors was not significant ($p>0.05$) until 45 DAE, showing that up to this period, CF rates and particle size had independent actions on plant height. After 60 DAE, the effect of rates varied with changes in particle size (Table 3). At 30 DAE, the control treatment displayed higher plant growth in comparison to treatments containing CF (Figure 1B), but this behavior changed over time. The treatments containing CF became more and more effective, in comparison to the control (Figure 1C-E), in such a way that at the last evaluation (90 DAE) control plants had the lowest height among all treatments (Figure 1F). In this last evaluation, the particle size of 2-4 mm enabled the plants to display their best performance, while plants cultivated with the other fractions displayed intermediate growth (Figure 1F). Substrates containing 50% and 75% of CF did not induce a high growth of plants during the initial development stages (Figure 1B-C), even though these treatments resulted in the best plant height at 90 DAE (Figure 1F).

The rate of CF applied to the soil was the only factor that led to a significant effect on carrot roots length (Rle) and roots diameter (Rdi). While other measured variables depended on the interactions of both studied factors (Table 4). When analyzing the effect of CF rate on Rle and Rdi, a quadratic response was observed, with maximum values occurring at rates of 44.5% and 45.6%, respectively (Figure 2A-B). The control treatment showed an average of 12.6 cm for root length, which is significantly ($p<0.01$) higher than the factorial data (within the range of 10.25 cm to 11,13 cm) (Table 4, Figure 2A). Nevertheless, plants subjected to control treatment presented no significant ($p>0.05$) difference in roots diameter compared to those containing CF (Table 4). Except for Rle and Rdi, a significant interaction between rate and particle size ($p < 0.05$) occurred for all other variables determined at 90 DAP (Table 4, Figure 3).

Concerning the fresh weight of the plant shoots (Figure 3A) and roots (Figure 3B), and the dry weight of plant shoots (Figure 3C) and plant roots (Figure 3D), plants cultivated with 2-4 mm CF, and at the rates of 45%-50% showed the highest performances.

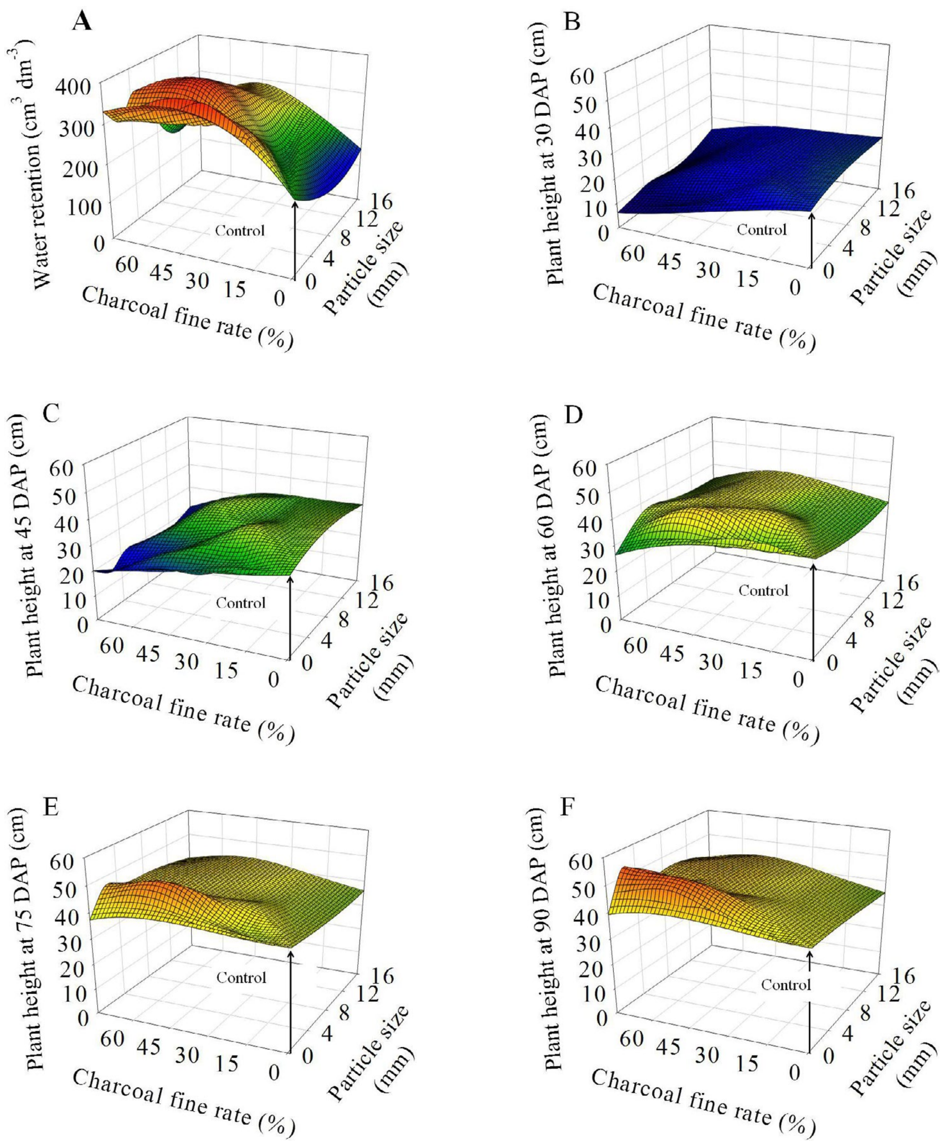

Figure 1: Effects of interaction between rate and particle size of charcoal fine on (A) soil and substrates (soil + charcoal fine) water retention; and plant height at 30 DAE (B); 45 DAE (C); 60 DAE (D); 75 DAE (E) and 90 DAE (F); cultivated in a red argisol, with charcoal fine rates of 25%(v/v), 50%(v/v) and 75%(v/v) and particle size of 1 (1-2 mm), 2 (2-4 mm), 4 (4-8 mm), 8 (8-16 mm) and 16 (16-32 mm). Control = soil without charcoal fine; DAE = days after plants emergence.

Table 4: Results of analysis of variance (F test) for roots length (Rle), roots diameter (Rdi), shoot fresh weight (Sfw), roots fresh weight (Rfw), shoots dry weight (Sdw) and roots dry weight (Rdw), measured at 90 days after plants emergence.

Causes of variation	Degree of freedom	F values of the variance analysis for each variable					
		Rle	Rdi	Sfw	Rfw	Sdw	Rdw
Particle size (S)	4	1.20 ns	1.67 ns	34.04**	7.03**	19.37**	4.99**
Rate (R)	2	3.40*	31.31**	68.03**	38.79**	45.43**	43.38**
Interaction (S×R)	8	2.09 ns	1.64 ns	8.41**	2.87**	5.85**	2.47*
Factorial	1	2.02*	5.89**	24.25**	9.19**	15.37**	9.03**
Control × Factorial	14	11.52**	0.01ns	50.09**	1.06 ns	24.18**	0.00 ns
Treatments	15	2.66**	5.50**	25.97**	8.65**	15.95**	8.43**
Residual variations	48	--------	--------	--------	--------	--------	--------
Total	63	--------	--------	--------	--------	--------	--------
CV (%)	--------	9.86	8.06	14.46	15.72	19.57	16.37

Significance of the F test: ** = p < 0.01; * = p < 0.05; ns = p > 0.05; CV = coefficient of variation.

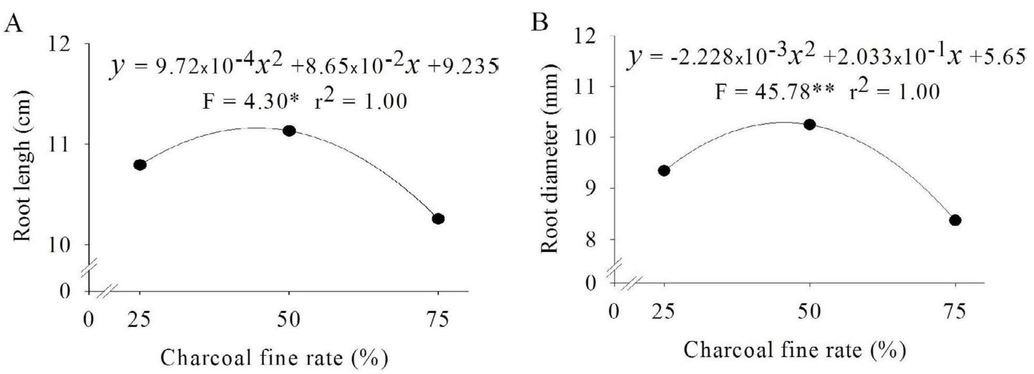

Figure 2: Mean effects of charcoal fine rates on roots length (A) and root diameter (B) of carrot plants cultivated in a red argisol with rates of 25%(v/v), 50%(v/v), and 75%(v/v) of charcoal fine.

About the fresh weight of shoots (Sfw) and roots (Rfw) and dry weight of shoots (Sdw) and roots (Rdw), the highest performances of plants were verified with the rate of 50% of CF, whilst at the application rates of 25% and 75% plants displayed similar results in comparison to the control, with significantly lower values than the rate of 50% (Table 4 and Figure 3).

The obtained data were also submitted to exploratory multivariate analysis, with the aim of better understanding the structure of results by analyzing the similarity among treatments to which plants were subjected (Figure 4).

Through a hierarchical approach (cluster analysis), treatments were classified in a dendrogram composed of excluding subgroups, which allowed the observation of the similarity between treatments. By analyzing data grouped as a function of treatments, it was observed the formation of three major groups (at a linkage distance of 8), as shown in Figure 5. It is noteworthy that the treatment 2/50 (combination between particle size of 2-4 mm applied at a rate of 50%) appeared on the far left (blue rectangle), in an opposite position compared to the group containing the control (marked with a red rectangle). This means that treatment 2/50 induced a very distinct effect in comparison to treatments located within the group including the control, while other treatments (in the black rectangle) showed intermediate effects (Figure 4).

To better understand what justified the separation of treatments into those groups shown in Figure 4, a non-hierarchical cluster analysis was performed, using the K-means method, and this method separated the treatments into five groups (Figure 5). Results indicated that the treatment 2/50 (blue line) showed the highest values for plant shoot and carrots (roots), both fresh and dry weight, while treatments including the control (red line) presented the lowest values for these variables, which explains why these treatments were in opposite positions in Figure 5.

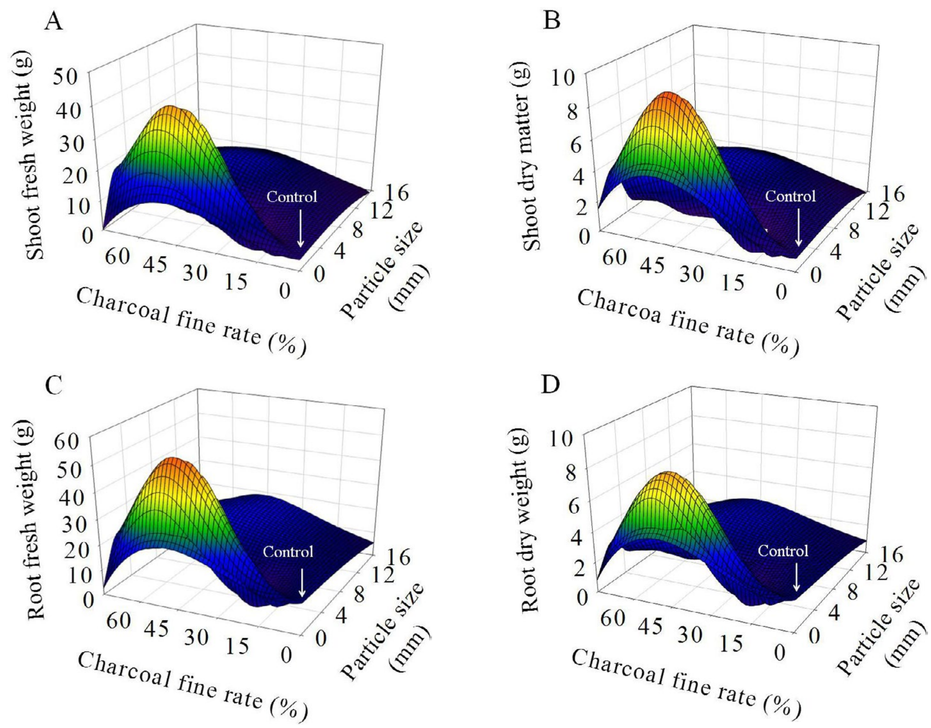

Figure 3: Effects of interaction between rate and particle size of charcoal fine on biometric variables: shoot fresh weight (A); shoot dry weight (B); root fresh weight (C), and root dry weight (D) of carrot plants cultivated in a clayey soil, with charcoal fine rates of 25%(v/v), 50%(v/v) and 75%(v/v) and particle size of 1 (1-2 mm), 2 (2-4mm), 4 (4-8 mm), 8 (8-16 mm) and 16 (16-32 mm). Control = soil without charcoal fine.

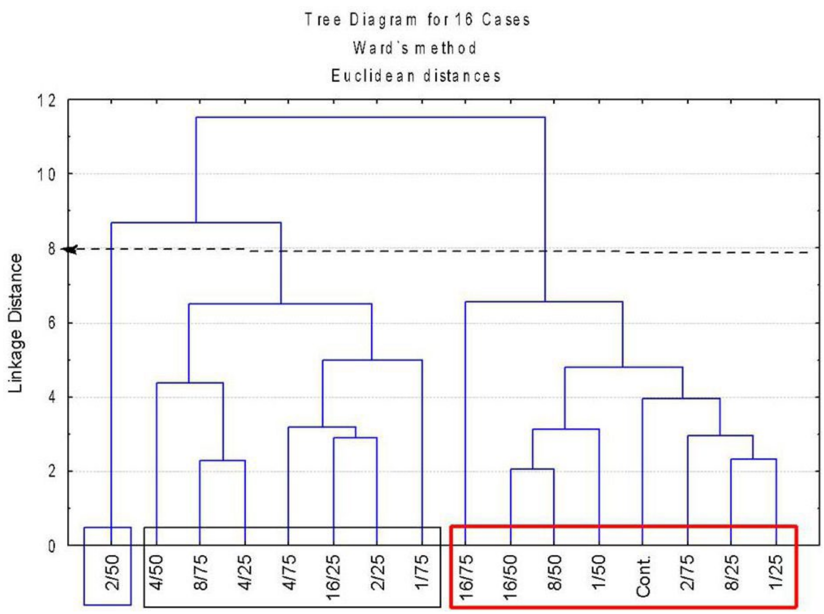

Figure 4: Dendrogram obtained by the multivariate analysis of hierarchic grouping (cluster analysis) of data by treatments, characterized by combinations of particle size (mm) / charcoal fine rate (%, in volumetric basis) added to a heavy clayey soil. The closer the proximity, the greater the similarity between groups.

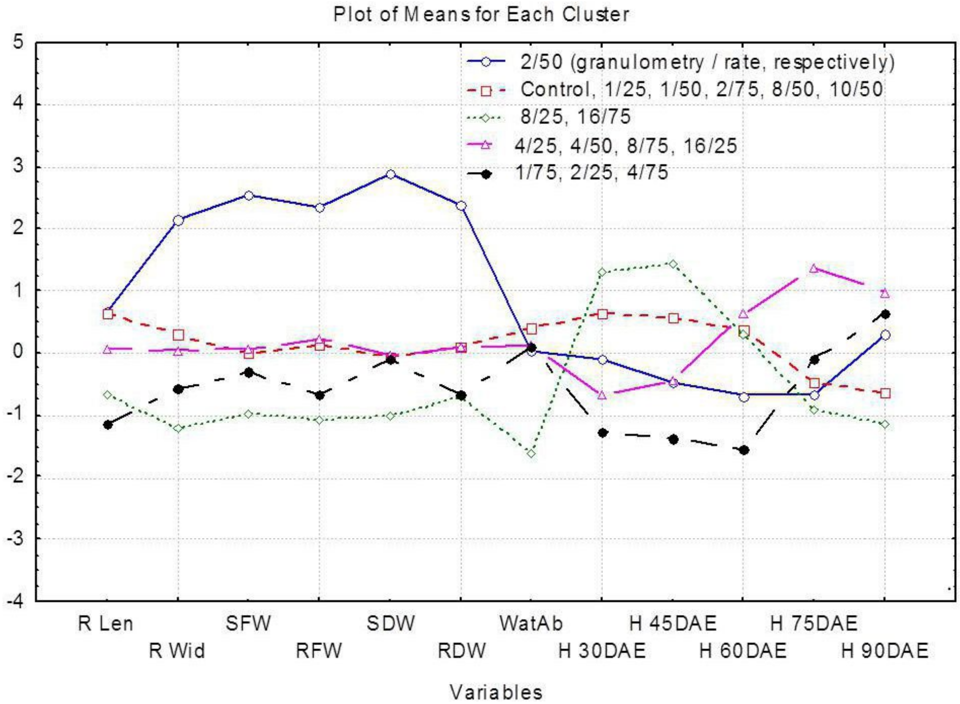

Figure 5: Multivariate cluster analysis made by the non-hierarchical method (K-means), using data from treatments (distinct combinations between particle size (mm) / rate (%, in volumetric basis) of charcoal fine added to the soil), and responses of the following variables: Rle (root length), Rdi (root diameter), Sfw (shoot fresh weight), Sdw (shoot dry weight), Rfw (root fresh weight), Rdw (root dry weight), Wab (soil or substrates water absorption), H-30 to H-90 (plants height measured at 30 to 90 days after plants emergence). Values plotted on the zero line correspond to the average of that variable, while values plotted on positive or negative lines correspond, respectively, to values above or below the average of that variable.

Although data of variables measured at the beginning of the assay (substrates water retention), and the intermediate plant growth stages (plant height from 30 DAE to 75 DAE) are important to understanding factors affecting culture development, the final plant growth (fresh and dry weight of plant shoot), and especially carrot production (root fresh and dry weight) at harvest time (90 DAE) are determinant variables to define what is the best dose and particle size to produce carrots in clayey soil. The final plant growth and carrot production depended on the interaction between CF rate and particle size (Table 4). Then, surface data analysis was developed to better understand the effects of this interaction. Results suggest that the best rate for carrot cultivation in clayey soil varied from 48% to 52% depending on the analyzed variable (Figure 3). While analyzing data in figure 3 some doubt may remain on what is the best CF particle size, results of cluster analysis (Figure 4) highlighted the best performance of treatment 2/50 (CF particle size of 2-4 mm, combined with a rate of 50%). Also, the results of the K-means analysis pointed out that this treatment showed differentiated performance mainly due to having induced values for plant shoot and roots above average (Figure 5). These results agree partially with the previous report mentioning that the best fraction of CF should be smaller than 8 mm, for better plant growth (Zanetti et al., 2003). By analyzing the chemical characteristics of CF (Table 2) it became clear that CF particle sizes of 2-4 mm showed the highest values for electrical conductivity, pH, and concentration of P, Ca, Mg, S, and Mn, compared to other particle sizes fractions. Then, this suggests that this CF fraction has the highest potential to release available nutrients in the soil, which would help explain the better growth and development of carrot plants in substrates containing this CF fraction (Figures 2-3). Because the amount of fertilizers added to each pot was exactly the same, then the good responses of plants cultured on the substrate with 2-4 mm particle size may be more related to the substrate water holding capacity and chemical changes, than physical alterations. This statement meets support in other studies reporting that biochar increases soil N, P, K, Ca, and Mg contents (Freitas et al., 2016; Agbede, 2021), and improves soil fertility (Gaskin et al., 2010; Agegnehu et al., 2015, Ronix et al., 2021), increases the nutrient use efficiency (Alburquerque et al., 2013), and balanced the ion charges in the soil (Major et al., 2010), besides increase in biological soil activity (Wang et al., 2012; Zhu et al., 2018). In this sense, the control treatment may have shown lower production of carrots measured at 90 DAE due to lower water retention (Figure 1A), and due to no additional nutrient intake, and no increase in nutrient use efficiency (Alburquerque et al., 2013), among other benefits.

Besides carrot production is most commonly measured by its fresh weight harvested, sometimes the quality is evaluated by carrots length and diameter, although the preference on specific carrots dimension may vary depending on consumer, or industry use. Different from other measured variables, carrot (root) length, and diameter, responded only to CF rates (Table 4). Once the clayey soil used in this research presented a high bulk density (Table 1), by increasing the CF rate from 25% to 75% in the soil a clear trend of reducing substrate (mixture of soil + CF) bulk density, was observed (Table 2). So, considering that no other variable depended exclusively on CF rate (Table 3-4), and that bulk density was directly related to CF rate, then it is reasonable to believe that carrot length and diameter depended only on the substrate bulk density. The highest values of carrot length and diameter occurred at CF rates of 44,5%, and 45,6%, respectively, and rates above or below these values led to lower carrot dimensions (Figure 2A-B). Thus, the highest carrot dimensions were obtained at rates lower than that suitable for the best plant, and root weight production. The high bulk density of this clayey soil (control) or substrates with CF rates lower than 45%(v/v) should have induced mechanical impedance to carrot growth (Adekiya et al., 2019, Agbede, 2021), while rates higher than 45% may have limited carrot growth due to reduction on the substrate holding water capacity (figure 1A). Souchie et al. (2011) studied a compost made of a blend between a red-yellow latosol of clayey texture and washed sand (3:1), with increasing rates of plant biochar, and evaluated the development of Tachigali vulgaris plants, and also observed good results for biochar rate of 50%. They verified that the rate of 50% reduced the senescence of seedling's leaves throughout the dry season, and improved plant height, stem diameter, roots and shoot dry matter, which agrees with the results found in our research. In the study of Petter and Madari (2012), it was found that biochar rates above 30%, blended with a commercial substrate, started to harm the development of Eucalyptus citriodora and E. urophylla, whilst a rate of 60% of biochar may have caused an induced deficiency of nitrogen, possibly due to the high C/N ratio found in the studied substrate. However, in our study, only rates above 50% harmed plant growth (Figures 1-3), and above 45% started to impair carrot root dimensions (Figure 2). The discrepancy between results may be due to distinct species and substrate used by Peter and Madari (2012), compared to the heavy clayey soil and carrot used in this study. Therefore, it seems that in this study, having only the clayey soil, a higher rate of CF was needed to provide similar results in comparison to mixtures with less dense substrates.

The origin of the material (Santos et al., 2013), and the carbonization temperature (Petter; Madari, 2012) used in charcoal production, among other factors, may determine the chemical and physical characteristics of obtained CF, or biochar. In this sense, not every material of pyrogenic origin is the same (Agbede, 2021) or leads to similar kinds of soil and plant responses, which justifies differences in results obtained by different research endeavors, which are sometimes contrasting. Because of that, there are risks of unfavorable responses to crop productivity when biochar is inappropriately used (Mukherjee; Law, 2014; Brtnicky et al., 2021). Considering that multiple variables related to soil, plant, and CF are involved in plant production on soil amended with CF (Adekiya et al., 2019; Agbede, 2021), further studies should be carried out with CF to improve the knowledge for better predict the responses of other plant species, and other soil types when facing the use of CF residue for soil amendment.

CONCLUSIONS

Findings of this research revealed that the highest production (fresh, and dry weight) of carrots cultivated in a heavy clayey soil was achieved with CF applied at a volumetric rate of 50% in particle size of 2-4 mm. The greatest carrot length and diameter was obtained at CF rates about 45%. Substrates containing CF delayed initial plant growth till 45 DAE, but increased plant development after 75 DAE, and improved plant, and carrot production at harvest time (90 DAE).

AUTHOR CONTRIBUTION

Conceptual Idea: Mendonça, A.R.; Cazetta, J.O.; Methodology design: Mendonça, A.R.; Cazetta, J.O.; Data collection: Mendonça, A.R.; Cazetta, J.O.; Data analysis and interpretation: Mendonça, A.R.; Cazetta, J.O.; Gonçalves, P.W.B., and Writing and editing: Mendonça, A.R.; Cazetta, J.O.; Gonçalves, P.W.B.

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