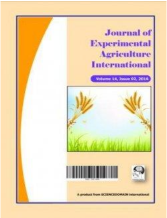

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Substrate Stabilization Using Humus with Tannery Sludge in Conilon Coffee Seedlings

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Authors' contributions

This work was carried out in collaboration with all authors. The authors SSB and APCGB designed the study, performed the statistical analysis and wrote the article. Authors LM and RAS performed the evaluation of developmental characteristics in the field, submitted the manuscript and were responsible for the corrections of the reviewers. The authors TMF, FAMMAF, WPR and SJF performed the physiological evaluations and wrote the physiological part in the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to evaluate the capacity to stabilize substrate with tannery sludge and soil in the development and physiology of Conilon coffee seedlings. The treatments consisted of fixed doses of tannery sludge (30% of volume) and different proportions of humus in the

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substrate (10, 20, 30, 40% and of humus), in addition to treatments with and without conventional soil fertilizer. The biometric and physiological evaluations were measured 210 days after planting the cuttings. Humus allowed the substrate to stabilize, causing the coffee seedlings to develop well, when compared to the conventional substrate, especially in the proportion of 30% of humus, with a higher Dickson quality index in coffee seedlings. Regarding the physiological aspects, there were no changes in the secondary compound indexes or in the nitrogen balance and leaf chlorophyll. Also, there was no damage to the photosynthetic apparatus evaluated by the quantum efficiency of Photosystem II. Treatment 6 composed of 100% of soil, presented the lowest mean of net photosynthetic rate, due to stomatal effects.

Keywords: Coffea canephora; substrate; stabilization; Dickson quality index; photosynthetic photons flux.

1. INTRODUCTION

Seedling production of different species, such as vegetables, forestry and fruit, provide a constant demand for raw materials necessary for substrates compositions, which can vary strongly according to the availability of each region [1,2]. Therefore, many regions should consider substrate components that are more readily available for low-cost agricultural use, such as tannery sludge.

Tannery sludge is a leather waste, which has the high potential for agricultural use due to a large amount of organic matter and essential nutrients for plants [3]. Several studies with different crops such as coffee, passion fruit and other forest species have shown the benefits of using tannery sludge as an alternative fertilizer or substrate component of plants [4-7].

Tannery sludge has a number of advantages when used as a substrate in plants, such as essential nutrients available for plants. However, there are restrictions on its use, as in the case of chromium and sodium, which are added in the leather processing and tanning in almost all Brazilian tanneries [8]. Since, tannery sludge has sodium and chromium, considerations regarding plant physiology, salinity and toxicity should be done; plant response to salinity is linked to the stomatal movement, directly affecting the secondary compounds production, for example, anthocyanins [9-11]. Depending on the species, increased salinity may reduce stomatal aperture by restricting CO_2 in chloroplasts and consequently photosynthetic assimilation [12,13].

The excess of chromium in the leaf tissue can alter the chloroplast ultrastructure, which has an underdeveloped lamellar system with widely spaced thylakoids and less granum, and it may affect the functioning of the photosynthetic apparatus [14,15]. Studies on the functioning of the photosynthetic apparatus related to the use of tannery sludge in plant substrates can show if chromium and sodium are affecting the plant development and the functioning of photosynthesis, as well as the production of secondary compounds, such as anthocyanins and flavonoids due to stresses underwent by the seedlings.

Sludge stabilization, such as tannery is normally required prior to using them as a fertilizer or organic matter sources, since some plants do not withstand the high loads of dissociated elements or the salinization caused by them, for example, coffee [16]. Humus has physical, chemical and biological characteristics capable of stabilizing tannery sludge [17,18].

Thus, due to the great importance that the coffee crop has for Brazilian agribusiness, which is the largest producer and exporter of grains in the world with a record harvest in 2016 of 56.1 million bags [19,20]. The objective of this study was to evaluate the substrate stabilization capacity associated with tannery sludge and soil in the development and physiology of Conilon coffee seedlings.

2. MATERIALS AND METHODS

The experiment was carried out at the Federal Institute of Education, Science and Technology of Espírito Santo - Campus Itapina, located in the municipality of Colatina, in the northwestern region of Espírito Santo, with geographic coordinates of latitude 19° 32 '22 "south latitude; 40° 37 '50 "west longitude and 71 meters altitude. The average maximum and minimum temperatures of the region are 19 and 31°C respectively. The experiment was carried out in a greenhouse with coffee seedlings propagation, using a randomized block design with six treatments and twelve replicates, with 17 seedlings per experimental plot for each treatment.

The treatments consisted of four different concentrations of humus from cow manure, dehydrated tannery sludge and subsoil, according to Table 1.

The genotype used for this study was the Conilon coffee Vitória Incaper 8142. This variety has 13 clones, however, for the purpose of the experiment, only the clone V8 was tested. The soil used for the substrate mixtures with the treatments is classified as a dystrophic red latosol [21] and its characteristics are described in Table 2.

Tannery sludge was provided by the company Capixaba Couros LTDA ME. After processing raw bovine leather, the sludge was released in a liquid-concentrated form and it was placed in evaporation tanks until it reached a humidity of approximately 13.8% (dry basis). The characteristics of dehydrated sludge and humus used in this experiment are presented in Tables 3 and 4.

The humus was obtained from the decomposition of cow manure obtained from animals confined in pens of the Federal Institute of Education, Science and Technology of Espírito Santo - Campus Itapina.

The seedlings were produced from cuttings obtained from the mature tissue of orthotropic branches, which were removed from crops with adequate phytosanitary and nutritional aspect. After removing the branches of the mother plants, they were sent to the greenhouse where 30 cm of orthotropic branch ends were eliminated. The cuttings were 6 to 8 cm high, with 1/3 of leaf blade, plagiotropic branches and above the insertion of the pair of leaves with 1 cm. The cuttings were planted in polyethylene bags with dimensions of 11x20 cm filled with the substrate 30 days before planting. The experiment was irrigated daily using a micro sprinkler, maintaining the field capacity of the substrates.

Two hundred and ten days after planting the cuttings, the seedlings were evaluated: number of leaves, seedling height (using a graduated ruler), stem diameter (using a digital caliper) and crown diameter of seedlings (using a graded ruler). To measure the stem diameter, the branch buds were considered, as well as the number of leaves, fresh shoot mass (FSM), fresh root mass (FRM) and total fresh mass (TFM), which were placed in a forced circulation oven at 70°C. The dry shoot mass (DSM), dry root mass (DRM) and total dry mass (TDM) were obtained. The Dickson quality index of seedlings [22] was calculated by the equation, DQI = [(DRM + DSM)/(Height/stem diameter + DSM/DRM)].

The aerial part of the seedlings was analyzed with a Multiplex (Force-A) fluorometer, with multiple sources of light excitation (ultraviolet, blue, green and red), estimating indexes of several compounds, such as total chlorophyll (SFR-G and SFR-R), anthocyanin (ANT-RG and ANT-RB) and flavonoids (FLAV). The indexes obtained by Multiplex (non-parameterized data) have more than one per characteristic, from the different combinations of wavelengths emitted by the equipment. All evaluations were performed in the morning between 8:00 and 11:00 a.m., evaluating only one side of the seedlings. To collect the data, the equipment was pointed to the top of the canopy at an angle of approximately 45 degrees.

Table 1. Treatment description containing different proportions of humus, tannery sludge, subsoil and conventional substrate fertilization

Treatment	Substrate component
H-10	10% of humus+ 60% of subsoil+ 30% of tannery sludge;
H-20	20% of humus+ 50% of subsoil+ 30% of tannery sludge;
H-30	30% of humus+ 40% of subsoil+ 30% of tannery sludge;
H-40	40% of humus+ 30% of subsoil+ 30% of tannery sludge;
T-C	144 liters of red subsoil; 625 g of P_2O_5; 200 g of limes; 200 g KCl; 18 liters of humus from cow manure.
T-T	100% of subsoil

Table 2. Soil chemical characteristics used as substrate component of seedlings

pH	P	K	Ca	Mg	Al	Na	C	O.M	SB	T	t	AS	BS	Fe	Cu	Zn	Mn	S	B
	-mg/dm³-		-----cmol$_c$/dm³-----				%	g/dm³	--cmol$_c$/dm³--			%		-------------mg/dm³-------------					
5	5	48	0.8	1.3	0	0.03	0.47	8.1	2.3	3.1	2.3	0	74	7	0.6	0.8	7.9	112	0.4

Obs: SB: sum base; AS: aluminum saturation; BS: base saturation, T: CEC a pH 7; t: effective CEC

Table 3. Characteristics of the dehydrated cow tannery sludge used in seedling substrate

pH	N	P_2O_5	K	Ca	Mg	C	Cr	Na	E.C	Fe	Cu	Zn	Mn
	-----------------------------------%--------------------------								dS/m	---------mg/dm^3--------			
12.30	3.7	0.20	0.08	2.70	0.1	0.93	4	0.80	17.30	57	1	1	1

Obs: E.C: electrical conductivity

Table 4. Chemical characteristics of humus used in seedling substrate

pH	M.O	Rem-P	K	Ca	Mg	Fe	Cu	Zn	Mn	AS	BS
	-------mg/dm^3-------			-----------------mmol/dm^3-------------						-----%----	
6.8	202	90	201	93.1	146	57	1	1	1	1.9	82

Obs: O.M: Organic matter; Rem- P: Remaining phosphorus; aluminum saturation; BS: base saturation; AS: aluminum saturation

The chlorophyll a fluorescence was measured using a fluorometer (Pocket PEA, Hansatech, King's Lynn, UK) in third pair of fully expanded leaves, which were placed in the dark for 30 minutes using forceps (Hansatech) to ensure that all "reaction centers" were "open" (quinone A oxidized) [23]. Parameters such as initial fluorescence (Fo), maximum photochemical efficiency (Fv/Fo), quantum yield of PSII (Fv/Fm) and photosynthetic index (PI) were used.

The gas exchanges (photosynthesis, stomatal conductance, transpiration and internal CO2 concentration) were measured using a portable infrared gas analyzer (model LI-6400, Li-COR, Lincoln, USA). They were measured at 10:00 a.m when sky was clear, with an air vapor pressure deficit of 2.44 ± 0.07. Photosynthetic photons flux of 1500μmol m^{-2} s^{-1} was used for this evaluation. Gas exchanges were evaluated in the same leaves used to measure the chlorophyll a fluorescence.

Statistical analyzes were performed with the aid of the Assistat program (version 7.7). All the characteristics were evaluated by the F test and the Scott-Knott test ($p < 0.05$) was used when significant differences were observed between the treatments. The degrees of freedom for the fresh mass and total dry mass were studied via regression analysis. The regressions were selected according to the level of significance (R^2).

3. RESULTS AND DISCUSSION

The use of humus promoted a high stabilization of the tannery sludge mixed with the substrate, therefore being favorable in the Conilon coffee seedlings production when compared to the conventional substrate. This result is considerably different from [5,10] when losses in the development and physiology of Conilon coffee seedlings were observed when using only the tannery sludge associated with the soil as a substrate.

Despite the stabilization provided by the humus, applying different concentrations of humus in the substrate promoted different development patterns, interfering in the Conilon coffee seedlings growth (Table 5). Furthermore, no statistical differences were observed for the crown diameter and number of leaves between conventional treatment and treatments that add humus and sludge in the substrate. For plant height and stem diameter, treatment with only 10% of humus in the substrate was inferior to the seedlings developed in conventional substrates, showing minimum values for the use of sludge with humus as a substrate of coffee plants.

In all the growth characteristics observed in Table 5, it was possible to observe that the humus and sludge in the substrate of Conilon coffee plants promoted growth gains when compared to the treatment that did not have fertilization, humus or tannery sludge (T-T).

The Dickson quality index of seedlings (DQI) in Table 6 shows that the best proportion tested between humus, tannery sludge and soil mixtures were obtained in the H-30 treatment, being higher than the other treatments. It is also possible to observe that all the treatments that received mixtures of sludge, humus and soil, presented seedling quality superior to the conventional treatment, even without chemical fertilizer. However, in Table 6, it is clear that the fresh mass and shoot dry mass of conventional treatment was superior to all the other treatments, showing the efficiency of chemical fertilization in the tissue development in plants, which may be due to the immediate availability of nutrients added by chemical fertilizers, even though it received slightly more than 12 % of humus in its composition. The DQI also reveals that adding humus to 30% of the substrate volume did not promote gain in seed quality.

In the root development, the best result was the treatment with 30% of humus in the substrate, with tannery sludge and soil, which may be due to a high proportion of humus in the substrate, conditioning the soil better and stabilizing the tannery sludge [24]. In addition, the dry or fresh mass of the conventional treatment did not differ from other sludge treatments, showing that humus, sludge and soil can promote root mass gain equivalent to conventional substrates. Studies done by Berilli et al. [4] using tannery sludge in Conilon coffee seedlings, without adding humus in the substrate, showed that the dry root mass was not affected by the sludge, and did not differ from the conventional treatment. Therefore, the use of humus stabilized the substrate and promoted the use of sludge as a component of this substrate.

The gravimetric and seedling quality data presented in Table 6, show that all the variables presented an increase of mass and quality with the increase of humus in the substrate, even in the treatment with 30% of the humus in the substrate. This can be better observed in Fig. 1, where a quadratic regression was estimated for the fresh and total dry mass of the plant, due to the humus in the substrate. Therefore, it was estimated that the maximum point, before where the curve fell for fresh shoot mass, was 38.7% of humus in the substrate, with a maximum weight of 12.1g per plant. On the other hand, for the total dry mass, the inflection point occurred with 31% of humus in the substrate with a maximum weight of 4.5g on per plant. This proportion estimated by the regression analysis for dry mass gain in the plant is very close to the H-30 treatment tested in this experiment, indicating a close to the ideal ratio for the use of these components in the substrate of Conilon coffee plants.

Table 5. Mean values of seedling height, number of leaves, crown and stem diameter of Conilon coffee seedlings grown in conventional substrate and with different proportions of humus (10; 20; 30 and 40%) with dehydrated tannery sludge and soil, 210 days after planting the cuttings

Treatment	Seedling height (mm)	Number of leaves	Crown diameter (mm)	Stem diameter (mm)
H-10	140 b	7.6 a	224 a	3.04 b
H-20	195 a	8.2 a	225 a	3.44 a
H-30	179 a	8.5 a	235 a	3.23 b
H-40	174 a	8.4 a	256 a	3.41 a
T-C	207 a	9.1 a	234 a	3.62 a
T-T	83 c	5.7 b	152 b	2.47 c
Mean	163	7.9	221	3.20
CV (%)	20.9	14.3	19.4	11.0

Mean values followed by the same letter do not differ statistically from each other by the Scott-Knott test at the 5% probability level

Table 6. Mean values of the Dickson quality index (DQI), fresh and dry shoot mass (FSM and DSM), fresh and dry root mass (FRM and DRM) of Conilon coffee seedlings grown in conventional substrate and with different ratios of humus (10; 20; 30 and 40%) with dehydrated tannery sludge and soil at 210 days after planting the cuttings

Treatment	DQI	FSM	DSM	FRM	DRM
		------------------------- g	--------------------------------		
H-10	0.44 b	5.8 c	1.8 c	2.2 b	0.78 b
H-20	0.40b	7.4 c	2.3 c	2.5 b	0.85 b
H-30	0.70 a	9.4 b	3.1 b	4.1 a	1.34 a
H-40	0.49b	9.0 b	2.8 b	2.8 b	0.95 b
T-C	0.29 c	12.6 a	4.0 a	2.2 b	0.83 b
T-T	0.24 c	3.2 d	1.0 d	1.1 c	0.34 c
Mean	0.42	7.9	2.5	2.5	0.85
CV (%)	43.2	34.9	39.7	40.1	41.3

Mean values followed by the same letter do not differ statistically from each other by the Scott-Knott test at the 5% probability level

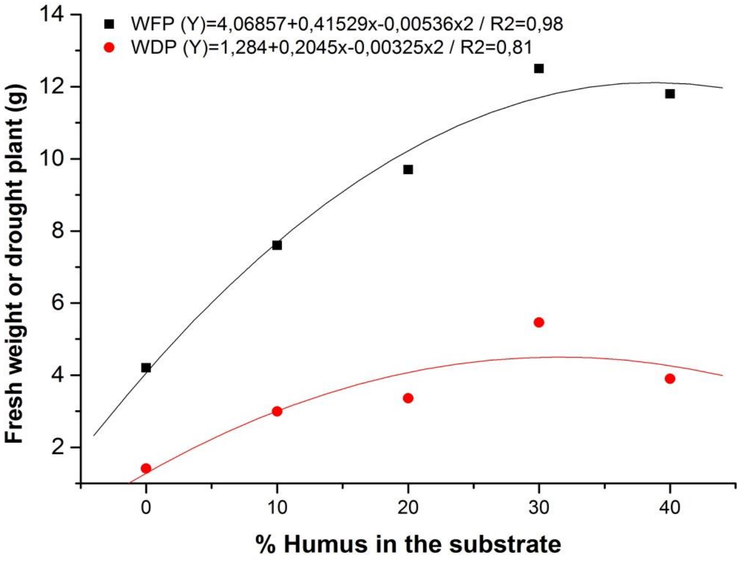

Fig. 1. Fresh and dry mass gain of Conilon coffee seedlings due to the percentage of humus in the substrate containing tannery sludge and soil

The inflection point of the dry mass shown in Fig. 1, close to 30% of humus in the substrate, may be related to a greater assimilation of chromium by the plant, since roots in the presence of organic acids allow chromium to enter the plant better and consequently, provide a greater accumulation of this element in the tissues. This can compromise its physiology and development, and the chloroplasts ultrastructure and the functioning of the photosynthetic apparatus [14,15,25].

Tannery sludge used in this experiment has a high chromium shell and this element in the tissues at high concentrations can damage the photosynthetic apparatus and influence flavonoid indexes [10,14,25] (Table 7). The results showed that the values of flavonoids and anthocyanins were stable in all treatments, showing low interference in the secondary compounds production due to the stabilization of the tannery sludge with the addition of humus to the substrate (Table 7).

Some plants are tolerant to heavy metals, controlling the absorption, accumulation and translocation of heavy metals in the tissues [26]. For coffee seedlings, chromium is an element with low mobility for the aerial part in relation to the amount of chromium allocated in the roots tissues and may contribute to a better functioning of the photosynthetic apparatus [5].

Table 7. Flavonoid indexes (FLAV), chlorophyll content (SFR-G and SFR-R), anthocyanin (ANT-RG and ANT-RB) and nitrogen balance (NBI-G and NBI-R), obtained using the multiplex equipment in Conilon coffee leaves grown in conventional substrates and with different concentrations of dehydrated tannery sludge at 210 days

Treatment	FLAV	SFR-G	SFR-R	ANT-RG	ANT-RB
H-10	0.399 a	3750 a	3561 a	0.313 a	-0.160 a
H-20	0.354 a	3966 a	3760 a	0.288 a	-0.180 a
H-30	0.373 a	3596 a	3456 a	0.316 a	-0.162 a
H-40	0.355 a	3945 a	3748 a	0.307 a	-0.158 a
T-C	0.366 a	3971 a	3781 a	0.316 a	-0.150 a
T-T	0.231 a	3176 b	3066 b	0.320 a	-0.188 a
Mean	0.346	3734	3562	0.310	-0.166
CV (%)	15.6	16.0	14.5	11.5	28.0

Mean values followed by the same letter do not differ statistically from each other by the Scott-Knott test at the 5% probability level

It was also possible to observe that the total chlorophyll index (SFR-G and SFR-R) measured by the fluorometer (Multiplex®) only differentiated the T-T substrate with lower indexes, revealing that the treatments with mixtures of humus, sludge and soil promoted indexes similar to those obtained in seedlings produced in conventional substrates.

The plants cultivated in a substrate composed of 100% soil (T-6) presented lower values of photosynthetic rate and the other treatments presented similar values in the control (Fig. 2), when evaluating the gas exchange. However, there were no significant differences between the treatments for stomatal conductance, transpiration and internal CO_2 concentration, although there was a trend towards lower conductance and transpiration values in treatment 6 (Fig. 2).

The values of the gas exchange obtained in this study were similar to the values obtained in other studies [27,28]. There were no differences between the treatments for chlorophyll a fluorescence parameters (Fig. 3), which indicated a good function of the electron transport system. Fv/Fm values between 0.75 and 0.80 are considered appropriate because they reflect good electron transport efficiency [23]. The photosynthetic performance of plants grown in substrate containing humus, sludge and soil, including plants grown on conventional substrates, do not seem to be related to the function of electron transport systems.

The Fv/Fo ratio is commonly used in studies of different species with different types of stress [29]. When studying gas exchange and chlorophyll fluorescence in six coffee cultivars under aluminum stress, Konrad et al. [30] found that the Fv/Fo ratio did not differ significantly between the six cultivars evaluated. It was observed that the mixture of humus with tannery sludge was positive, since no abnormal physiological behavior was detected in this characteristic (Fig. 3B), such as the stress caused by chromium and sodium elements in the present study.

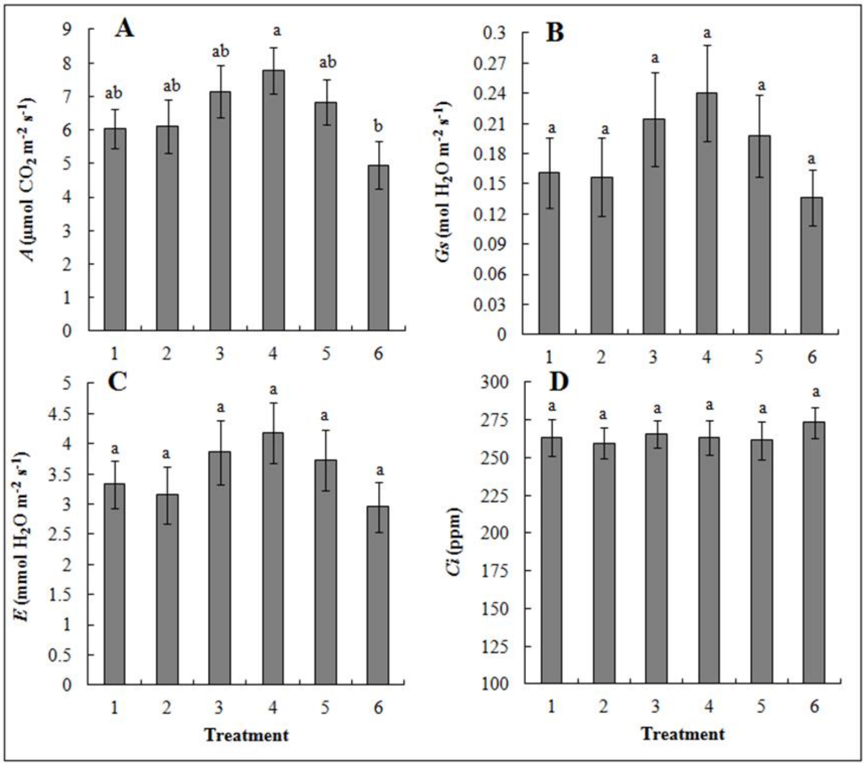

Fig. 2. Photosynthetic rate (A), stomatal conductance (B), transpiration (C) and internal CO_2 concentration (D) of Conilon coffee plants grown in substrates with different concentrations of tannery sludge and humus

Mean values followed by the same letter do not differ statistically from each other by the Scott-Knott test at the 5% probability level

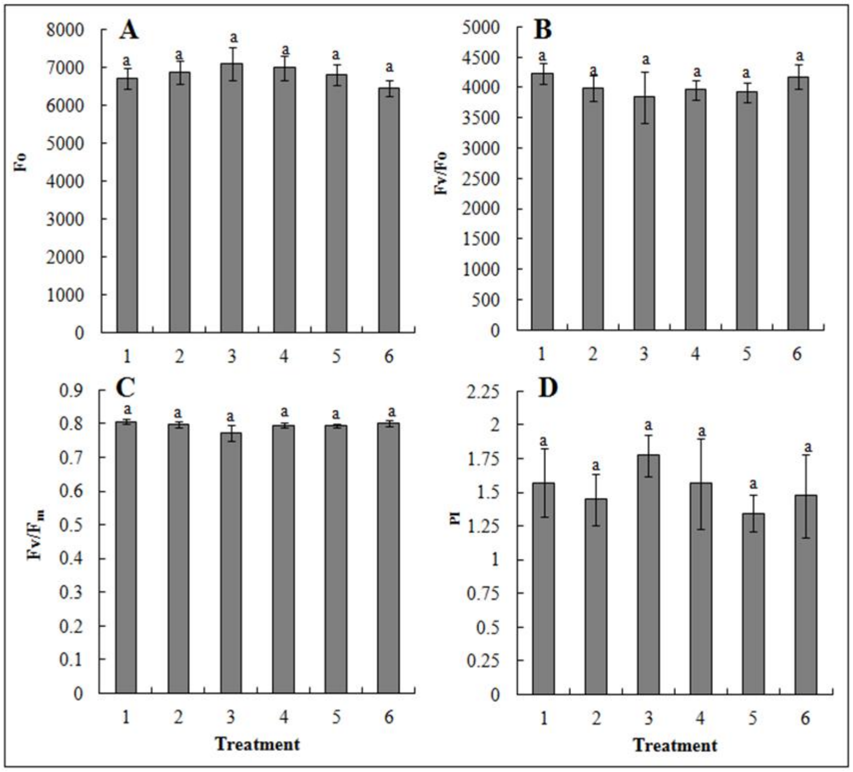

Fig. 3. Initial fluorescence (A), maximum photochemical efficiency (B), maximum quantum yield of PSII (C) and photosynthetic index (D) of Conilon seedlings grown in substrates with different concentrations of tannery sludge and humus

Mean values followed by the same letter do not differ statistically from each other by the Scott-Knott test at the 5% probability level

In general, the results presented in this study show the importance of adding humus to the substrate with the use of dehydrated tannery sludge. The data indicated that humus stabilized different substrates, since the tannery sludge has elements that in excess can cause problems in the plant growth, such as chromium and sodium, even though it is rich in nutrients [8,10], making the plants aggressive, and mixing with the humus can reduce these deleterious effects by stabilizing the substrate. Also, humus favored better nutrient distribution, substrate aeration and increased the soil microbiota, due to the peculiar characteristics of this component, including high cation and ion exchange capacity, slow availability of nutrients and regulation of the pH [17,18]. These results showed that Conilon coffee had better plants and physiological development, even with tannery sludge, showing that there is a potential for its use since it stabilized the substrate and reduced organic waste and environmental pollution.

4. CONCLUSION

The humus is an appropriate conditioner to use together with tannery sludge in the substrate for the Conilon coffee seedlings production.

The best proportion of humus observed in this experiment was 30%, in the mixture with 30% of tannery sludge and 40% of soil, presenting better seedling quality, no deleterious effects of chromium and sodium in the seedlings growth and physiology.

There were no significant differences in relation to the secondary compounds indexes or total chlorophyll of the leaves, between the treatment with a conventional substrate and the use of sludge mixed with soil and humus.

There were no significant differences between the treatments for the gas exchange variables, expect for the substrate composed of 100% soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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