Original Article

INFLUENCE OF TANNERY WASTEWATER SLUDGE DOSES ON BIOMETRIC AND CHLOROPHYLL FLUORESCENCE PARAMETERS IN CONILON COFFEE

INFLUÊNCIA DE DOSES DE LODO DE CURTUME NOS PARÂMETROS BIOMÉTRICOS E DE FLUORESCÊNCIA DA CLOROFILA EM CAFÉ CONILON

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ABSTRACT: Tannery wastewater sludge is an industrial residue that can be used in the pure form or associated to other residues, such as byproduct production from cultivated plants. The objective of the present study was to assess substrate composition for producing conilon coffee stem cuttings, varying increasing tannery wastewater sludge doses. A randomized block design was used with four treatments, three increasing doses of tannery wastewater sludge and one conventional treatment. The stem cuttings were assessed for parameters related to photosystem II functioning and biometry. The results indicated that substrates consisting of tannery wastewater sludge, humus and subsoil promote, in general, improvement in plant growth compared to conventional substrates, highlighting the Dickson quality index. Both chlorophyll fluorescence parameters and chlorophyll contents estimates were not affected by using tannery wastewater sludge in the mixture to formulate substrate for conilon coffee stem cuttings.

KEYWORDS: *Coffea canephora*. Sustainability. Chlorophyll contents. Heavy metals

INTRODUCTION

Tannery wastewater sludge has been used in Brazil as soil corrective or as an alternative nutrient source for plants, mainly because of its high reducing power and wealth of nutrients, such as calcium, nitrogen, magnesium and sulfur and therefore among other forms of use, it is an alternative source for plant substrate composition (POSSATO et al., 2014; BERILLI et al., 2014; BERILLI et al., 2018).

In spite of the beneficial potential of tannery wastewater sludge for use in substrate for plants, the pure mixture of dried tannery wastewater sludge and soil does not present good results, as shown in studies by Berilli et al. (2014). These authors observed that this mixture used as substrate for conilon coffee stem cuttings was inefficient compared to conventional fertilization, which uses cured cattle manure as organic fertilizer source.

Unsuccessful use of tannery wastewater sludge mixed with soil and without other associated organic matter sources may be related to the presence of high sodium and chrome contents, which are added in leather processing and curing. High contents of these elements may disturb the osmotic balance of the plants and alter the chloroplast structure and, in turn, damage the photosynthetic apparatus (FERREIRA et al., 2003; SHANKER et al., 2005).

Factors such as those presented previously showed that the addition of a further component is necessary in order to use this residue as substrate component to improve the chemical, physical and biological conditions of the root environment, for example humus, which has peculiar characteristics that act on these three critical points (MEDEIROS et al., 2015). With this, is expected that humus added to other substrate compounds works as a stabilizer, and can reduce or even cancel the phytotoxic effects that may come from other substrate components, as is the case of tannery wastewater sludge. This effect already occurs in the natural environment, where the high interactive capacity of humus with soil particles promotes stable conditions and improves soil fertility (BALDOTTO; VELLOSO, 2014).

Chlorophyll fluorescence analysis gives an accurate assessment of the photosynthetic potential, because qualitative and quantitative information can be obtained on the physiological condition of the photosynthesis apparatus (FALQUETO et al., 2007; THOREN et al., 2010). Furthermore, chlorophyll

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fluorescence can be affected by several types of stress, including nutrient lack or excess (STRASSER et al., 2004). It has been possible from chlorophyll fluorescence measurements to characterize, quantify and detect plant stress before the symptoms are visible in the leaves (CHRISTEN et al., 2007).

Bearing in mind the need for advances in studies on residue use, the objective of the present study was to assess the influence of increasing tannery wastewater sludge doses in substrate formulation for conilon coffee stem cuttings on both growth and chlorophyll fluorescence parameters.

MATERIAL AND METHODS

The experiment was carried out at the Espírito Santo Federal Education, Science and Technology Institute (IFES) - Itapina Campus, located in the municipality of Colatina, Northwestern Espírito Santo state, Brazil, geographic coordinates 19° 32' 22" latitude south; 40° 37' 50" longitude west, 71 meters altitude and mean maximum and minimum temperatures of 31 and 19 ^{0}C, respectively. The experiment was carried out in a coffee plant propagation nursery in a randomized block design with four treatments and 12 replications, considering 17 stem cuttings per replication for each treatment.

The genotype used for this study was the conilon coffee variety, clone V8, Vitória Incaper 8142 according to Fonseca et al. (2004). Four treatments were established: T-10 - 10% tannery wastewater sludge + 30% humus + 60% soil; T-20 - 20% tannery wastewater sludge + 30% humus +50% soil; T-40 - 40% tannery wastewater sludge + 30% humus + 30% soil and; T-C - 144L soil, 625g simple superphosphate, 200g lime, 200g KCl and 18L humus.

The soil used for the substrate mixtures with the tannery wastewater sludge treatments and conventional treatment is classified as a dystrophic red latossoil. Dried tannery wastewater sludge was obtained after processing raw cattle hide, in the liquid concentrate form with 97% moisture (dry base). The liquid tannery wastewater sludge was placed in evaporation tanks until it reached approximately 13.8% moisture (dry base). The humus used in the treatment and substrate mixtures was obtained from the IFES-Itapina vermiculture center, prepared with cattle manure. Table 1 shows the characteristics of the soil, dried tannery wastewater sludge and humus.

Table 1. Chemical characteristics of the soil, tannery wastewater sludge and humus used as substrate components for the stem cuttings

Soil

pH	P	K	Ca	Mg	Al	Na	C	M.O.	SB	T	t	m	V	Fe	Cu	Zn	Mn	S	B
	-mg/dm^3-		------cmol$_c$/dm^3-----				%	g/dm3	--cmol$_c$/dm^3--			%		-------------mg/dm^3-------------					
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

Tannery wastewater sludge

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

Humus

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

SB: sum of bases; m: aluminum saturation; V: base saturation; T: CTC at pH 7; t: effective CTC. C.E.: electric conductivity; M.O.: organic material; P-rem: remaining phosphorus; aluminum saturation V: base saturation

The plants were produced from stem cuttings obtained from adult orthotropic branch tissue, taken from plantations with good plant health and nutritional aspect. The branches were removed from the mother plants and taken to a greenhouse, where 30 cm were cut off the ends of the orthotropic branches (branches with several stem cuttings). The stem cuttings were then standardized to 6 cm length and one pair of leaves with one third of the 1/3 of the leaf limbo each. The stem cuttings were planted in 11 x 20 cm (1.9 dm^3) polyethylene bags, previously filled with the substrates. They were irrigated daily, throughout the experiment, by a microsprayer, keeping the substrate always at field capacity.

The experiment lasted 210 days, counted from the stem cutting planting. The stem cuttings were assessed for number of leaves, height (using a graded ruler), stem diameter (using a digital pachymeter), canopy diameter (using a graded ruler) and leaf area (Li-Cor, mod: Li-3100C). The stem diameter was measured considering the branching that emerged from the original stem cutting as were the number of leaves, the canopy (CFM), root (RFM) and total (TFM) fresh matter, that were later taken to a forced air chamber at 70 ^{o}C until they reached constant weight on digital precision scales, and the values were obtained of the canopy (CDM), root (RDM) and total (TDM) dry matter. The Dickson quality index was also calculated (DICKSON et al., 1960), by the formula, IQD = [(RDM + CDM) / (height/stem diameter + CDM/RDM)].

The a chlorophyll fluorescence was measured on the third pair of fully opened leaves using a Pocket fluorometer model PEA (Hansatech Instruments Ltd, United Kingdom). Some parameters were then analyzed such as initial fluorescence (F_o), proportionality of the water oxidation activity in the donor side of the photosystem II (F_v/F_o), maximal quantum yield of photosystem II (F_v/F_m) and the photosynthetic index (PI). For this analysis, part of the sampled leaf was dark-adapted for *ca*. 30-40 min. using leaf clips (Hansatech) to turn the reaction centres into an "open" (oxidised QA) state (BOLHÁR-NORDENKAMPF et al., 1989)..

The chlorophyll content was estimated by a portable chlorophyll meter, SPAD-502 (Minolta, Japan). The determinations, five measurements per plant, were taken on the same leaves that the photochemical efficiency measurements were taken.

Table 2 shows the data for temperature, luminosity and relative humidity monitored in the greenhouse throughout the experiment. The data was submitted to analysis of variance and the means differentiated by the t test at 5% probability. The statistical analyses were made by the Assistat program (version 7.7).

Table 2. Data for maximum minimum temperature, luminosity (accumulated in the day) and relative humidity (RH) of the months relative to the period of applying the experiment in the greenhouse

Month/2015	Temperature Max. (^{0}C)	Temperature Mín. (^{0}C)	Luminosity (Lux)	RH (%)
March	42,2	23,5	5270,2	57,7
April	40,5	15,1	2829,5	75,2
May	40,7	14,0	3279,1	74,8
June	37,0	14,6	1353,2	74,8
July	39,6	13,4	1555,4	71,4
August	43,0	15,7	1926,7	65,2
September	45,0	17,5	2301,4	60,4
October	45,5	19,4	2643,6	61,8
Mean	41,7	16,6	2644,9	67,7

RESULTS AND DISCUSSION

Overall, differences were observed among the treatments for growth characteristics assessed (except for canopy diameter) showing that there was influence from the addition of tannery wastewater sludge at different doses in the substrate, especially when compared to the conventional substrate. In general, conventional treatment values were either equal or less than the treatments with the addition of dried tannery wastewater sludge.

Plant height is one of the main characteristics observed by producers and researchers as well as one of the most relevant visual parameters of development and vigor. In this sense it was observed that the treatments that presented the biggest means for plant height were the treatments with 10% (T-10) and 20% (T-20) tannery wastewater sludge in the substrate (Table 3).

Covre et al. (2013) characterized morphological aspects of stem cuttings of different clones of conilon coffee Vitória, at 210 days after planting the stem cuttings and reported heights ranging from 13 to 24 cm, with an average of 24.02 cm for clone 8 (the same as used in the present experiment). These results show that the values observed in the present experiment for plant height, in all the treatments, are within the normal ranges for the clone tested. However, it was observed that higher doses of dried tannery wastewater sludge in the substrate tended to reduce the plant size. This fact also was observed in other growth traits such as number of leaves, leaf area and canopy and root fresh matter, and also root and total plant dry matter (Tables 3 and 4).

Table 3. Means of plant height, number of leaves, canopy diameter, stem diameter and leaf area of conilon coffee stem cuttings cultivated in conventional substrate and with different concentrations of dried tannery wastewater sludge at 210 days after planting.

Treatment	Plant height (cm)	Number of leaves (und)	Canopy diameter (cm)	Stem diameter (mm)	Leaf area (cm^2)
T-10	23,5 a	8 a	25,53 a	4,16 a	327,33 a
T-20	22,7 a	7 b	24,81 a	3,97 ab	302,61 a
T-40	20,3 b	7 b	24,16 a	3,85 bc	293,54 a
T-C	19,4 b	6 c	24,19 a	3,65 c	244,58 b
Mean	21,5	7,2	24,7	3,9	292,0
CV (%)	12,8	15,2	10,9	8,7	18,1

Means followed by different letters in the column differ statistically by the t test at the level of 1%; CV – coefficient of variation.

The use of tannery wastewater sludge in conilon coffee cutting propagation was first studied by Berilli et al. (2014) but with treatments that differed from the present study by the addition of 30% humus to the substrate, to improve the ryzosphere conditions and stabilize the substrate components. Collectively, these experiments shows that the addition of tannery wastewater sludge and humus to substrate increased the plant growth compared to conventional substrate in most of the characteristics assessed. This fact did not occur in the experiments carried out by Berilli et al. (2014), because substrate contained only tannery wastewater sludge and soil without adding another organic matter source. Therefore, we can infer that the humus contributed for stabilizing the substrate with the use of tannery wastewater sludge and soil.

Table 4. Means of canopy fresh matter and dry matter (MFPA and MSPA), root fresh and dry matter (MFR and MSR), plant total fresh and dry matter (MFT and MST) and Dickson quality index (IQD) of conilon coffee stem cuttings cultivated in conventional substrate and with different concentrations of dried tannery wastewater sludge at 210 days after planting

Treatment	MFPA (g)	MSPA (g)	MFR (g)	MSR (g)	MFT (g)	MST (g)	IQD
T-10	14,7 a	4,1 a	14,7 a	1,185 a	19,8 a	5,3 a	0,579 a
T-20	13,7 ab	3,7 ab	13,7 ab	1,003 a	18,1 a	4,8 ab	0,503 a
T-40	12,8 b	3,6 b	12,8 b	1,004 a	19,2 a	4,6 b	0,517 a
T-C	10,8 c	3,1 c	10,8 c	0,755 b	13,9 b	3,8 c	0,410 b
Mean	13,0	3,6	13,0	0,99	17,8	4,6	0,50
CV (%)	15,8	17,4	15,8	23,5	19,5	17,6	18,9

Means followed by different letters in the column differ statistically by the t test at the level of 1%; CV – coefficient of variation

The capacity to enhance the use of tannery wastewater sludge as an extra nutritional source in conilon coffee plant substrate, promoted by adding humus to the substrate, became clear when the growth traits from the treatments with the addition of tannery wastewater sludge associated to humus were compared with the conventional treatment, that also had 30% humus. There was greater development in the stem cuttings promoted by adding tannery wastewater sludge associated to humus. The characteristics that best represent this synergistic effect of tannery wastewater sludge with humus are leaf area and the Dickson quality index. Stem cuttings cultivated with the addition of tannery wastewater sludge in the substrate show higher values for such variables than conventional substrate (Table 4). The Dickson index is a good reference to indicate plant quality, because it takes into account several growth characteristics (DICKSON et al., 1960).

In addition, root system dry matter (MSR) was also another interesting characteristic that differentiated the stem cuttings cultivated in substrates with the addition of tannery wastewater sludge compared to the conventional substrate, . The stem cuttings with higher mass gains in the root system were cultivated with the addition of tannery wastewater sludge in the substrate. Berilli et al. (2014) observed that many stem cutting growth characteristics were damaged with the addition of pure tannery wastewater sludge associated to subsoil as substrate for conilon coffee plant production, except for the roots, which it was similar to the conventional substrate. Here, the association of tannery wastewater sludge, soil and humus promoted more root system growth in the stem cuttings compared to the traditional substrate, with chemical fertilization, humus and soil (T-C).

During the 210 days of cultivation, the stem cuttings cultivated in conventional substrates presented slower growth rhythm than the stem cuttings cultivated in substrate containing tannery wastewater sludge, soil and humus (Figure 1). This fact was observed for plant height, stem diameter, number of leaves and canopy diameter. It can also be observed that characteristics such as height, stem diameter and number of leaves show exponential growth curves that are more expressive 120 days after stem cutting planting (Figure 1). Results observed by Berilli et al. (2015), who studied the growth of conilon coffee stem cuttings cultivated in substrates with tannery wastewater sludge and conventional substrates, showed that for this same clone, there were linear growth rhythms up to 120 days after stem cutting planting, confirming the results of the present experiment, that exponential plant growth begins after this period.

There was no significant difference between the treatments for any of the fluorescence parameters (Table 5). Further, it was observed that the values were adequate, as for example, the F_v/F_m ratio was close to 0.8 (Figure 1C), which indicates adequate photosystem II functioning (BOLHÀR NORDENKAMPF et al., 1989). The F_v/F_m ratio reflects the maximum photochemical capacity and is related to the number of active PSII complexes.

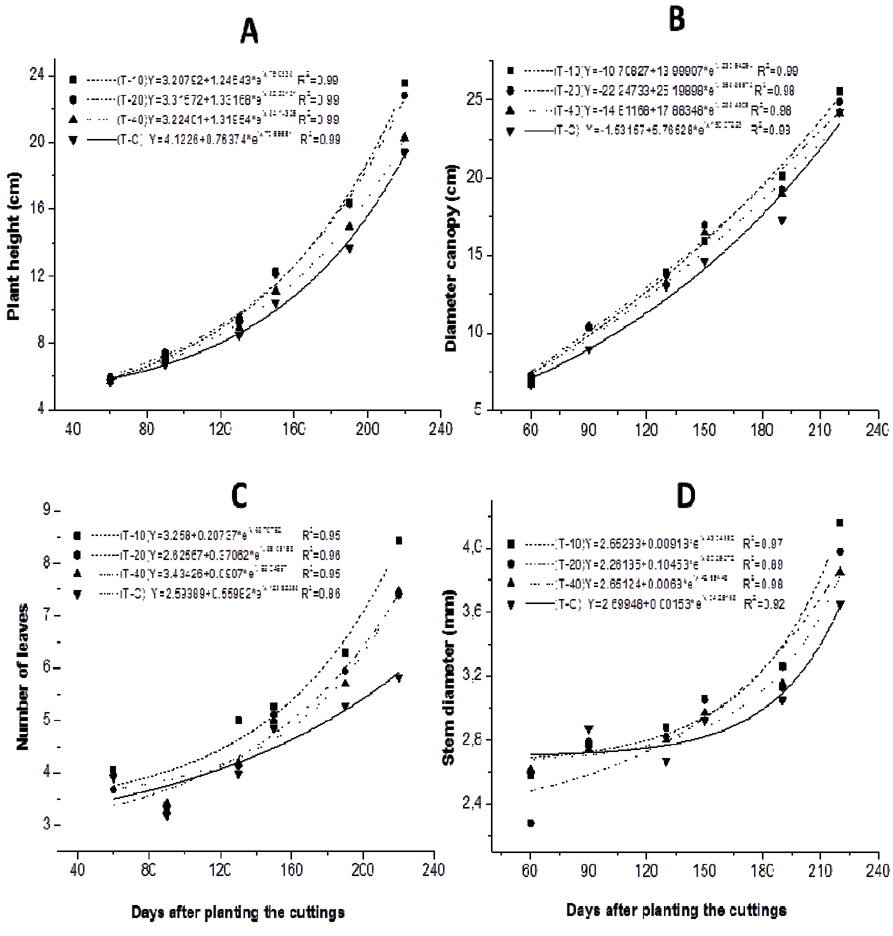

Figure 1. Height (A), canopy diameter (B), number of leaves (C) and stem diameter (D) of conilon coffee stem cuttings, grown in substrates with different tannery wastewater sludge doses (T-10; T-20; T-40) and conventional substrate (T-C), over 210 days post stem cutting planting

The PI values (Table 5) were between 1.18 and 1.64 that also showed good functioning and regulation of plant photosystems. This variable is very efficient in detecting the effects of environmental stress in plants (STRASSER et al., 2004), and is considered more sensitive to the effects of stress than the F_v/F_m ratio due to the combination of three steps of the photosynthetic activity of the PSII reaction centers: the density of the active reaction centers based on the quantity of chlorophyll molecules, that is, the efficiency in light absorption, capacity to reduce quinone *a*, that is related to the activity of the oxygen evolution complex on the PSII donor side (KALAJI et al., 2011); and the electron transport rate that represents luminous energy conversion to electron transport in the PSII, that is, the contribution of biochemical reactions. The high values of the PI variable indicate high efficiency in the photochemical processes reported above (STRASSER et al., 2004). The green intensity measured by the portable chlorophyll meter gave a similar response to the fluorescence parameters (Table 5).

Table 5. Initial fluorescence (A), proportionality of the water oxidation complex activity on the donor side of photosystem II (B), photosystem II maximum quantic yield (C), photosynthetic index (D) and green intensity (E) of conilon coffee stem cuttings grown in substrates with different tannery wastewater sludge and humus concentrations.

Treatment	Fo	Fv/Fo	Fv/Fm	PI	SPAD
T-10	7109 a	3955 a	0.80 a	1.18 a	34.07 a
T-20	7405 a	3938 a	0.79 a	1.64 a	39.66 a
T-40	7587 a	3368 a	0.79 a	1.59 a	38.15 a
T-C	7345 a	3422 a	0.80 a	1.24 a	37.26 a
Mean	7361	3671	0.80	1.41	37.29
CV%	15.49	11.64	2.74	40.70	26.50

Means followed by different letters in the column differ statistically by the t test at 1%; CV – coefficient of variation

Taken all together, it is evident that low tannery wastewater sludge doses associated to humus can contribute to improving conilon coffee stem cutting development, probably due to the large amounts of nutrients essential to the plants (TAVARES et al., 2013). Furthermore, the humus dose used was sufficient to neutralize the heavy metals present in the tannery wastewater sludge, since excessive chrome absorption could damage the chloroplast ultrastructure as well as total chlorophyll and carotenoid contents, affecting electron transport and, in turn, photosynthesis (AGASTIAN et al., 2000; SHANKER et al., 2005). The chlorophyll *a* fluorescence data and green intensity suggest that such damages do not occur (Table 5). Furthermore, the humus may positively influence phosphorus capture and metabolism (ZANDONADI et al., 2013).

Regarding the growth data, the positive phenotypic correlations between plant height and diameter (Table 3) indicate that there was no excess growth in height (etiolation) that could have been caused by low luminous intensity, or also by excess fertilizer (MARANA et al., 2008). Furthermore, the large number of leaves contributed to the greater leaf area, as was expected. Probably the larger leaf area, observed in T-10 compared to T-C, contributed to better initial plant development after planting and could also result in greater initial yields.

Based on the results obtained, tannery wastewater sludge associated to humus presents potential for conilon coffee plant production and could reduce the cost for nurseries located close to slaughterhouses. In addition, it also contributes to reducing organic waste accumulation. However, long-term studies, under field conditions, including development, productivity and grain quality assessments are essential, as well as the assessment of the potential risks of soil heavy metals contamination.

CONCLUSIONS

Tannery wastewater sludge associated to humus and soil promoted greater growth of conilon coffee stem cuttings when compared to conventional substrate. Some relevant characteristics of plant growth, such as height, shoot and root fresh and dry matter were negatively affected by treatment with the highest tannery wastewater sludge dose, revealing a certain limit regarding using this component in preparing substrate for conilon coffee plant production.

Fluorescence parameters and chlorophyll content estimates were not affected by using tannery wastewater sludge in the mixture to formulate conilon coffee plant substrate. In the present experimental conditions, the dose of 10% tannery wastewater sludge was shown to be the most indicated for conilon coffee plant production.

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RESUMO: O lodo de curtume é um resíduo industrial que pode ser aproveitado de forma pura ou associado a outros resíduos, como na produção de substratos de plantas cultivadas. Neste sentido, o objetivo desse trabalho foi avaliar a composição de substrato para a produção de mudas de café conilon, variando doses crescentes de lodo de curtume. O delineamento utilizado foi em blocos casualizados, com quatro tratamentos, sendo três doses crescentes de lodo e um tratamento convencional. As mudas foram avaliadas em relação aos parâmetros relacionados ao funcionamento do fotossistema II e a biometria. Os resultados indicam que substratos compostos por lodo, húmus e terra de subsolo promoveram, em geral, melhoria no crescimento das mudas quando comparados aos substratos convencionais, destacando-se o índice de qualidade de Dickson. Os parâmetros de fluorescência e estimativas do teor de clorofila não foram afetados pela utilização do lodo de curtume na mistura para formulação de substrato de mudas de café conilon.

PALAVRAS-CHAVE: *Coffea canephora*. Sustentabilidade. Teor de clorofila. Metais pesados

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