



# Influence of chromium and sodium on development, physiology, and anatomy of Conilon coffee seedlings

Leonardo Martineli<sup>1</sup> · Sávio da Silva Berilli<sup>2</sup> · Ramon Amaro de Sales<sup>3</sup> · Maura da Cunha<sup>4</sup> · Paola Afonsa Vieira Lo Monaco<sup>5</sup> · Sílvio de Jesus Freitas<sup>4</sup> · Maristella Martineli<sup>6</sup> · Ana Paula Candido Gabriel Berilli<sup>2</sup> · Saulo Pireda<sup>4</sup> · Dhiego da Silva Oliveira<sup>4</sup> · Lucas Louzada Pereira<sup>7</sup>

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## Abstract

Some components found in the composition of the tannery sludge are nutrients for the plants; it can be considered an alternative source of fertilization as they have favorable agronomic characteristics. However, it is reported in some studies that the presence of chromium and sodium in this residue causes physiological and anatomical disturbances that inhibit the development of the plants. The objective of this study was to evaluate the influence of chromium and sodium on the physiology, anatomy, and development of Conilon coffee seedlings grown on substrates produced with tannery sludge and equivalent doses of chromium and sodium. The experiment was carried out in nursery using randomized block design, containing 5 treatments and 7 repetitions. The treatments consisted of the application of a 40% tannery sludge dose and equivalent doses of chromium and sodium mixed with a conventional substrate. Notably, the presence of sodium in the substrate caused greater damage to the plants, negatively influencing the physiology, anatomy, and, consequently, development of the plants, while the presence of chromium suggests that it does not influence much the evaluated characteristics. The treatment with tannery sludge, on the other hand, despite containing the same chromium and sodium contents, revealed a more pronounced negative influence on the physiology, anatomy, and development patterns of the seedlings. This shows that sodium and chromium alone are not the only factors responsible for the lowest growth indicators studied.

**Keywords** *Coffea canéfora* · Chromium · Residue · Sodium · Sludge · Tannery

## Introduction

The management of residues has become an international concern as industrialization and populations continue to increase (Bhat et al. 2018). In this context, studies involving the use of agro-industrial residues in farming have become increasingly common, since these residues have a high amount of organic matter and essential nutrients for plants (Berilli et al. 2018a, b; Quarteza et al. 2018a), and its use can be recommended as a fertilizer and soil conditioner.

Several residues are recommended for farming as they are used as soil improvers, fertilizers, and conditioners, considering the ability of the soil macro- and microbiota

in decomposing organic materials. However, it is necessary to study changes in soil properties and the response of the plants to assess the fertilizing potential of these residues, and the possible contamination of the environment by toxic elements (Ferreira et al. 2003).

Among the various existing residues, the use of tannery sludge in farming has gained notoriety and has been the subject of several studies, especially as a substrate for the production of Conilon coffee seedlings in Brazil (Quarteza et al. 2018b; Sales et al. 2018a; Berilli et al. 2020a). However, such studies revealed a certain degree of toxicity to plants, attributed to chromium and sodium found in this residue, influencing the levels of secondary compounds such as flavonoids and anthocyanins or even the development of seedlings.

Cr is a toxic heavy metal, with negative effects on animal and human health, besides affecting plant growth, production, and metabolic activities (Singh et al. 2021) when present in high amounts. Cr stress is known to generate excess

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✉ Ramon Amaro de Sales  
ramonamarodesales@hotmail.com

Extended author information available on the last page of the article

ROS ( $H_2O_2$  and superoxide) and cause lipid peroxidation (Singh et al. 2020). Lipid peroxidation caused by ROS damages biological membranes by breaking the mitochondrial respiratory chain and makes the membranes vulnerable to oxidative damage, besides acting as a cell death signal, inducing programmed cell death (Su et al. 2019; Singh et al. 2020).

Na is another element that has deleterious effects to plants when present in high amounts in the soil. Salt stress negatively affects growth, development, and production, limiting plant metabolic activities, such as osmotic potential, enzymatic activities, and ionic imbalance (Vinocur & Altman 2005; Khan et al. 2021).

Tannery sludge consists of organic and inorganic materials. A high amount of chromium and sodium is found in the inorganic matter, which is a cause for concern due to the possibility of causing environmental pollution. However, the agronomic use of this residue has been considered an option of utilization as most of them consists of effective organic material in the fertilization and neutralization of acidic soils (Gödecke et al. 2012; Malafaia et al. 2015).

The challenge of this experiment with tannery sludge is to elucidate the role of chromium and sodium as the agent that causes disturbances to plants. Therefore, it is necessary to identify the effects caused by the presence

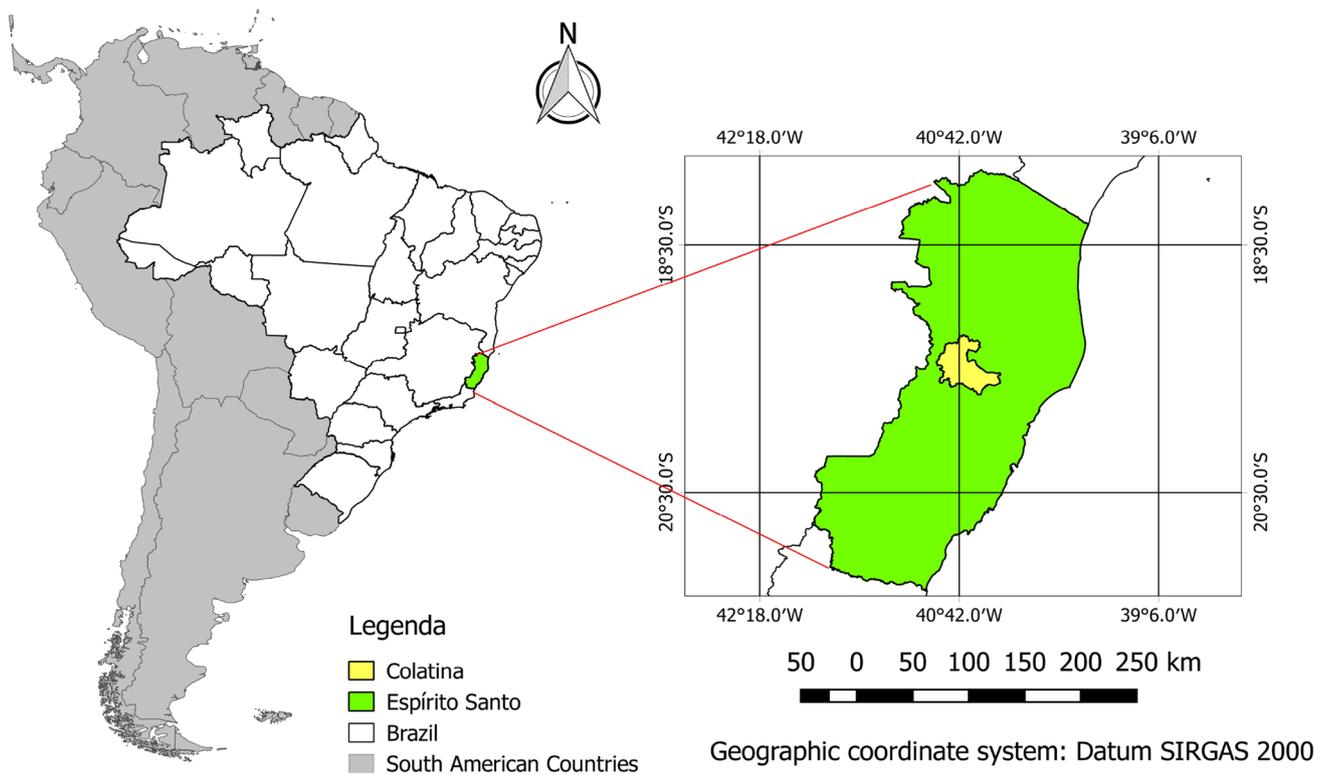
of chromium and sodium, exclusively or in combination in order to reveal the real influence that these elements cause to plants, serving as a basis for further studies on the mitigation of the harmful effects on plant development.

Thus, the objective of this study was to evaluate and to identify the possible effects of chromium and sodium in the dehydrated tannery sludge in relation to the physiology, anatomy, and development of Conilon coffee seedlings (*Coffea canephora*).

## Materials and methods

### Study area location

This study was conducted at the Federal Institute of Education, Science and Technology of Espírito Santo – Itapina Campus, located in the municipality of Colatina, north-western Espírito Santo state, Brazil, within the geographic coordinates  $19^{\circ} 32' 22''$  south latitude and  $40^{\circ} 37' 50''$  west longitude and altitude of 71 m (Fig. 1). The climate in the region is Tropical Aw, according to the Köppen's climate classification, with a well-defined rainy season from October to January and an average climatological rainfall of 1029.9 mm (Alvares et al. 2013; Sales et al. 2018b).



**Fig. 1** Geographic location of Colatina, located in the state of Espírito Santo, Brazil

**Table 1** Description of the evaluated treatments and their respective components

Treatment	Substrate component
CT	100% conventional substrate
CT+Na	100% conventional substrate + 46.5 g sodium
CT+Cr	100% conventional substrate + 390 g Cr <sup>+3</sup>
CT+Na+Cr	100% conventional substrate + 465 g sodium + 390 g Cr <sup>+3</sup>
CT+TS	60% conventional substrate + 40% dehydrated tannery sludge

Where CT, conventional treatment; Na, sodium; Cr, chromium; and TS, tannery sludge

## Experiment design and characterization of the residues

The experiment was carried out in a nursery for the propagation of irrigated Conilon coffee seedlings, in a randomized block design made up by five treatments with different concentrations and mixtures of substrates, seven repetitions, each plot consisting of 10 plants, totaling 50 seedlings per block and a total of 350 seedlings in the experiment. The treatments applied in the experiment are described in Table 1.

The conventional substrate used in the composition of the treatments was a mixture recommended by the Capixaba Institute of Research, Technical Assistance and Rural Extension (INCAPER), for the production of quality Conilon coffee seedlings: for each cubic meter prepared (m<sup>3</sup>), the substrate showed in its composition 75% of sieved subsoil earth, 25% bovine manure, 1.5 kg of dolomitic limestone, 5.0 kg of simple superphosphate, and 0.5 kg of potassium chloride (Ferrão et al. 2012). The sodium and chromium contained in the CT+Na, CT+Cr, and CT+Na+Cr treatments represented the amounts equivalent to a 40% dose of dehydrated tannery sludge, corresponding to the dosage present in the CT+TS treatment. The tannery sludge dose was determined from studies carried out by Berilli et al. (2014), in which they reported physiological losses that weaken the development

of Conilon coffee seedlings, when subjected to a dosage equal to or greater than 40% of tannery sludge in the substrate, which were mainly attributed to the presence of chromium and sodium in the substrate.

The dehydrated tannery sludge used in this study was previously crushed and sieved, and a sample was analyzed to determine the nutritional characteristics and levels of chromium and sodium present in the residue. The results found were as follows: potential of hydrogen, 12.3; nitrogen, 37.0 g dm<sup>-3</sup>; phosphorus, 2.0 g dm<sup>-3</sup>; potassium, 0.8 g dm<sup>-3</sup>; calcium, 27.0 g dm<sup>-3</sup>; magnesium, 1.0 g dm<sup>-3</sup>; carbon, 9.3 g dm<sup>-3</sup>; chromium, 37.0 g dm<sup>-3</sup>; sodium, 4.4 g dm<sup>-3</sup>; electrical conductivity, 17.3 dS m<sup>-1</sup>; iron, 57.0 mg dm<sup>-3</sup>; copper, 1.0 mg dm<sup>-3</sup>; zinc, 1.0 mg dm<sup>-3</sup>; and manganese, 1.0 mg dm<sup>-3</sup>.

Considering that the tannery sludge used in this study had a concentration of 4.4 g dm<sup>-3</sup> of sodium and 37.0 g dm<sup>-3</sup> of chromium in its composition, 46.5 g of sodium was applied to the treatments CT+Na and CT+Na+Cr (in the form of 107.2 g of sodium carbonate Na<sub>2</sub>CO<sub>3</sub>) and 390 g of chromium (in the form of 570 g of trivalent chromium oxide Cr<sub>2</sub>O<sub>3</sub>) to treatments CT+Cr and CT+Na+Cr. These substances were homogenized into the conventional substrate forming the respective treatments. After, they were placed in black polyethylene bags, with a volume of 500 mL, punctured in their lower half, recommended for the production of Conilon coffee seedlings. The treatments were prepared and packed 30 days before planting of the cuttings.

Conilon coffee (*Coffea canephora* Pierre ex Froehner) seedlings from the clonal cultivar Vitória Incaper 8142 (clone V8) were used, produced from cuttings obtained from adult tissue from orthotropic branches. These seedlings were removed from the crops at adequate phytosanitary and nutritional aspect. The recommended procedures for seedling preparation followed the recommendations of Ferrão et al. (2012).

Samples of each treatment were collected at planting and sent to the soil analysis laboratory to determine the nutritional characteristics of each treatment considering the pH levels, macronutrients, and electrical conductivity (Table 2).

**Table 2** Characterization of the mixtures of the treatment substrates used in the experiment regarding some chemical and physical attributes

Treatment	pH	O.M g dm <sup>-3</sup>	rem P mg L <sup>-1</sup>	P —g dm <sup>-3</sup> —	K —g dm <sup>-3</sup> —	Ca —cmol dm <sup>-3</sup> —	Mg —cmol dm <sup>-3</sup> —	Al	E.C dS m <sup>-1</sup>
CT	5.9	16.5	44.0	0.66	0.58	4.3	1.4	0.0	1.98
CT+Na	5.9	16.1	41.0	0.44	0.55	3.7	1.9	0.0	3.84
CT+Cr	6.2	16.7	42.0	0.44	0.52	3.5	2.1	0.0	1.80
CT+Na+Cr	6.2	19.1	41.0	0.50	0.40	3.7	2.1	0.0	3.85
CT+TS	7.8	28.2	21.0	0.37	0.26	36.4	1.0	0.0	4.03

pH potential of hydrogen, O.M. organic matter, remP remaining phosphorus, P phosphorus, K potassium, Ca calcium, Mg magnesium, Al aluminum, E.C. electrical conductivity

## Evaluated characteristics

Evaluations on the development, physiology, and anatomy of the plant were carried out 180 days after planting (DAP). Regarding plant development, the following characteristics were evaluated: plant height (PH, cm), stem diameter (SD, mm), crown diameter (CrD, cm), number of leaves (NF), leaf area (LA, cm<sup>2</sup>), aerial part dry matter (APDM, g), root dry mass (RDM, g), total dry mass (TDM, g), and root length (RoL, cm).

To obtain the height of the aerial part, canopy diameter, and the length of the roots, a millimeter ruler was used. The height of the aerial part was obtained by measuring from the base of the neck to the apical bud that result in the last pair of leaves. For the crown diameter, the distance between the ends of the largest pair of leaves was measured. The stem diameter was obtained with the aid of a precision digital caliper. The leaf area was obtained using a leaf area measuring device (Li-cor®, mod: Li-3100C). The dry mass of the aerial part was obtained by cutting the seedlings close to the substrate. To obtain the root dry mass, the roots were carefully washed in tap running water, using a sieve. Afterwards, the materials were packed in paper bags and placed in an oven with forced air circulation at 65 °C for 72 h. Then, the materials were weighed on a 0.01-g precision electronic scale.

From the aforementioned parameters, it was possible to determine the Dickson's quality index (Dickson et al. 1960) and the leaf area ratio (LAR, cm<sup>2</sup> g<sup>-1</sup>), according to Eqs. 1 and 2.

$$DQI = [TDM/(PH/SD) + (APDM/RoDM)] \quad (1)$$

$$LAR = (LA/TDM) \quad (2)$$

Physiological evaluations were performed using a fluorometer (Force-A®, mod: MULTIPLEX 330), with multiple sources of light excitation (ultraviolet, blue, green, and red), estimating indices of various compounds, such as nitrogen balance (NBI-G and NBI-R), total chlorophyll (SFR-G and SFR-R), anthocyanin (ANTH-RG and ANTH-RB), and

flavonoids (FLAV). The indices obtained by the MULTIPLEX 330 resulted from the different combinations of wavelengths emitted by the equipment, where the indices with the letter "G" are obtained with the green light, the indices with the letter "B" by the blue light, and the indices with the letter "R" by the red light (Sales et al. 2018c). The evaluation with multiplex was carried out in the morning, between 9h00 and 11h00 a.m., in situ, and on only one side of the seedlings, pointing the equipment towards the canopy from top to bottom at an angle of approximately 45 degrees, following the methodology of Berilli et al. (2020b).

The anatomical evaluation was carried out to check possible damage to the cell structure and to determine the thickness of the different groups of cells in the leaves. It is performed by means of optical microscopy (Leica®, mod: DM500). Completely expanded leaves were collected and fixed in the experimentation field, and the subsequent steps took place in a Plant Science Laboratory. The median part of the leaves was fixed in a solution containing 2.5% glutaraldehyde, 4.0% formaldehyde, and 0.05 M cacodylate buffer at pH 7.2 and stored under refrigeration until processing. In the processing, cross-sections of the leaves were made freehand, and then the following parameters were evaluated: leaf thickness (LTCK), adaxial epidermis (AD EP), palisade parenchyma (PALI), lacunous parenchyma (LACU), and abaxial epidermis (AB EP).

## Data analysis

Before the analysis of variance, the attendance of the assumptions of normality of errors and homogeneity of the variances was checked through the Shapiro–Wilk and Bartlett tests, respectively. The data obtained were submitted to analysis of variance by the Scott-Knott test at the level of 5% significance ( $p < 0.05$ ). The entire statistical procedure was performed using the open-source R® software.

**Table 3** Means of the physiological indexes, obtained through a multiplex fluorometer on leaves of Conilon coffee seedlings grown on different substrates, 180 days after planting

Treatment	NBI-G	NBI-R	FLAV	SFR-G	SFR-R	ANTH-RG	ANTH-RB
CT	1.11 a	1.06 a	0.12 b	1.33 a	1.30 a	0.041 a	-0.57 a
CT+Na	0.95 b	0.74 b	0.20 a	1.37 a	1.32 a	0.039 a	-0.58 a
CT+Cr	0.92 b	0.74 b	0.18 a	1.43 a	1.37 a	0.041 a	-0.55 a
CT+Na+Cr	0.96 b	0.87 b	0.17 a	1.41 a	1.36 a	0.042 a	-0.57 a
CT+TS	0.95 b	0.86 b	0.16 a	1.22 b	1.19 b	0.034 a	-0.60 a
CV (%)	11.68	17.42	23.39	8.73	8.28	31.99	4.67

Means followed by the same letter in the column do not differ statistically from each other by the test of Scott-Knott at the 5% level of significance. *FLAV* flavonoids, *SFR-G* and *SFR-R* chlorophyll content, *ANTH-RG* and *ANTH-RB* anthocyanin, *NBI-G* and *NBI-R* nitrogen balance

**Table 4** Morphological parameters in Conilon coffee seedlings submitted to different types of treatments with chromium and sodium, 180 days after planting

Treatment	LN Un	PH ——cm——	CaD	SD mm	LA cm <sup>2</sup>	RL cm
CT	9.72 a	14.31 a	22.54 a	3.48 a	219.45 a	21.97 a
CT+Na	7.86 b	11.09 b	20.86 a	3.06 b	176.68 b	19.25 b
CT+Cr	9.24 a	14.58 a	23.10 a	3.64 a	211.18 a	20.26 a
CT+Na+Cr	7.97 b	12.51 a	22.91 a	3.46 a	187.96 b	19.20 b
CT+TS	5.61 c	8.53 b	17.44 b	2.69 c	108.72 c	17.75 c
CV %	17.64	23.12	11.61	8.87	16.61	10.61

Means followed by the same letter in the column do not differ statistically from each other by the test of Scott-Knott at the 5% level of significance. *LN* leaf number, *PH* plant height, *CaD* canopy diameter, *SD* stem diameter, *LA* leaf area, *RL* root length.

## Results and discussion

The results from the evaluation of several physiological indices of the plants using the multiplex apparatus (Table 3) showed that for the nitrogen balance indices, the treatments with chromium and sodium in their composition were lower than the conventional treatment. This is likely to be related to the inhibitory effect that chromium performs on the main enzymes of N metabolism, such as nitrate reductase, nitrite reductase, glutamine synthetase, glutamate dehydrogenase, and urease (Kumar and Joshi 2008). In addition, salinity increases competition at the root surface between nitrate (NO<sub>3</sub><sup>-</sup>) and chloride (Cl<sup>-</sup>), as well as ammonium (NH<sub>4</sub><sup>+</sup>) and sodium (Na<sup>+</sup>), as observed by Hessini et al. (2019).

On the other hand, it can be seen in the analysis of the phenolic compounds that the treatments that have chromium and sodium in their composition presented higher levels of flavonoids when compared to the conventional one (Table 3). This greater production of flavonoids in the treatments CT+Na, CT+Cr, CT+Na+Cr, and CT+TS may be linked to the defense of plants to the accumulation of the elements chromium and sodium in their compositions (Silva et al. 2015). Chromium toxicity alters the physiology of plants by producing reactive oxygen species (ROS) and causes changes in the absorption of mineral elements, while the accumulation of excess intracellular sodium ions affects the bioenergetic processes of photosynthesis, causing peroxidation of membranes, therefore increasing the flavonoid content as a way to fight against the oxidative stress caused by these elements (Mastalerczuk et al. 2019; Ramos-Sotelo et al. 2019; Din et al. 2020).

Regarding the chlorophyll index shown in Table 3, the treatment containing the tannery sludge (CT+TS) showed the lowest results, being the only one that differed from the conventional treatment. This may be related to the smaller thickness of the palisade and lacunous parenchyma (Table 6). In addition, the high pH of the substrate containing tannery sludge may have reduced the absorption of N by plants. At the same time, these plants had to invest some

of this N, which was already in smaller quantities, in the biosynthesis and metabolic regulation of polyphenols (flavonoids), and some in the primary metabolism, which use the same precursor, the amino acid phenylalanine. As observed by Martines et al. (2010), the tannery sludge applied to the soil can favor the loss of nitrogen caused by ammonia volatilization due to its high pH and the high concentration of ammonium nitrogen.

Although plants produced on conventional substrate with sodium (CT+Na), chromium (CT+Cr), and sodium + chromium (CT+Na+Cr) have had a lower nitrogen balance than the conventional treatment (CT), they managed to invest in chlorophyll likewise. Therefore, it is possible that the lower chlorophyll in the treatment with tannery sludge is the outcome of the lower amount of N and micronutrients available to plants, in response to its high pH value (7.8) (Novais et al. 2007). The activity of microorganisms, as well as the solubility and availability of nutrients are some of the most important processes that depend on pH, as observed by Gentili et al. (2018). Thus, the high pH may have considerably reduced the absorption of various nutrients and reduced the activity of the substrate microbiota.

**Table 5** Morphological parameters in Conilon coffee seedlings submitted to different types of treatments with chromium and sodium, 180 days after planting

Treatment	APDM	RoDM	TDM	DQI	LAR
	——g——				cm <sup>2</sup> g <sup>-1</sup>
CT	3.07 a	0.75 a	3.82 a	0.47 a	57.44 a
CT+Na	2.29 b	0.48 b	2.76 b	0.33 b	64.01 a
CT+Cr	2.88 a	0.69 a	3.57 a	0.44 a	59.15 a
CT+Na+Cr	2.42 b	0.65 a	3.07 b	0.42 a	61.22 a
CT+TS	1.46 c	0.34 b	1.79 c	0.24 c	60.73 a
CV %	19.18	22.16	18.50	17.06	12.82

Means followed by the same letter in the column do not differ statistically from each other by the test of Scott-Knott at the 5% level of significance. *APDM* aerial part dry matter, *RoDM* root dry matter, *TDM* total dry mass, *DQI* Dickinson's quality index, *LAR* leaf area ratio

**Table 6** Anatomical parameters of Conilon coffee leaves, grown in different treatments containing chromium and sodium, 180 days after planting

Treatment	LTCK	AD EP	PALI	LACU	AB EP
	—————µm—————				
CT	204.74 a	19.59 a	48.62 a	115.64 a	15.43 a
CT+Na	192.84 b	18.63 a	42.46 b	113.64 a	14.56 a
CT+Cr	206.57 a	18.75 a	50.56 a	118.51 a	15.52 a
CT+Na+Cr	203.12 a	19.07 a	44.88 b	117.02 a	15.97 a
CT+TS	165.79 c	18.98 a	33.66 c	89.44 b	15.22 a
CV %	4.61	7.54	12.25	8.66	11.13

Means followed by the same letter in the column do not differ statistically from each other by the test of Scott-Knott at the 5% level of significance. *LTCK* leaf thickness, *AD EP* adaxial epidermis, *PALI* palisade parenchyma, *LACU* lacunous parenchyma, *AB EP* abaxial epidermis

In relation to the growth (Table 4) and gravimetric (Table 5) analyses of the seedlings, significant differences were observed between the treatments applied, revealing different response patterns in the plants. As it can be seen in Table 4, for the characteristic number of leaves, leaf area, stem diameter, and root length, all treatments containing sodium in their composition (CT+Na; CT+Na+Cr; CT+TS) showed lower results than the conventional treatment. Therefore, the treatments that contained sodium in their composition had considerable negative effects on the growth of the plants, especially for the CT+TS treatment, which, in addition to sodium and chromium, presented a high pH in the substrate (Table 2), further reducing plant growth.

The leaf area presented reductions of 24% (CT+Na), 17% (CT+Na+Cr), and 100% (CT+TS) when compared to the conventional treatment (CT). As for root length, the reductions were 14%, 14%, and 23% for the treatments CT+Na, CT+Na+Cr, and CT+TS, respectively, when compared to CT.

At the evaluation of the plant height and canopy diameter (Table 4), a reduction was observed in plants treated with tannery sludge when compared to those treated with conventional substrate. The plant height variable in the CT+TS treatment reached 67% less than the conventional treatment.

The leaf area was severely impaired in the CT+TS treatment (108 cm<sup>2</sup>), and as a consequence, there was less intercepted solar radiation, which reduced net photosynthesis and, consequently, carbon assimilation, resulting in smaller plant sizes (Table 4), lower dry matter gains of the aerial part (stem + leaves), root and consequently a lower seedling quality index (DQI), which encompasses all growth variables (Table 5). The leaf area is responsible for intercepting solar radiation and, through the light harvesting complex

and a series of photochemical and biochemical processes, produces photoassimilates for the plant, which will be responsible for maintenance and growth (Sales et al. 2017). In this study, the DQI clearly demonstrates the results found in Tables 4 and 5, showing the best quality indexes for the treatments CT, CT+Cr, and CT+Cr+Na, which ranged from 0.42 to 0.47, followed by the CT+Na treatment with 0.33 and, finally, CT+TS treatment with 0.24.

The different treatments did not cause any differences in the LAR (Table 5). Thus, the net photosynthetic capacity in relation to the increase in the total mass of the plant was not affected. However, when the total carbon gain by plants (TDM) was evaluated, the division of three groups was observed, where the first was formed by CT and CT+Cr, with higher averages. This result reveals that the presence of chromium exclusively in the substrate, in the applied dose, did not affect the carbon accumulation by the plants; in addition, the same was observed for all growth characteristics (Table 4) and evaluated gravimetry (Table 5). The accumulation of chromium in Conilon coffee seedlings occurs mainly in the root tissues, as observed by Berilli et al. (2015). Thus, it is possible to suggest that this greater accumulation in the roots of coffee plants reduces the toxic effects of this element on the photosynthetic organs.

The second group, made up by the treatments CT+Na and CT+Na+Cr, showed results with lower values than the first group (CT and CT+Cr) and greater than the third group, formed by the treatment with tannery sludge (CT+TS). This fact shows that the presence of sodium or the combination of sodium+chromium in the substrate in the applied proportion inhibited the normal growth of the plants. The adverse effects induced by salinity and stress by metals in the development of plants involve biochemical disturbances particularly caused by oxidative damage (Monteiro et al. 2011). Excess of sodium in the soil competes with Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, and other cations, reducing the availability of these essential elements for crops. Therefore, soils with high levels of exchangeable sodium (Na<sup>+</sup>) may affect plant growth by dispersing soil particles, deficiencies, nutritional imbalances, and specific toxicity for sodium-sensitive plants, reducing plant growth (Machado and Serralheiro 2017).

However, for the characteristics of plant height, stem diameter, root dry matter, and quality of the seedling, the combination sodium+chromium (CT+Na+Cr) showed better results than the treatment with sodium (CT+Na). Thus, it is assumed that there is a positive influence related to the presence of the chromium element in the substrate, which reduces the negative effects of sodium on the development of Conilon coffee seedlings, either by stimulating growth or even by inhibiting sodium absorption.

Although there is no conclusive evidence for the essentiality of chromium in plant metabolism, some studies show that small additions of this element have

stimulating effects on plant growth (Zayed and Terry 2003; Paiva et al. 2009). These reports help to explain the better performance of the CT + Cr + Na treatment in relation to the CT + Na. However, there was no evidence of the recommended levels or even tolerance of this element for Conilon coffee seedlings, revealing the need for further studies.

For the treatment containing tannery sludge in its composition (CT + TS), a lower response pattern of the plants is observed in all evaluated characteristics when compared to conventional treatment (Tables 4 and 5). Similar results have been reported by Berilli et al. (2014) when studying different doses of dehydrated tannery sludge in the substrate composition of Conilon coffee seedlings.

It is suggested in this study that the negative results of CT + TS treatment are related to some parameters found in the analysis of the substrates (Table 2), notably, the high electrical conductivity value ( $4.03 \text{ dS m}^{-1}$ ), associated with the low potassium concentration ( $0.26 \text{ g dm}^{-3}$ ), high pH value (7.8), and the harmful effects of sodium. The electrical conductivity values found in the experiment reveal a saline substrate (Novais et al. 2007). Salinity (excessive accumulation of toxic ions sodium and chloride ions) reduces the capacity of the plant in absorbing water, causes disturbances in homeostasis and ion toxicity in the soil, and competes with the absorption and metabolism of essential mineral nutrients for plants, such as phosphorus, potassium, nitrogen, and calcium (Parihar et al. 2015; Machado and Serralheiro 2017; Alam et al. 2021). Due to the low potassium content in the chemical analysis of the CT + TS treatment (Table 2), associated with the salinity and the high pH of the substrate, it can be inferred that potassium has become a limiting factor to the growth of these plants.

Kämpf (2005) described the pH values in water in nine distinct bands, ranging from extremely low ( $< 4.5$ ) to extremely high ( $> 6.9$ ), whereas in substrates, the pH range considered ideal for plants varies between 5.2 and 5.5. In this case, the high pH found in the substrate (7.8) may have impaired the absorption of several other nutrients, both macro- and micronutrients (Novais et al. 2007). According to Martinez (2004), the indirect effect of pH is related to the solubility of nutrients, and at pH higher than 6.0, precipitation may occur, which is no longer available to plants, mainly nitrogen, which is closely linked to the formation of chlorophyll molecules. Jiang et al. (2017) report that many characteristics of plants, such as height, lateral propagation, biomass, size and number of flowers, and pollen production, among others, are influenced by the pH value.

Regarding the anatomical characteristics of the leaf evaluated in this study (Table 6), no differences were observed in the thickness of the epidermis on the abaxial and adaxial surfaces. However, a reduction in leaf thickness was observed in the treatment containing tannery sludge (CT + TS) and in

the treatment containing sodium (CT + Na) when compared to conventional (CT).

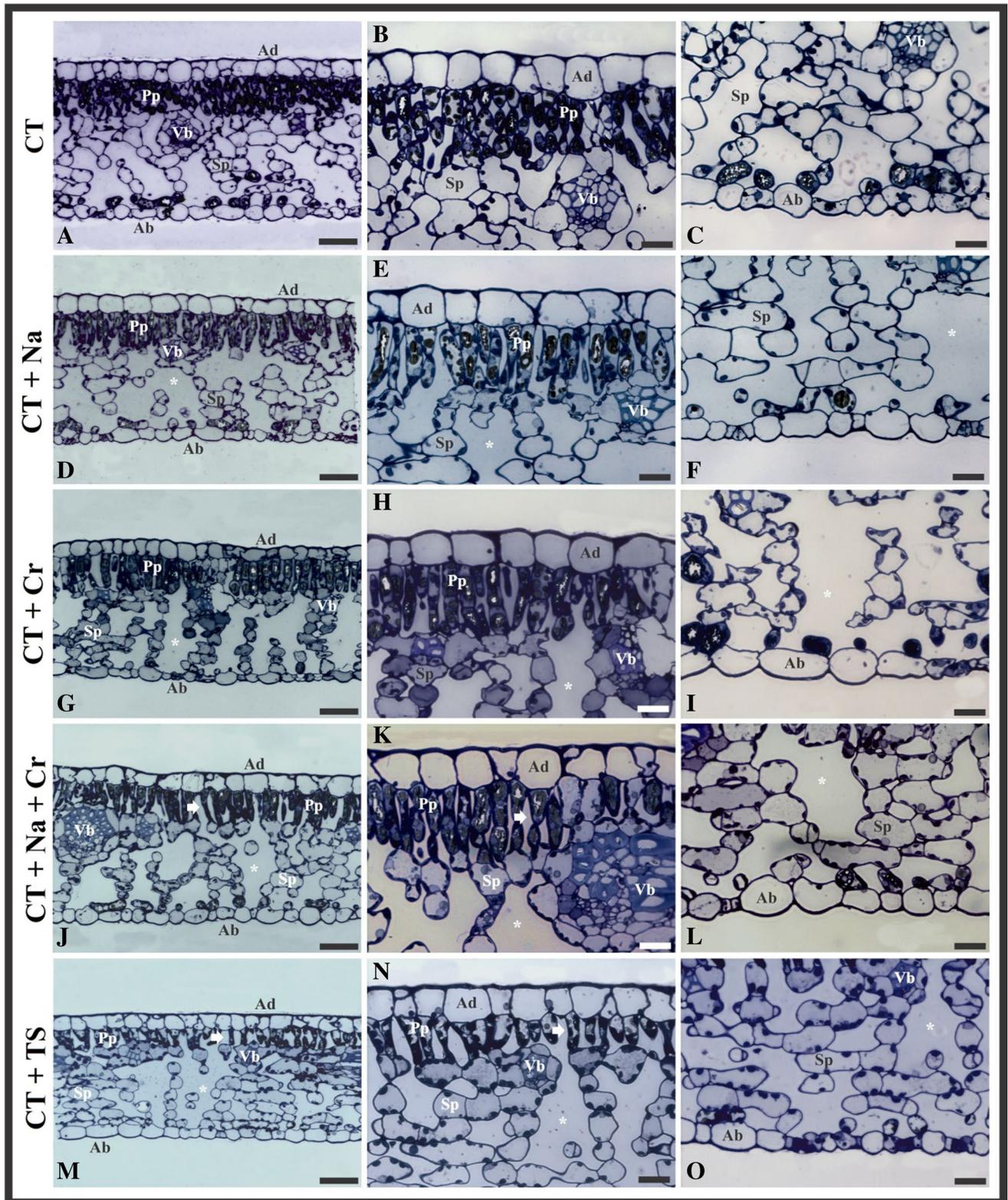
Leaf thickness (LTCK) of coffee seedlings submitted to treatments CT + TS and CT + Na were inferior to treatment CT in 23% and 6%, respectively. This fact may be especially related to the reduction of the palisade parenchyma in these treatments. Changes in chloroplasts and disorganization of the palisade parenchyma in Conilon coffee leaves were observed by Berilli et al. (2018b) in Conilon coffee seedlings on substrates with tannery sludge. Han et al. (2004) showed that, in the presence of trivalent chromium ( $\text{Cr}^{3+}$ ), the plants of *Sinapis alba* L.—Brassicaceae present a decrease in both the palisade and spongy parenchyma.

The combination of sodium + chromium (CT + Na + Cr) showed better results than sodium treatment (CT + Na) for leaf thickness (Table 6), showing a positive influence of chromium on the substrate, reducing some of the harmful effects of sodium. The inhibitory effects of salinity on the leaf structure may have been caused by the inhibition of the growth of vascular elements, the inhibition of pro-cambium activity, and the decrease in the size of the mesophyll cells, inhibiting plant growth and cellular functions (Reinhardt and Rost 1995; Gupta et al. 2021).

In the evaluation of the salt stress in *Coffea arabica* L. plants, Lima et al. (2014) enabled to observe that after 25 days of salt stress, the plants had a reduction in the leaf thickness and the cells of the palisade parenchyma were further from each other, with thinner walls and less thickness in relation to plants that were not under salt stress. Bastías et al. (2013) reported that young leaves of *Zea mays* L. in the presence of salinity showed a reduction in the size of the leaf mesophyll cells and an irregular shape. Similar to the results obtained in this study in particular, with coffee seedlings, the authors Barhoumi et al. (2007) and Agami (2014) observed that salinity led to a reduction in leaf mesophyll and in the leaf thickness, as consequence.

Regarding the palisade parenchyma (Table 6), it was observed that the treatments containing sodium differed from the conventional, showing a reduction in the thickness of this tissue, which can be better observed in Fig. 2. The presence of sodium seems to be the main cause in the reduction of thickness of the palisade parenchyma, shown by all treatments containing this element. It is likely that the osmotic stress caused by sodium caused damage to cell division and expansion, reducing the cells of the palisade parenchyma. In relation to the treatment containing tannery sludge, this negative effect of sodium was possibly aggravated by the high pH of the substrate. In this case, it is suggested to reduce the availability of several nutrients, resulting in the reduction in the palisade parenchyma.

A study by Parida et al. (2004) reports that the thickness of the mesophyll and the intercellular spaces decreased with the salinity in *Bruguiera parviflora* leaves, due to the



**Fig. 2** Compared anatomy of the leaf of *C. canephora* in different filters viewed under optical microscopy up to 180 days after planting. **A, B, C** 100% conventional Substrate; **D, E, F** 100% conventional substrate + 46.5 g sodium; **G, H, I** 100% conventional

substrate + 390 g of chromium; **J, K, L** 100% conventional substrate + 46.5 g sodium + 390 g chromium; and **M, N, O** 60% conventional substrate + 40% dehydrated tannery sludge

reduction in the length of the cells of the palisade parenchyma and the number of layers of the lacunous parenchyma, suggesting that the sodium treatment substantially reduced the CO<sub>2</sub> diffusion since the stomatal and mesophyll conductance decreased in the *B. parviflora* leaves in salinity conditions.

Cells with low mesophyll conductance can be the result from the contraction of the cell walls, resulting in a high density and low porosity of the mesophyll (Lima et al. 2014). For the species *Solanum lycopersicum* L. cv. (moneymaker), Albaladejo et al. (2017) observed that after 7 days of treatment with salt (NaCl), the leaf cells of this species became smaller, and both the palisade parenchyma and the lacunous parenchyma and, mainly, the cells of the palisade parenchyma became thinner and contracted, which resulted in a higher cell density.

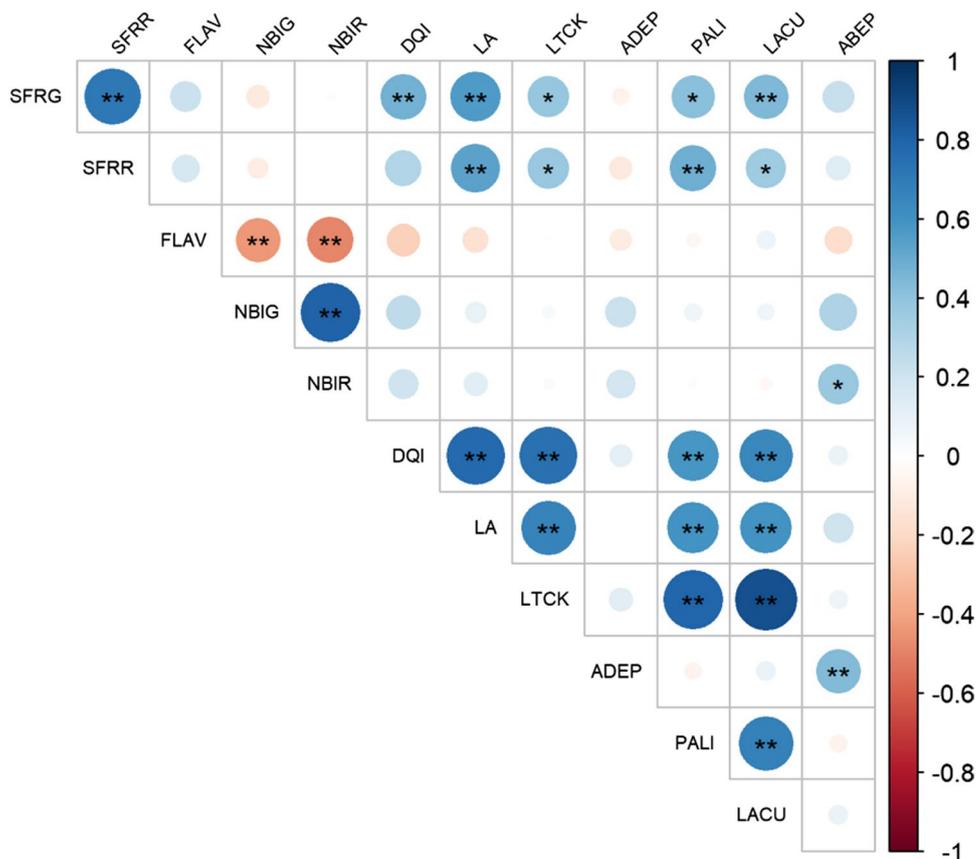
This study showed an inverse relationship (negative correlation) between the flavonoid indices and nitrogen balance, as observed in Fig. 3. This response was also observed by Sales et al. (2018c) in *Schinus terebinthifolius* Raddi (Anacardiácea) seedlings and by Ghiasy-Oskoe et al. (2018) in the species *Cnicus benedictus* L. (Asteraceae). This fact is apparently related to the pathways of biosynthesis of secondary metabolites in response to differences in growth environments, as reported by Radušienė et al. (2019). Several

hypotheses have been developed to explain the effect of nitrogen on the exchange between plant growth and the production of secondary carbon-based metabolites, which means changes in the partition of carbon skeletons between primary and secondary metabolism (Ghiasy-Oskoe et al. 2018; Radušienė et al. 2019).

It is possible to observe through the correlation analysis that the changes in the leaf area strongly interfere in the quality of seedlings (DQI). This fact is corroborated by the significant and positive correlation observed between leaf area and DQI ( $r=0.77$ ). Therefore, the reduction in the leaf area in the CT + TS treatment resulted in a decrease in all other variables used in the calculation of the DQI, in addition to decreasing the chlorophyll index of the plant.

This study in particular also showed that leaf thickness was positively correlated with chlorophyll indexes, DQI, leaf area, and palisade and lacunous parenchyma. As there was no significant correlation in the epidermis between the adaxial and abaxial surfaces, it can be inferred that the changes in leaf thickness are mainly related to the two fundamental tissues of the mesophyll. According to Zhang et al. (2020), the thickness of palisade and lacunous parenchyma are responsible for determining the variations in leaf thickness, in especial.

**Fig. 3** Association (Pearson’s correlation) among the principal analyzed variables. Significant at \* $p < 0.05$  and \*\* $p < 0.01$



The palisade and lacunous parenchyma showed a positive correlation with the chlorophyll index. Therefore, the increment in the thickness of these tissues promoted the increase in the chlorophyll index. As observed in the CT + TS, these tissues were less thick and, consequently, a reduced chlorophyll index was observed in these plants. Besides being the main molecules involved in photosynthesis, chlorophylls accumulate mainly in the palisade parenchyma (Asmar et al. 2013).

Both chlorophyllan parenchyma (palisade and lacunous) have many chloroplasts, which are the principal tissues responsible for photosynthesis in plants. Its ability to respond to light stimuli is particularly high, influencing leaf thickness, consequently (Castro et al. 2005).

Overall, it could be seen in this study that despite being a heavy metal, chromium was not able to reduce the carbon gain of coffee plants at the proportion that it was used in it, even maintaining levels of chlorophyll similar to that shown in the conventional treatment. In addition, chromium has a positive influence when used in combination with sodium, in order to reduce part of the harmful effects from sodium, which was the most harmful element in the growth, physiology, and anatomy of Conilon coffee plants. Based on these results, it is suggested that the tannery sludge utilized as a substrate has a high use potential. However, before being used, ways of reducing the harmful effects resulting from sodium and high pH should be investigated, as they were the principal causes of the negative results obtained in this study.

## Conclusion

The results indicate that the Cr alone had a low impact on the physiology, anatomy, and, consequently development of Conilon coffee seedlings at the dose used. On the other hand, exposure to Na had negative impacts on normal plant growth.

The summation effects of the elements chromium and sodium show an intermediate response pattern, which reveals a possible positive effect of the presence of chromium, which is supposed to attenuate the negative effects of sodium on Conilon coffee seedlings.

The adverse results of the application of dehydrated tannery sludge are somatic effects that go beyond the parameters Cr and Na, revealing that they are not the only factors responsible for the lower growth indicators studied.

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## Declarations

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## Authors and Affiliations

Leonardo Martineli<sup>1</sup>  · Sávio da Silva Berilli<sup>2</sup>  · Ramon Amaro de Sales<sup>3</sup>  · Maura da Cunha<sup>4</sup>  ·  
 Paola Afonsa Vieira Lo Monaco<sup>5</sup>  · Sílvio de Jesus Freitas<sup>4</sup>  · Maristella Martineli<sup>6</sup>  ·  
 Ana Paula Candido Gabriel Berilli<sup>2</sup>  · Saulo Pireda<sup>4</sup> · Dhiego da Silva Oliveira<sup>4</sup> · Lucas Louzada Pereira<sup>7</sup> 

Leonardo Martineli  
leonardo.martineli@ifes.edu.br

Sávio da Silva Berilli  
savio.berilli@ifes.edu.br

Maura da Cunha  
maura@uenf.br

Paola Afonsa Vieira Lo Monaco  
paolalm@ifes.edu.br

Sílvio de Jesus Freitas  
freitassj@yahoo.com.br

Maristella Martineli  
maristella.martineli@unimontes.br

Ana Paula Candido Gabriel Berilli  
ana.berilli@ifes.edu.br

Saulo Pireda  
saulopireda@hotmail.com

Dhiego da Silva Oliveira  
diego\_oliveira\_3586@yahoo.com.br

Lucas Louzada Pereira  
lucas.pereira@ifes.edu.br

<sup>1</sup> Federal Institute of Espírito Santo – Itapina Campus, Rodovia Br-259, Km 70, IFES Campus Itapina, Colatina, ES CEP: 29.717-000, Brazil

<sup>2</sup> Federal Institute of Espírito Santo - Alegre Campus, Rodovia BR-482, Km 47, Alegre, ES CEP: 29500-000, Brazil

<sup>3</sup> Federal University of Viçosa, Av. Peter Henry Rolfs, Campus Universitário, Viçosa, MG CEP: 36570-900, Brazil

<sup>4</sup> State University of the North Fluminense Darcy Ribeiro, Av. Alberto Lamego, 875 - Parque California, Campos Dos Goytacazes, RJ CEP: 28013-600, Brazil

<sup>5</sup> Federal Institute of Espírito Santo - Santa Teresa Campus, Rodovia ES-080, Km 93, Santa Teresa, ES 29660-000, Brazil

<sup>6</sup> Department of Agricultural Sciences of the State University of Montes Claros – Janaúba Campus, Av. Reinaldo Viana, 2630, Janaúba, MG CEP: 39.440-000, Brazil

<sup>7</sup> Federal Institute of Espírito Santo – Venda Nova Campus, Avenida Elizabeth Minete Perim, nº 500, São Rafael, Venda Nova Do Imigrante, ES CEP 29375-000, Brazil