Plant Physiol. Rep. (October–December 2021) 26(4):722–728
https://doi.org/10.1007/s40502-021-00634-9

ORIGINAL ARTICLE

Growth and physiological parameters in conilon coffee seedlings fertilized through foliar application of tannery sludge

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Received: 22 May 2020 / Accepted: 1 November 2021 / Published online: 15 November 2021

Abstract Currently, industries are increasingly concerned about the destination of their waste, and one of the solutions found may be the reuse of certain waste in the form of organic fertilizers. Thus, the aim of this work was to evaluate the growth and physiology of conilon coffee seedlings under foliar application of liquid tannery sludge as an alternative to fertilization. The experiment was conduced in a greenhouse. The experimental design was in randomized blocks, with eight replications and seven treatments, which consisted of different concentrations of tannery sludge (6.20, 8.80, 11.47, 14.10 and 17.60 mL of sludge tannery diluted in 1 L of water), a conventional treatment with urea and a control treatment using only water. Growth and physiological characteristics were evaluated through gas exchange, fluorometric analysis and color analysis using the Colorimeter. The conilon coffee seedlings fertilized with 14.10 and 17.60 mL L^{-1} showed a satisfactory growth pattern. The doses of tannery sludge used in this study did not promote changes in color analysis and indices obtained by fluorimetry, except for flavonoids, in which the highest dose promoted greater synthesis of this secondary metabolite. Doses below 14.10 mL L^{-1} promoted changes in gas exchange, however, leaf photosynthesis was only compromised with doses equal to or less than 8.80 mL L^{-1}.

Keywords *Coffea canephora* · Tannery waste · Plant growth · Waste management · Secondary metabolism

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Introduction

With an area of more than 400 thousand hectares and with an estimated production for 2019 varying between 860 and 980 thousand tons, Brazil stands out worldwide for the production of Conilon coffee (CONAB, 2019). Only in the State of Espírito Santo, the largest national producer, the typical family activity generates about 250 thousand direct and indirect jobs and it is responsible for 35% of the State's agricultural GDP being very important in socioeconomic development (Incaper, 2020). The drink made of coffee beans is consumed in several countries by millions of people and with the population growth, an excessive demand is created which leads to an increase in the value of the product and induces the planting of new coffee plantations. Projections related to coffee show that production and consumption in 2023 will increase 22.1% and 29.8% respectively compared to 2016 (Mapa, 2018).

Despite the appreciation of the product, one of the biggest barriers of the current agriculture is the high cost of production, making agricultural production increasingly difficult. Thus, techniques have been used by several producers aiming to reduce expenses, mainly in seedling production systems, such as the use of bio fertilizers in order to control diseases, pests and for the nutritional supply of plants through foliar application. This practice has generated positive results in the development of several cultures, such as *Passiflora edulis* f. *flavicarpa* and *Coffea canephora* (Berilli et al., 2018a; Sales et al., 2018a).

Among the great variety of residues with potential use in agriculture, the tannery sludge has gained notoriety due to its composition rich in organic compounds and inorganic salts essential for the development of plants. In this context, this would be an alternative in order to reduce production costs considering the increase in the cost of agricultural inputs (Malafaia et al., 2016).

The use of tannery sludge in the various segments of coffee cultivation, such as seedling production, seems to be a promising alternative when considering plant nutrition and cost reduction resulting from the lower use of inputs (Berilli et al., 2015). Despite this, some components present in this residue, mainly chromium (Cr) and sodium (Na), can interfere with the development and physiology of the coffee plantation, becoming a limiting factor to its use (Berilli et al., 2018b; Korndörfer, 2006).

Another interesting aspect in the use of tannery sludge in agriculture is related to the disposal of this residue, reducing the environmental impact of its disposal. Most of these tailings cause damage to the environment when wrongly disposed of. Therefore, in addition to the economic point of view, the reuse of this waste contributes to increased sustainability (Silva et al., 2019).

Due to incipient studies about this subject, the present work aims to evaluate the growth and physiology of conilon coffee seedlings under the application of liquid tannery sludge in the aerial part as an alternative source of fertilizer.

Material and methods

Characteristics of the area under study and experimental design

The experiment was conduced in a seedling propagation nursery of the Federal Institute of Education, Science and Technology of Espírito Santo—Campus Itapina, located in the municipality of Colatina (19° 32′ 22″ S 40° 37′ 50″ W and 71 m). The region's climate is Tropical Aw, according to the Köppen climate classification, with a well-defined rainy season between October and January and an average climatological precipitation of 1029.9 mm (Peel et al., 2007). For the execution of the experiment, a randomized block design was used, with eight replications and seven treatments, being considered 21 plants per experimental plot. The irrigation management throughout the experiment was performed daily by microsprinkler, always maintaining the substrate field capacity. The treatments consisted of five concentrations of tannery sludge, a urea solution, considered as conventional foliar fertilization (T-C) by Gonzaga (2000), and a solution considered control, with pure water (T-0).

The treatments consisted of the application of 1 L of solution with different concentrations of liquid tannery sludge diluted in water. The concentrations used were: Conventional treatment (TC), 0.0135 g of nitrogen in the form of urea (0.3 g of urea) diluted in one liter of water; T1, 6.20 mL of liquid sludge diluted in one liter of water (0.62%); T2, 8.80 mL of liquid sludge diluted in one liter of water (0.88%); T3, 11.47 mL of liquid sludge diluted in one liter of water (1.15%); T4, 14.10 mL of liquid sludge diluted in one liter of water (1.41%); T5, 17.60 mL of liquid sludge diluted in one liter of water (1.76%) and Control treatment (T0), consisting of pure water.

Origin of clones and preparation of seedlings

In this experiment, conilon coffee seedlings of the clonal variety "Vitória Incaper—8142" were used, more specifically the V8 genotype. To perform the experiment, seedlings of conillon coffee (Coffea canephora Pierre) were used, produced from cuttings obtained from adult tissue of orthotropic branches, taken from crops with good phytosanitary and nutritional aspect of the Federal Institute of Education, Science and Technology of the Espírito Santo Santo—Itapina Campus. After removing the branches from the mother plants, they were taken to the greenhouse, where 30 cm from the ends of the orthotropic branches were removed. Then, the standardization of cuttings was carried out, with 6 to 8 cm in height, leaves with 1/3 of the leaf blade, plagiotropic branches above the insertion of the pair of leaves with 1 cm. After immersing the cuttings in a solution of fungicide based on dithiocarbamates, they were planted in 600 ml polyethylene bags previously filled with the substrate.

The substrates used for the production of seedlings in this experiment followed a mixture considered traditional by the producers of conilon coffee seedlings, and for each 80 L of red earth of underground were added: 625 g of P_2O_5, 200 g of calcium, 200 g of KCl and 20 L of humus of cattle manure. The soil used had the following chemical characteristics: pH: 6.20; phosphorus: 3.0 mg dm^{-3}; potassium: 44.0 mg dm^{-3}; calcium: 14.1 mmolc dm^{-3}; magnesium 10.5 mmolc dm^{-3}; potential acidity (H + Al) 7.2 mmolc dm^{-3}; organic matter: 2.2 g dm^{-3}; sum of bases (SB): 25.7 mmolc dm^{-3}; pH 7 CEC (T): 32.9 mmolc dm^{-3}; effective CEC (t): 25.7 mmolc dm^{-3} and base saturation: 78.1%.

The amount of liquid tannery sludge added to each solution was based on the amount of nitrogen (N) present in the initial concentration of liquid sludge, containing the following composition: organic carbon 9.9%; total organic matter 8.03 g L^{-1}; nitrogen 2200 mg L^{-1}; phosphorus 55 mg L^{-1}; potassium 110 mg L^{-1}; calcium 8930 mg L^{-1}; magnesium 1370 mg L^{-1}; sulfur 1508 mg L^{-1}; boron 14 mg L^{-1}; sodium 1700 mg L^{-1}; chromium 3500 mg L^{-1}.

The application of the solutions corresponding to each treatment was started after emerging the first pair of fully expanded leaves and was repeated monthly until the seedling reached commercial size, which occurred at 228 days after planting cuttings (DAP). Fertilization was carried out at the end of the day and the plants remained with the solution until the next day, using sprinklers to apply the effluent.

Growth and physiological characteristics assessed

Six evaluations were performed during the experiment (66, 97, 126, 154, 193 and 228 DAP), measuring the plant height (cm), measured with the aid of a graduated ruler; stem diameter (mm), measured with the aid of a digital caliper; crown diameter (cm), measured with the aid of a graduated ruler; and number of leaves, by counting. At the end of the experiment, color analyzes were performed using the Minolta Colorimeter equipment (model CR-400 in DL/65 configuration), determining the luminosity (L*), chromaticity (a* and b*) and hue angle (h0), in addition to the SPAD portable chlorophyll meter. In the same period, the aerial part of the seedlings were analyzed with a fluorometer model Multiplex® (Force-A), estimating indices of nitrogen balance (NBI-G and NBI-R), of chlorophyll (SFR-G and SFR- R), anthocyanins (ANT-RG and ANT-RB) and flavonoids (FLAV). The indices obtained by the Multiplex® were derived, sometimes, from different combinations of wavelengths emitted by the equipment, and the equipment has light emitting diodes (LED), with four excitation bands, ultraviolet (UV) and three of red, green, blue (RGB), in addition to three detection bands, which are yellow (YF) of 590 nm, red (RF) of 685 nm and far red (FRF) of 735 nm.

The rates of leaf photosynthesis (A, µmol CO_2 m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), vapor pressure deficit between leaf and air (VPD leaf-air, kPa) and leaf temperature (CT leaf, °C) were measured between 09:00 and 11:00 a.m. in fully developed leaves of the second pair using an infrared gas analyzer (model LI-6400, Li-COR, Lincoln, Nebraska, USA).

Statistical analysis

The data were subjected to analysis of variance, and when the treatments were significant, the averages were subjected to Dunnett's test, at the level of 5% probability. R software was used for the analyses.

Results and discussion

The application of tannery sludge as foliar fertilization can provide the seedlings of Conilon coffee with different responses in their developmental and physiological characteristics. When the number of leaves was evaluated (Fig. 1) it was possible to notice that the different applications of tannery sludge showed the same response pattern over the different evaluation periods, always differing treatments T-0, T-1, T-2 and T-3 when compared to conventional treatment (T-C). The treatments T-0, T-1, T-2 and T-3 were inferior to the conventional treatment up to 24% for leaf number at 66 DAP, reaching more than 50% at 228 DAP. This difference found in the emission of leaves from the first evaluation to the final evaluation in these treatments is mainly due to the smaller amount of nutrients received by them, since T-0 received only water in the foliar application and T-1, T-2 and T-3 received applications of 6.20, 8.80 and 11.47 mL sludge L^{-1} of water, respectively, being these ones the lowest doses. The responses to treatments T-4 and T-5 were identical to the conventional treatment in all periods of evaluation, indicating that the application of 14.10 and 17.60 mL sludge L^{-1} of water was satisfactory in the emission of leaves.

For the plant height characteristic (Fig. 2), it is clear that at 66 DAP only treatments T-0 and T-1 produced results inferior to the conventional treatment (T-C). From 97 DAP

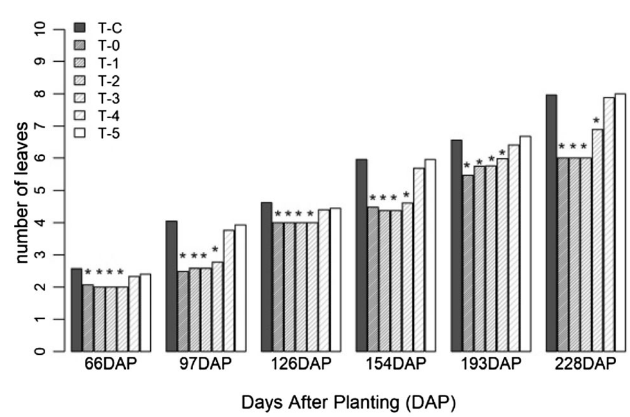

Fig. 1 Response of the number of leaves to the seven treatments, during six evaluations. Averages accompanied by the symbol * differ from conventional treatment (T-C) at the 5% probability level by Dunnett's test

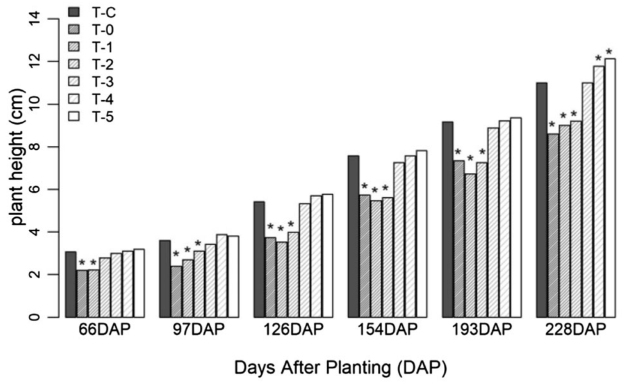

Fig. 2 Response of the plant height to the seven treatments, during six evaluations. Averages accompanied by the symbol * differ from conventional treatment (T-C) at the 5% probability level by Dunnett's test

until the end of the experiment, that is, 228 DAP, the treatments inferior to the conventional ones were T-0, T-1 and T-2. The highest doses of tannery sludge, 14.10 and 17.60 mL L^{-1}, provided larger plants than the conventional treatment at 228 DAP, with gains of 7 and 10%, respectively. This gain was probably obtained due to the benefits that the residue brings, such as nitrogen, potassium, magnesium, sulfur, among others, contributing to greater plant growth.

Both macronutrients and micronutrients play several roles in plants, being linked to photosynthesis, respiration, enzymatic cofactors and in the constitution of DNA and RNA proteins, which can accelerate the development of plants (Carneiro et al., 2015; Okumura et al., 2011). Therefore, despite the amount of nitrogen being higher in conventional treatment, treatments T-4 and T-5 were compensated by other mineral elements, contributing to their development. When the crown diameter is evaluated (Fig. 3), it is observed that in the first two measurements (66 and 97 DAP), the treatments that provided a significant difference when compared to the conventional treatment were T-0 and T-1, both of them being lower. At 228 DAP, the treatments that provided crowns of smaller diameter are T-0, T-1 and T-2, while T-5 produced a larger crown diameter. Thus, it can be seen that even the T-5 presenting results equal to the conventional treatment for the number of leaves (Fig. 1), the same did not occur with the crown diameter, obtaining a gain in photosynthetic area due to the greater expansion of tissue cells, thus compensating for leaf emission, which may contribute to increased photosynthesis.

In contrast to these results, Berilli et al. (2014) found in conilon coffee seedlings results inferior to the conventional treatment for the crown diameter from the use of tannery sludge. However, the aforementioned authors used the residue in the solid phase along with the substrate, in which the amount of nutrients supplied to the plants was higher, including Cr and Na, which may have contributed to the higher values for toxicity. According to Malafaia et al. (2016), the use of tannery sludge residue is an attractive alternative in soil fertilization, promoting greater crop productivity, and it was observed in this experiment that its use also promoted greater seedling development. Stem diameter (Fig. 4) did not show significant differences between treatments at 66 DAP. Significant difference was observed only at 97 DAP, in which treatments T-0, T-1 and T-2 had the smallest stem diameters in compared to conventional treatment. At the end of the experiment (228 DAP), treatments T-3 and T-4 also had significantly smaller diameters than T-C. At the end of the experiment (228 DAP), treatments T-3 and T-4 also revealed diameters significantly smaller than conventional treatment. The treatment that used 17.60 mL of liquid tannery sludge L^{-1} (T-5) was the only one that did not differ from the

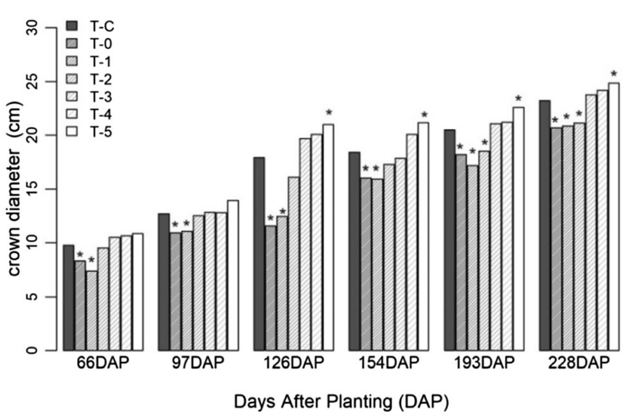

Fig. 3 Response of crown diameter (cm) to the seven treatments, during six evaluations. Averages accompanied by the symbol * differ from conventional treatment (T-C) at the 5% probability level by Dunnett's test

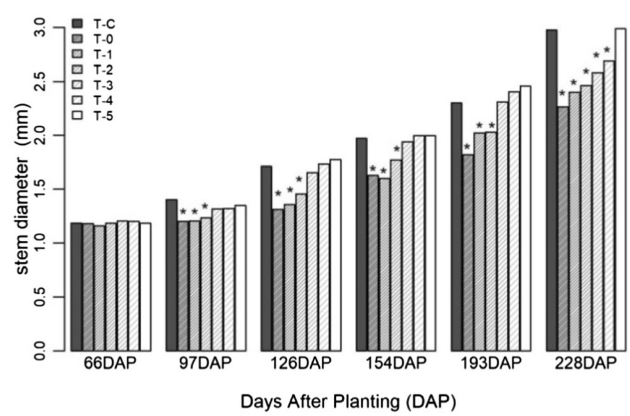

Fig. 4 Response of stem diameter (mm) to the seven treatments, during six evaluations. Averages accompanied by the symbol * differ from conventional treatment (T-C) at the 5% probability level by Dunnett's test

conventional treatment. In this way, the potential for using the residue is noticed, since the results presented in the highest doses promoted satisfactory results for the conilon coffee seedlings.

According to Silva et al. (2017), the use of the colorimeter equipment provides important information about the coloration of plant tissues, with the T-0 treatment for parameter L* being significantly higher than those of conventional treatment (Table 1). This reveals that there was greater reflectance in the leaf tissues of plants that received only water in the aerial part, which may be linked to a greater thickening of the cuticle and a lower amount of chlorophyll, as observed by the SPAD indices in Table 1 and chlorophyll in Table 2.

According to the authors Rossato and Kolb (2010) and Schmidt et al. (2017), the increase in cuticle thickness has, among other functions, the ability to reflect light, in order to maintain optimal leaf temperature levels and favor physiological processes. In addition, as observed in parameter b* and the hue angle closest to 90°, there was a greater yellow color for the plants at T-0, which may be related to the greater exposure or production of carotenoids, when compared with conventional treatment.

The higher yellow color intensity of these plants (T-0), as indicated by the hue angle, may be related to the lower amount of nutrients, mainly nitrogen, the basic constituent of chlorophylls, since the conventional treatment received fertilization with urea. The yellow color observed in leaves, according to Lima et al. (2012), is a typical symptom of nitrogen deficiency, corroborating the nitrogen balance index (NBI-R) in Table 2, in which the T-0 was lower than conventional treatment.

For the characteristics obtained by the fluorometer in Table 2, it can be seen that there were no significant differences for the anthocyanin index. For the flavonoid index (Table 2), it is clear that T-5 differed from conventional treatment, being more than 30% higher in the production of this metabolite. For these same treatments, there was no difference in the nitrogen and chlorophyll balance indexes. This reveals that the production of the secondary compound (flavonoids) is not due to competition for nitrogen, as observed by Treutter (2010) and Becker et al. (2015), but rather due to some stress associated with ROS, which may be caused by the toxic effect of Cr and Na, present in the tannery sludge. The authors Sales et al. (2018b), evaluating different substrates in the production of Schinus Terebinthifolius Raddi seedlings, found higher levels of flavonoids in the substrate constituted by the mixture of soil with dehydrated tannery sludge.

The control treatment (water) and the treatment with the lowest dose of tannery sludge, that is 6.20 mL L^{-1}, showed lower values of $VPD_{leaf\text{-}air}$, with the respective values of 3.02 and 2.82 kPa (Table 3). These results reflected a lower transpiratory rate, resulting in a reduction in leaf photosynthesis. Therefore, due to the lower mineral nutrition received by these plants, there was a serious impairment of carbon assimilation. Several mineral nutrients when restricted to plants can impair photosynthesis. P levels affect photophosphorylation, rubisco enzyme activity and the reactions of the Calvin cycle (Neocleous & Savvas, 2019), while the k ion acts directly on the opening and closing of stomata. N limitation can induce to a higher proportion of N from leaves allocated to cell walls, which increases leaf mass per area, reducing the photosynthetic efficiency of N use and decreasing maximum photosynthesis (Hidaka & Kitayama, 2009; Zhang et al., 2018).

When observing the leaf temperature (Table 3), it is clear that the treatments T-0, T-1, T-2 and T-3 showed higher values when compared to conventional treatment, contributing to a lower stomatal conductance. The seedlings obtained in this experiment have always remained in the soil with the moisture close to the field capacity, being

Table 1 Averages of the SPAD index and L*, a*, b* and h0 staining parameters, in leaves of conilon coffee seedlings under different leaf applications of tannery sludge, at 228 days of age

Treatment	SPAD	L*	a*	b*	h^0
C-T	33.633	36.567	−11.348	16.346	118.790
T-0 (Water)	26.601*	44.432*	−11.368	20.033*	112.935*
T-1 (6.20 mL L^{-1})	26.554*	40.040	−11.374	17.876	115.774
T-2 (8.80 mL L^{-1})	27.883*	40.247	−11.916	18.973	115.123
T-3 (11.47 mL L^{-1})	29.340	38.265	−11.718	16.645	118.525
T-4 (14.10 mL L^{-1})	29,231	39.631	−11.780	18.043	116.010
T-5 (17.60 mL L^{-1})	29.375	40.190	−11.816	18.135	116.265
MSD	4.497	4.124	1.152	3.581	5.813
CV (%)	10.88	6.58	6.33	12.67	3.22

Averages accompanied by the symbol * differ from conventional treatment (T-C) at the 5% probability level by Dunnett's test. MSD = minimum significant difference

Table 2 Average indices of Flavonoids (FLAV), anthocyanin (ANTH-RG and ANTH-RB), chlorophyll content (SFR-G and SFR-R) and nitrogen balance (NBI-G and NBI-R), obtained using Multiplex® equipment on conilon coffee seedlings under different leaf applications of tannery sludge, at 228 days of age

Treatment	FLAV	SFR-R	NBI-R	ANTH-RG	ANTH-RB
C-T	0.561	1.330	0.315	−0.089	−0.686
T-0 (Water)	0.632	1.074*	0.218*	−0.078	−0.704
T-1 (6.20 mL L^{-1})	0.627	1.276	0.326	−0.109	−0.696
T-2 (8.80 mL L^{-1})	0.633	1.329	0.358	−0.102	−0.691
T-3 (11.47 mL L^{-1})	0.730	1.278	0.333	−0.088	−0.695
T-4 (14.10 mL L^{-1})	0.614	1.339	0.351	−0.084	−0.694
T-5 (17.60 mL L^{-1})	0.739*	1.439	0.385	−0.106	−0.698
MSD	0.177	0.254	0.095	0.039	0.028
CV(%)	17.96	12.76	19.26	26.88	2.65

Averages accompanied by the symbol * differ from conventional treatment (T-C) at the 5% probability level by Dunnett's test. MSD = minimum significant difference

Table 3 Average values of net photosynthetic rate (*A*), stomatal conductance (g_s), transpiration rate (*E*), vapor pressure deficit between leaf and air (VPD leaf-air) and leaf temperature (CT leaf) in Conilon coffee seedlings grown under different sources of organic matter, at 228 days of age

Treatment	*A*	*gs*	*E*	VPD $_{\text{air-leaf}}$	CT leaf
C-T	7.984	0.306	5.016	2.172	31.282
T-0 (Water)	4.674*	0.119*	3.113*	3.024*	34.487*
T-1 (6.20 mL L^{-1})	6.259*	0.149*	3.732*	2.822*	32.772*
T-2 (8.80 mL L^{-1})	6.543	0.156*	3.812	2.731	33.316*
T-3 (11.47 mL L^{-1})	6.903	0.231*	4.232	2.573	32.329*
T-4 (14.10 mL L^{-1})	6.741	0.239	4.497	2.582	31.768
T-5 (17.60 mL L^{-1})	7.293	0.286	5.199	2.083	30.719
MSD	1.716	0.068	1.210	0.614	0.990
CV (%)	17.13	18.78	17.7	15.24	1.95

Averages accompanied by the symbol * differ from conventional treatment (T-C) at the 5% probability level by Dunnett's test. MSD = minimum significant difference

not water availability the limiting factor for these characteristics. In this case, nitrogen was considered the main reason for the reduction of these characteristics, as observed by Simões et al. (2015) in Jatropha curcas L., in which the authors state that the nitrogen doses and the availability of water in the soil significantly interfere in the production and physiology of these plants.

Conclusion

It is concluded through this research that the treatments T-4 (14.10 mL L^{-1}) and T-5 (17.60 mL L^{-1}) presented averages equal to or superior to the conventional treatment for the parameters number of leaves, plant height, canopy diameter and stem diameter at 228 DAP. In addition, there were higher values of L* and b*, and lower values of h0, SPAD, SFR-R and NBI-R in the leaf tissues of plants that received only water in the shoot, indicating greater cuticle thickness and yellow colored leaves. The FLAV index was higher in the treatment that received 17.60 mL L^{-1}. Finally, doses below 8.80 mL L^{-1} reduced air-leaf A, E and VPD, while gs and CT leaf were compromised with doses equal to or lower than 11.47 mL L^{-1}.

Acknowledgements The authors would like to thank the Espírito Santo Research and Innovation Support Foundation (FAPES) and the Federal Institute of Education, Science and Technology of Espírito Santo (IFES) for their financial and institutional support for research.

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