ISSN 2175-6813

Revista Engenharia na Agricultura

Viçosa, MG, DEA/UFV - DOI: 10.13083/reveng.v29i1.9970 V.28, p.549-557, 2020

EFFECT OF FOLIAR-APPLIED TANNERY SLUDGE ON GROWTH AND PHYSIOLOGY OF *PASSIFLORA EDULIS* SEEDLINGS

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Keywords:
Yellow passion fruit
Secondary metabolism
Propagation
Sustainability

ABSTRACT

Today, industries are increasingly concerned about the disposal of their waste. One of the proposed solutions to deal with certain wastes is through agriculture in the form of fertilizers. Thus, the objective of this study was to evaluate the effect of liquid tannery sludge as foliar fertilizer on the growth and physiology of passion fruit seedlings. The experiment was conducted in a greenhouse, in a randomized block design with six replications. Six treatments were applied to leaves: tannery sludge diluted in water (132, 263, 395 and 527 mL L^{-1}); only water (control); and the conventional treatment. The following growth characteristics were evaluated: leaf production, plant height, crown diameter, stem diameter, total fresh matter and dry matter of the plants. The physiological characteristics evaluated were the indices chlorophyll, nitrogen balance, flavonoids, anthocyanins, and plant color. The flavonoid index increased in plants that received foliar application of 527 mL L^{-1} without compromising the other characteristics evaluated. The doses 263, 395 and 527 mL L^{-1} showed the best results for the growth characteristics, equaling those of the conventional treatments. Foliar application of tannery sludge can be recommended for yellow passion fruit seedlings.

Palavras-chave:
Maracujá-amarelo
Metabolismo secundário
Propagação
Sustentabilidade

DESENVOLVIMENTO E FISIOLOGIA DE MUDAS DE *PASSIFLORA EDULIS* EM FUNÇÃO DA APLICAÇÕES FOLIARES DE LODO DE CURTUME

RESUMO

Atualmente as indústrias estão cada vez mais preocupadas como a destinação de seus resíduos e, uma das soluções encontradas que podem ser aderidas a determinados dejetos, está voltada à agricultura na forma de fertilizantes. Deste modo, o objetivo deste trabalho foi avaliar o efeito da aplicação de lodo de curtume líquido na parte aérea como adubo, no desenvolvimento e fisiologia de mudas de maracujá-amarelo. O experimento foi realizado em casa de vegetação, em um delineamento experimental em blocos casualizados com seis repetições e seis tratamentos, consistindo da aplicação de lodo de curtume via foliar diluídos em água (132, 263, 395 e 527 mL L^{-1}), um tratamento só com água (controle) e um convencional. Foram avaliadas as seguintes características: emissão de folhas, altura da planta, diâmetro da copa, diâmetro do caule, matéria fresca e seca total das plantas. Também avaliou-se a fisiologia através dos índices de clorofila, balanço de nitrogênio, flavonoides e antocianinas, bem como a coloração das plantas. Houve aumento no índice de flavonoides nas plantas que receberam 527 mL L^{-1} via foliar sem comprometer as demais características avaliadas. As melhores doses aplicadas foram de 263, 395 e 527 mL L^{-1}, apresentando resultados iguais ao convencional para as características de desenvolvimento. O lodo de curtume aplicado via foliar pode ser recomendado para mudas de maracujá-amarelo.

Recebido para publicação em 07/04/2020 • Aprovado em 07/10/2020 • Publicado em 29/12/2020

INTRODUCTION

The Brazilian leather industry is one of the major drivers of the national economy. It is estimated that the tannery production chain generates US $ 3.5 billion annually (NEHRING & FABRE, 2020). However, the activity is responsible for the generation of significant amounts of waste (GUIMARÃES *et al.*, 2015), so alternative uses for this waste is crucial to preserve environmental quality.

The agricultural activity has undergone deep changes due to the increase in production costs, thus, the use of tannery sludge as fertilizer can be an alternative for agriculture, mainly due to its chemical composition, with high organic matter and nutrient content (SALES *et al.*, 2018). Tannery sludge can act as corrective and fertilizer. It has potential as fertilizer because of the high contents of several essential nutrients such as nitrogen, phosphorus, potassium, calcium, sulfur, and magnesium (TEIXEIRA *et al.*, 2015; QUARTEZANI *et al.*, 2018). However, because of the high amount of sodium and chromium in its composition, the use of this waste in farming areas must have defined application criteria.

Heavy metals may alter the metabolic routes in plants and there is great variation in sensitivity among species (SILVA *et al.*, 2007). In environments with problematic levels of these metals, one of the possible responses of plants is the production of secondary metabolites, as for example, flavonoids (BERILLI *et al.*, 2016). These metabolites can increase the probability of survival of a species, since they play several biological activities (FUMAGALI *et al.*, 2008) aiming to inactivate or immobilize toxic elements within plants.

Due to the problems caused by chromium and salinity, research has continuously focused on finding alternative and appropriate uses of tannery sludge in agriculture. It gave satisfactory responses when tested as a seedling substrate (SILVA *et al.*, 2019; BERILLI *et al.*, 2019), supplying plants with nutrients and increasing yield (OLIVEIRA *et al.*, 2013; GONÇALVES *et al.*, 2014). However, little is known about the use of this waste as foliar fertilizer and the response of passion fruit seedlings, a crop cultivated in several regions of Brazil and of great economic importance (MIYAKE *et al.*, 2016).

In response to the lack of information on the use of this waste as foliar fertilizer in passion fruit, as well as the absence of regulations in the state of Espírito Santo (BERILLI *et al.*, 2014), this line of research is essential to contribute to the knowledge bases and promote sustainable agriculture. Therefore, the objective of this study was to evaluate the effect of liquid tannery sludge as foliar fertilizer on the growth and physiology of yellow passion fruit seedlings.

MATERIAL AND METHODS

The study was developed at the Federal Institute of Espírito Santo - Campus Itapina, located at coordinates 19° 29'52.7 "S and 40º45'36.9" W, 71 m altitude, in the municipality of Colatina-ES. The experiment was conducted in a greenhouse with 50% shading rate and automated irrigation to keep the substrates close to the field capacity, in a randomized block design with six treatments, six replications, and six experimental plots per treatment.

The treatments consisted of four different concentrations of liquid tannery sludge, a control using pure water, and a conventional treatment, as follows: T1 – conventional application of 0.44 g urea + 0.44 g K_2O per dm^{-3} of water; T2 – application of pure water; T3 – 132 mL of tannery sludge completed to 1 dm^{-3} with water; T4 – 263 mL of tannery sludge completed to 1 dm^{-3} with water; T5 – 395 mL of tannery sludge completed to 1 dm^{-3} with water; T6 – 527 mL of tannery sludge completed to 1 dm^{-3} with water.

The seedling substrates of all treatments were prepared with 75% of soil and 25% of composted cattle manure sieved, 5 kg m^{-3} of simple superphosphate, and 1 kg m^{-3} of KCl. All the formulations were prepared 15 days before sowing. The soil chemical characteristics are as follows: pH - 6.2; P - 3.0 mg dm^{-3}; K - 44.0 mg dm^{-3}; remaining-P - 12.0 mg ml^{-1}; Ca - 14.1 $mmol_c$ dm^{-3}; Mg - 10.5 $mmol_c$ dm^{-3}; H + Al - 7.2 $mmol_c$ dm^{-3}; cation exchange capacity - 32.9 $mmol_c$ dm^{-3}; effective cation exchange capacity - 25.7 $mmol_c$ dm^{-3}; sum of bases - 25.7 $mmol_c$ dm^{-3}; organic matter - 2.2 g dm^{-3}; base saturation - 78.1%.

The tannery sludge was provided by the

company Capixaba Couros LTDA ME, located in the municipality of Baixo Guandu – ES. Manure was collected from cattle confined in feedlots in the IFES campus Itapina. The characteristics of tannery sludge and cattle manure are listed in Table 1.

Table 1. Characterization of the tannery sludge and cattle manure used in the experiment (dry basis)

Parameter	Unity	Tannery sludge	Cattle manure
N	g dm^{-3}	2.20	21.00
P	g dm^{-3}	0.02	11.90
K	g dm^{-3}	0.09	7.50
Ca	g dm^{-3}	8.93	21.80
Mg	g dm^{-3}	1.37	5.40
S	g dm^{-3}	1.51	4.50
B	g dm^{-3}	14.00	16.10
Na	g dm^{-3}	1700.00	3.70
Cr	g dm^{-3}	3500.00	20.00
Total organic matter	g dm^{-3}	8.30	460.30
Organic carbon	g dm^{-3}	99.00	200.20

N: Nitrogen; P: Phosphorus; K: Potassium; Ca: Calcium; Mg: Magnesium; S: Sulfur; B: Boron; Na: Sodium; Cr: Chromium

For the production of seedlings, two seeds of *Passiflora edulis* f. *flavicarpa* were planted per perforated polyethylene bag (11 x 20 cm). Micro sprinkler automatic irrigation was carried out for 10 seconds every 10 minutes, keeping the substrate always close to the field capacity.

Seedling emergence started 15 days after planting and foliar fertilization was carried out weekly with a watering can 12 days after emergence. During the experiment, the climatic data inside the greenhouse were measured, with average temperatures of 28.39 °C in September and 28.27 °C in October. The average relative air humidity for September was 59.77% and light intensity of 2292.03 (accumulated Lux), while in October the average relative air humidity was 59.32% and light intensity of 2336.33 (accumulated Lux).

After 58 days, the seedlings reached the commercial size, and the growth evaluations were carried out at intervals of 34, 41, 49, and 58 days after planting. The parameters evaluated were: leaf number (count), plant height from collar to apex with a metric ruler, crown diameter measured the largest distances between leaves of the same crown with a metric ruler, and stem diameter measured with a digital caliper. Total fresh and dry matter were obtained using destructive measurements. For the dry matter, plant samples were placed in a forced circulation oven at 72 °C for 72 hours and weighed in a precision analytical balance.

At the end of the experiment, physiological analyses assessed color using a Minolta Colorimeter (model CR-400) and determined the indices lightness (L*), chroma (a* and b*), and hue angle (h^o). In the same period, a Multiplex® fluorometer (Force-A, France) was used to estimate the indices nitrogen balance (NBI-G and NBI-R), chlorophyll (SFR-G and SFR-R), anthocyanins (ANT-RG and ANT-RB), and flavonoids (FLAV) in leaves of the seedlings.

The Multiplex® indices were calculated by the mean of 4 excitation wavelengths: ultraviolet (UV) and three in red, green, blue (RGB); and 3 detection wavelengths: yellow (YF, 590 nm), red (RF, 685 nm), and far-red (FRF, 735 nm). All measurements were carried out in the morning, between 9:00 am and 11:00 am, on only one side of the seedlings, with the sensor pointing to the canopy from top to bottom with an angle of approximately 45°. After the measurements, the data were parameterized.

The data were analyzed by the F test in the analysis of variance, and whenever significance was found, the means were compared by the Dunnett test at 5% ($p < 0.05$) of probability. The Pearson's correlation test was performed between the physiological variables. Statistical analyses were performed using the R Core Team statistical program.

RESULTS AND DISCUSSION

Among the treatments applied, the growth characteristics showed significant difference by the F test in at least one evaluation period (34, 41, 49, and 58 days after planting), except for the number of leaves (Figure 1 a), which did not differ in any of the evaluations. Other authors found no differences in the production of leaves in passion fruit seedlings in response to fertilization, such as Prates *et al*. (2010) and Sousa *et al*. (2013), at 70 days after sowing, since it is a genetic trait and, hence, more difficult to be changed.

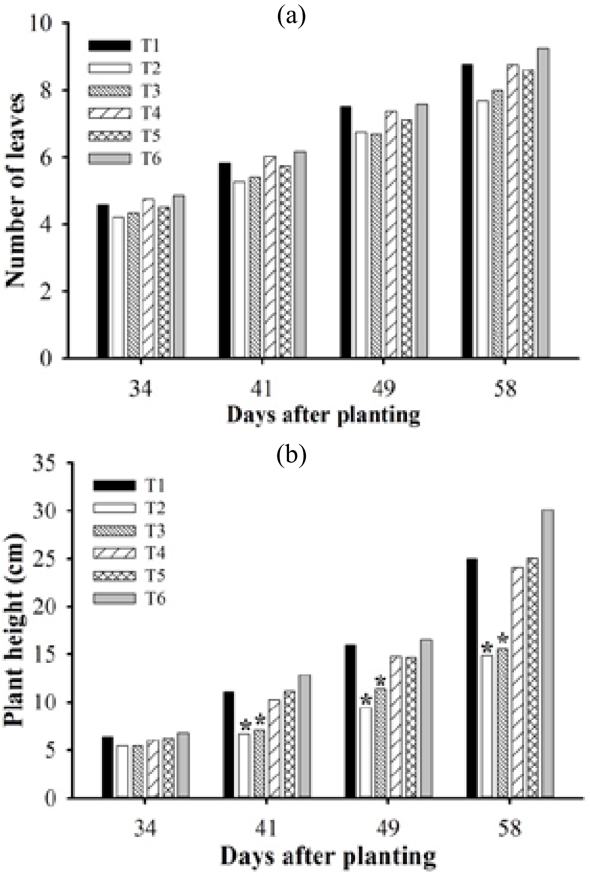

Figure 1. Leaf number (a) and plant height (b) of *Passiflora edulis* f. *flavicarpa* seedlings under six treatments in four evaluations. Means followed by * are significantly different from conventional treatment (T1) at 5% (p <0.05) probability level by the Dunnett's test

Plant height (Figure 1 b) clearly showed no difference between treatments at 34 days after sowing. However, at 41 days after sowing, the treatments T2 and T3 significantly differed from the conventional treatment (T1). At 41 days after sowing, the conventional treatment was 60% higher than T2 and T3. This difference remained in the subsequent evaluations (49 and 58 days after sowing). The difference in height between these plants and the plants from the conventional treatment is related to the less amount of nitrogen applied, since T2 consisted of pure water and T3 consisted of the lowest concentration of tannery sludge (132 mL L^{-1}). The growth and development of plants is highly dependent on nitrogen. Nitrogen participates in protein synthesis, ion absorption, photosynthesis, cell differentiation, and several other functions in plants (OKUMURA *et al*., 2011).

The stem diameter (Figure 2 a) at 34 and 41 days after sowing showed no difference between treatments. After 49 days, T2 and T3 differed from the conventional treatment. At 58 days after sowing, only T2 was different from the conventional treatment, whereas the difference previously found at 49 days after sowing in T3 was not observed at the end of the experiment. This is associated with the beneficial effects of the tannery sludge, providing nutrients such as phosphorus, potassium, calcium, and magnesium, all very important for plant development.

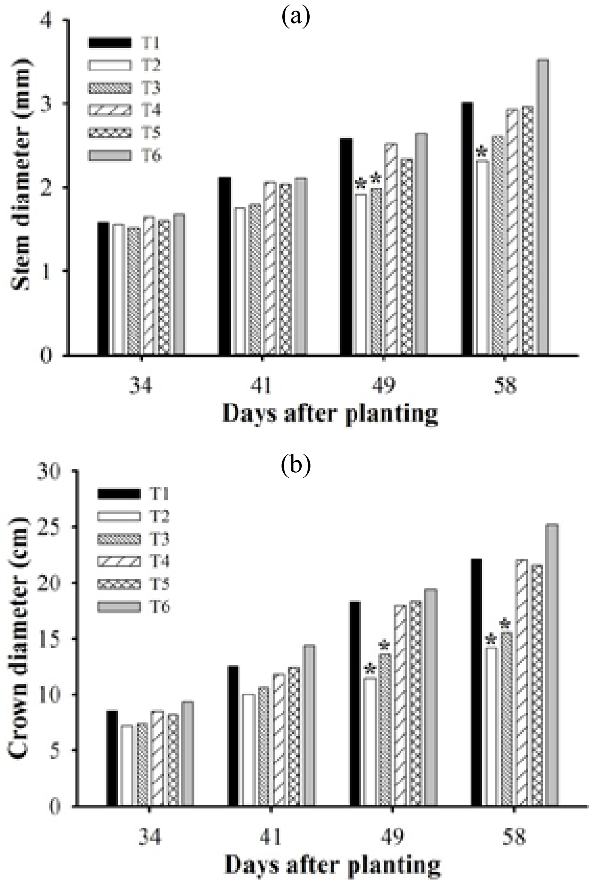

Figure 2. Stem diameter (a) and crown diameter (b) of *Passiflora edulis* f. *flavicarpa* seedlings under six treatments in four evaluations. Means followed by * are significantly different from conventional treatment (T1) at 5% (p <0.05) probability level by the Dunnett's test

Although leaf number did not present any significant difference at any evaluation time, the crown diameter (Figure 2 b) showed differences at 49 and 58 days after sowing. The conventional treatment showed more than 50% gain in crown diameter when compared to T2 in the evaluations carried out at 49 and 58 days after sowing. This shows that there was a gain in leaf area due to the greater expansion of the plant tissue, thus compensating for the leaf production and increasing the photosynthetic area of these plants. In addition, the doses of 263, 395, and 527 mL of tannery sludge as foliar fertilization showed results to the conventional treatment in all growth evaluations. This shows that the tannery sludge from the dose of 263 mL L^{-1} was efficient in supplying nutrients to the plants, ensuring a response pattern similar to the conventional treatment.

Consequently, it is apparent that the application of doses of tannery sludge provided the plants with a good development, which is also noted in the total fresh and dry mass of the plants (Table 2). These beneficial effects were reported by Sales *et al*. (2016) with the use of tannery sludge in seedlings of *Passiflora morifolia* Mast. However, the authors applied the sludge in the solid phase, as 25% of the substrate, and obtained total fresh and dry biomass equal to the conventional treatment. The dose 527 mL of tannery sludge provided a 55% gain of total fresh mass (Table 2) compared with the conventional treatment, however, this difference is not observed in the dry mass of the plant, which was the same as in the conventional treatment. The results of T2 and T3 were different from the conventional treatment in dry mass gain, 179% and 120% lower, respectively.

The color characteristics assessed by the colorimeter (Table 2) showed no significant differences. This shows that the instrument did not detect variations in the intensities of green, red, or yellow of the leaves of passion fruit seedlings under conventional foliar fertilization and different concentrations of tannery sludge foliar fertilization, even with relatively low coefficients of variation.

There were no differences in the anthocyanin index (ANT-RG and ANT-RB) and in the SFR-G, SFR-R and NBI-R indexes obtained by the respective green and red light excitation (Table 3). This shows that there was no greater synthesis of anthocyanins, chlorophyll, and nitrogen balance excited by red light, showing a lower sensitivity of the instrument at this wavelength.

Table 2. Means of color parameters L*, a *, b * and h^o of *Passiflora edulis* f. *flavicarpa* seedlings grown under conventional foliar fertilization and different levels of leaf-applied tannery sludge at 58 days of age

Treatment	TFM	TDM	L*	a*	b*	h^o
T1 - Conventional	8.81	1.48	36.15	-11.49	15.94	118.57
T2 – w/ fertilization	4.27*	0.53*	37.59	-11.10	15.85	118.05
T3 - 132 mL sludge	4.74*	0.67*	36.36	-11.03	15.12	119.66
T4 - 263 mL sludge	9.90	1.51	35.94	-11.09	15.09	119.76
T5 - 395 mL sludge	9.58	1.47	35.46	-10.96	15.24	120.18
T6 - 527 mL sludge	13.68*	2.10	35.52	-11.00	15.25	119.44
CV(%)	24.80	30.26	2.11	3.28	4.15	0.82

Means followed by * are significantly different from the conventional treatment at 5% ($P < 0.05$) probability level by the Dunnett's test. CV: coefficient of variation; TFM: total fresh mass; TDM: total dry mass; L*: lightness; a* and b*: chroma; h^o: hue angle

Table 3. Mean indices of flavonoids (FLAV), anthocyanins (ANT-RG and ANT-RB), chlorophyll (SFR-G and SFR-R), and nitrogen balance (NBI-G and NBI-R) calculated with Multiplex® measurements in leaves of yellow passion fruit seedlings grown under conventional foliar fertilization and different levels of leaf-applied tannery sludge

Treatment	SFR-G	SFR-R	FLAV	NBI-G	NBI-R	ANTH-RG	ANTH-RB
T1 - Conventional	1.44	1.37	0.53	0.28	0.34	-0.18	-0.83
T2 – w/ fertilization	1.32	1.21	0.57	0.22*	0.29	-0.18	-0.83
T3 - 132 mL sludge	1.30	1.20	0.59	0.22*	0.28	-0.17	-0.84
T4 - 263 mL sludge	1.41	1.35	0.58	0.25	0.33	-0.20	-0.86
T5 - 395 mL sludge	1.54	1.40	0.63	0.29	0.33	-0.18	-0.83
T6 - 527 mL sludge	1.62	1.47	0.84*	0.25	0.33	-0.21	-0.79
CV(%)	8.93	7.90	15.15	14.86	19.55	20.05	6.09

Means followed by * are significantly different from the conventional treatment at 5% ($P < 0.05$) probability level by the Dunnett's test. CV: coefficient of variation; nitrogen balance indices (NBI-G and NBI-R), chlorophyll (SFR-G and SFR-R), anthocyanins (ANT-RG and ANT-RB), and flavonoids (FLAV)

For the NBI-G index, the treatment that did not receive foliar fertilization (T2) and the treatment with the lowest dose of leaf-applied tannery sludge (T3) were the only ones that differed from the control treatment (Table 3). Thus, the Multiplex® sensor had a greater sensitivity for this characteristic, showing that the non-application of foliar fertilization and the lowest dose of tannery sludge (132 mL L^{-1}) were inferior to the conventional treatment for nitrogen balance; it is likely that the protein synthesis of these plants was reduced, since there was less dry mass produced.

The flavonoid index in Table 3 only showed difference in T6, with the highest sludge dose of 527 mL L^{-1} that favors a greater absorption of chromium and sodium by the stomata of the leaf tissue and promotes a greater production of this secondary metabolite. Chromium toxicity disturbs the plant physiology by producing reactive oxygen species (ROS) and causes changes in the absorption of mineral elements. Concurrently, the accumulation of excess intracellular sodium ions affects the bioenergetic processes of photosynthesis, causing peroxidation of membranes and increasing the flavonoid content to fight the oxidative stress caused by salinity (MASTALERCZUK *et al*., 2019; RAMOS-SOTELO *et al*., 2019; DIN *et al*., 2020).

Berilli *et al*. (2015) reported chromium accumulation in conilon coffee seedlings using tannery sludge in solid phase as substrate, while Sales *et al*. (2018b) found an increase in the flavonoid index in *Schinus terebinthifolia* seedlings grown in substrates with tannery sludge. Therefore, the chromium and sodium contained in the tannery sludge in the highest dose applied may have induced ROS in leaf tissues and, consequently, increased levels of flavonoids, given this compound intercepts and neutralizes ROS in stressful situations for the plant. Nevertheless, the plants responded satisfactorily, and the growth, color, or chlorophyll synthesis of plants grown with the dose of 527 mL L^{-1} of tannery sludge was not compromised.

The correlation analyses (Figure 3) showed a negative linear correlation between SFR-G and the L* parameter. Therefore, the higher the chlorophyll index, the lower the L* value. Lower L* (darker) associated with increased chlorophyll levels may be related to greater absorption of light by chlorophylls. Chlorophylls are green pigments found in oxygenated photosynthetic organisms, such as plants, algae and cyanobacteria, with essential roles of light energy absorption in the photosynthesis process (LI; CHEN, 2015; FERREIRA; SANT'ANNA, 2017).

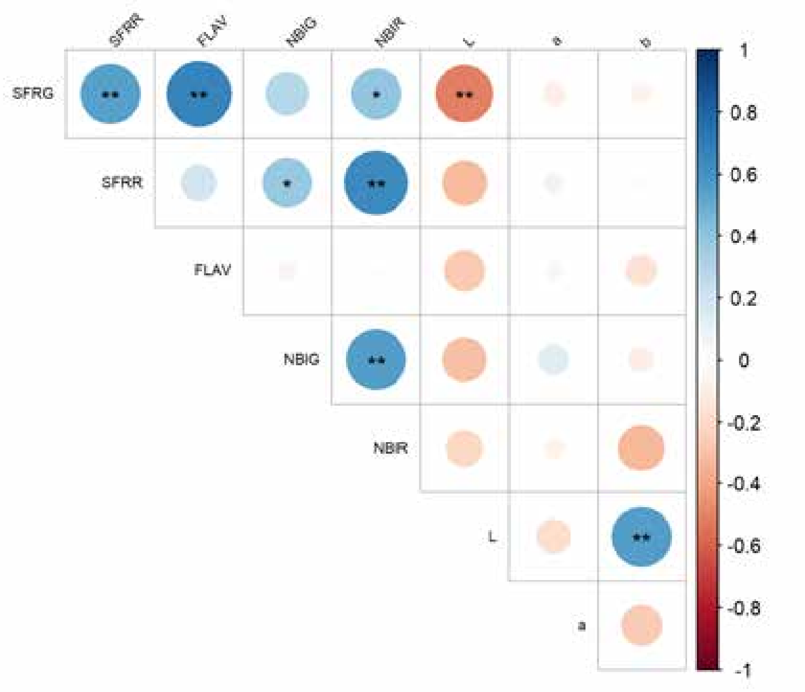

Figure 3. Association (Pearson's correlation) between the main variables studied. Significant at * $p < 0.05$; ** $p < 0.01$

In this study, a positive correlation was found between the parameters L* and b*. Thus, the greater the lightness provided by high L* values, the more yellow the color of the leaf (high b* value). This reveals that the more yellow leaves have greater reflectance among the leaf tissues of plants and may be linked to a greater thickening of the cuticle combined with a lower amount of chlorophyll, as previously mentioned, less chlorophyll reduces the absorption of light and, this means that more light will pass through the leaf tissues.

The chlorophyll index (SFR-G) showed a positive correlation with the flavonoids, contrasting with the reports by Coelho *et al.* (2012). This shows that there was no competition between the nitrogen used by the polyphenols and chlorophyll biosynthesis, indicating that the increase in flavonoids was not caused by the lack of nitrogen, but another toxic effect such as chromium and sodium present in the tannery sludge. In addition, the toxicity of these elements did not interfere in chlorophyll synthesis, which consequently did not interfere in the development of these plants.

CONCLUSIONS

- Tannery sludge has high potential as an alternative source of foliar fertilization in yellow passion fruit seedlings.
- The leaf application of doses between 263 and 527 mL L^{-1} of tannery sludge can be recommended for the production of yellow passion fruit seedlings.

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