Rooting biostimulants for *Coffea arabica* L. cuttings

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ABSTRACT

In the rooting process of Arabica coffee cuttings, plant hormones are used for inducing root formation. However, synthetic molecules cannot be used to form organic seedlings. The objective of this work was to evaluate the effect *Cyperus haspan* extract and different concentrations and types of humic substances on Arabica coffee cuttings. Arabica coffee shoots were collected, and cuttings containing at least two buds were taken. The experiment had a three-factor design, 2 (presence or absence *Cyperus haspan* extract) x 2 (humic or fulvic acid) x 4 (0, 10, 25 and 50 mg dm^{-3} concentrations). The following vegetative data were evaluated: shoot height (cm), survival, remaining leaves, leaf pairs, vigor, number of shoots,root length, area root, volume root, diameter root and fresh and dry mass were also evaluated. The enzyme activity of H+-ATPase, catalase, superoxide dismutase, alcohol dehydrogenase and esterase was also quantified. The results showed that the Arabica coffee cutting rooting rate was increased when treated with humic acid at a concentration of 10 mg dm^{-3} in the presence of *Cyperus haspan* extract. The lowest oxidative stress was observed in the 10 mg.dm^{-3} humic and fulvic acid treatments, regardless of the use of the extract.

Key words: Root system; IBA; humic acid; fulvic acid; shoots.

1 INTRODUCTION

Arabica coffee is entirely propagated via seeds since it is a crop with approximately 90% autogamy. Because it is a perennial crop, healthy and well-developed seedlings are vital for the successful establishment of new crops.

The number of studies on the vegetative propagation of Arabica coffee cuttings has increased recently (Pereira et al., 2018; Rezende et al., 2017), but this method is still little used due to the difficulty of establishing a standardized methodology because there is a large variation among the cultivars in how they respond to the rooting process (Carvalho, 2008).

The current methodologies used for the rooting of Arabica coffee cuttings use auxins for inducing root formation, and these cuttings activate vascular cambium cells to promote the formation of adventitious roots in the cuttings (Hartmann et al., 2011). IBA (indole-3-butyric acid) is a auxin growth regulator group, have been preferentially used because they are nontoxic and are the most effective for most species. However, synthetic molecules cannot be used for forming organic seedlings.

In the context of organic agriculture it is necessary to find alternatives to promote rooting. *Cyperus haspan*, considered the most important weed worldwide, is known for its allelopathic effects (Andrade; Bittencourt; Vestena, 2009). According to Lorenzi (2000), *C. haspan* has high level of IBA hormone, specific for plant root formation. IBA has been widely used to stimulate the rooting of cuttings of several species (Cruz Silva; Alves Neto; Viecelli, 2011). These

authors have reported that *Cyperus haspan* tubers contain a greater amount of auxins, like IBA, than any other herbaceous species.

An interesting alternative for promoting the rooting of coffee cuttings is the use of humic substances (HS). Humic substances are present in the decomposed organic fraction in the soil. They may have similar effects to auxins on plant metabolism. For example, the development of lateral roots is increased upon treatment with humic substances and is related to the cell division mechanisms regulated by auxins (Trevisan et al., 2010). Moreover, they are able to induce the exudation of several of chemical compounds in the root region, they also influence the size and structure of the microbial community, having an important role in plant nutrition (Canellas, 2019).

The aim of this study was to evaluate the effect of *Cyperus haspan* extract and different concentrations and types of humic substances in the rooting of Arabica coffee cuttings to enable the production of clonal seedlings for organic crops.

2 MATERIAL AND METHODS

The experiment was conducted at the Federal University of Lavras in the municipality of Lavras, Minas Gerais at the Department of Agriculture, Coffee Farming Sector where shoots (suckers) of Arabica coffee plants, cultivar Topázio MG1190, were collected. After the shoots were collected, 5 to 7 cm cuttings were taken that contained at least two buds; a pair of leaves was left on each cutting, which was then washed in pure water (Oliveira et al., 2010).

The cuttings were treated with *Cyperus haspan* extract by dipping their bases in the extract solution for 60 minutes and were then placed in substrate for rooting. The *Cyperus haspan* was made with 800 g of plant tubers collected at the Universidade Federal de Lavras campus according to the methodology proposed in Dias et al. (2012). The extract was prepared by grinding the tubers and dissolving them in a solution composed of 665 mL of distilled water and 335 mL of grain alcohol (Roncatto et al., 2008).

The humic substances were placed in the substrate (2:2:1 sand, clay, vermiculite) via water at a concentration of 0, 10, 25 and 50 mg dm^{-3}. The experiment was carried out in a greenhouse that had controlled relative air humidity (between 85-90%) and temperature (24 °C), with 50% shade cloth roofing and irrigation by an automated mist microsprinkler system.

At the end of the experiment (at 130 days) the following vegetative data were evaluated: shoot height (cm), survival, remaining leaves, leaf pairs, vigor and number of shoots. The root length, area, volume and diameter were also evaluated, and the fresh and dry root masses were quantified. The enzyme activities of H+-ATPase and the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), alcohol dehydrogenase (ADH) and esterase (EST) were also quantified.

To analyze the length, area, volume and diameter of the root, photographs of the cuttings were taken for analysis in the software SAFIRA, 2010 - Fiber and Root Analysis System (Jorge; Rodrigues, 2010). The vegetative data were analyzed by counting and measuring with centimeter rulers. The roots samples were washed and then weighed on an analytical balance to obtain the fresh mass. After weighing the samples, they were placed individually in labeled paper bags and placed in a forced-air oven at 65 ºC where they remained until reaching a constant weight, after which they were weighed on an analytical balance to obtain the dry mass. The leaves were removed and stored at -80 ºC for further evaluation of enzyme activity.

The Arabica coffee seedlings were macerated in polyvinylpyrrolidone (PVP) and liquid nitrogen. Afterwards, 100 mg samples were collected for analysis of each enzyme and stored in microtubes at -86 ºC.

The CAT activity was analyzed using a reaction medium consisting of 200 mM potassium phosphate (pH 7.0), water and 250 mM H2O2, which was then incubated at 30 °C while the hydrogen peroxide consumption was monitored (Havir; Mc Hale, 1987).

For SOD activity analysis, the reaction medium consisted of 100 mM potassium phosphate (pH 7.8), 70 mM methionine, 10 µM EDTA, water, 1 mM nitrotetrazolium blue (NBT) and 0.2 mM riboflavin. The cuvette containing the reaction medium and sample was illuminated for 7 minutes. For the control, the same reaction medium without sample was illuminated, while the blank was kept in the dark. SOD was assessed by the ability of the enzyme to inhibit NBT photoreduction. The readings were carried out at 560 nm, considering that one unit of SOD corresponded to the amount of enzyme capable of 50% inhibition of NBT photoreduction under the assay conditions (Giannopolitis et al., 1977).

For ADH assessment, 250 µL of extraction buffer (0.2 M Tris-HCl, pH 8.0) and 0.1% β-mercaptophenol were added. The samples were left overnight and were subsequently centrifuged at 14,000 rpm for 30 minutes at 4 °C. A 60 µL aliquot of supernatant was collected and applied to a 7.5% polyacrylamide separating gel with a 4.5% stacking gel. The electrophoresis was performed at 150 volts for 6 hours, upon which the gels were stained according to the methodology described by Alfenas (2006).

For EST assessment, protein extraction was performed by adding 320 µL of extraction buffer (0.2 M Tris) to 100 mg of seed powder, vortexing and storing for one hour in a refrigerator. The samples were centrifuged at 16,000 rpm at 4 ºC for 60 minutes, and 60 µL of the supernatant was applied to a polyacrylamide gel. A Tris-glycine (pH 8.9) gel/electrode buffer system was used. Electrophoresis was performed at 110 V for 5 hours, and the gels were stained for esterase activity, as described by Alfenas (2006). The interpretation of the results was based on a visual analysis of the electrophoresis gels, taking into consideration the intensity of each of the electrophoretic bands in the evaluated system.

For analysis of the H+-ATPase enzyme, the macerated samples were homogenized in 20 mL of ice-cold extraction medium containing 250 mmol L^{-1} sucrose, 10% glycerol (w:v), 0.5% PVP-40 (polyvinylpyrrolidone-40 kDa), 2 mmol L^{-1} EDTA, 0.2% BSA (bovine serum albumin) (w:v) and 0.1 M Tris-HCl, pH 7.5. The total protein concentration in the preparation was measured by the method in Bradford (1976).

The reaction was initiated with the addition of the protein (present in isolated vesicles) and stopped by adding trichloroacetic acid (TCA) to a final concentration of 10% (v:v).

A randomized block design with 4 replicates and 5 cuttings per plot was used. The experiment had a three-factor design, 2 (presence or absence *Cyperus haspan* extract) x 2 (humic or fulvic acid) x 4 (0, 10, 25 and 50 mg dm^{-3}), that is, 16 treatments and 64 plots with total use of 320 cuttings. Analysis of variance was performed for all of the evaluated characteristics, and when significant, qualitative variables were analyzed by the Scott-Knott test at 5% and quantitative variables by regression analysis. The SISVAR software was used for the analyses (Ferreira, 2014).

3 RESULTS AND DISCUSSION

In the analysis of variance there was no significant effect found for the studied sources regarding survival, vigor,

root length, root area and root volume. However, for the number of shoots, shoot height and number of leaf pairs there was a significant effect at the 5% significance level for the factor concentrations of humic substances. For the remaining leaves, there was a significant effect of the interaction between the concentrations and the types of humic substances.

There was a triple interaction for the characteristics of fresh and dry root mass, H^+-ATPase activity and antioxidant enzymes, that is, there were changes in the behavior of these variables in the presence and absence of *Cyperus haspan* extract with the increase of the concentration of humic substances.

The shoot height and number of shoots showed similar curves with increasing concentrations of humic substances. Peaks were obtained at the highest and lowest concentrations (0 and 50 mg dm^{-3}) (Figure 1). The promotion of plant growth by humic substances is well documented (Nardi et al., 2009). In general, the growth response of monocotyledons to HS appears to be higher than that of dicotyledons, although the molecular and physiological basis for this difference remains obscure (Canellas et al., 2011).
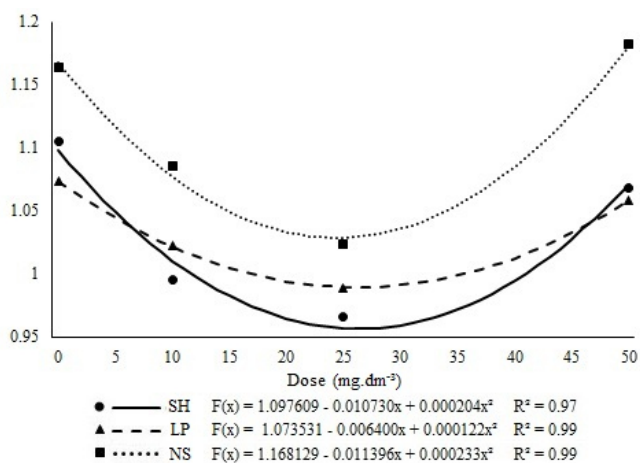
Figure 1: Shoot height (SH) in centimeters, number of leaf pairs (LP) and number ofshoots (NS) according to humic concentrations.

However, in the case of Arabica coffee, the response of the shoots was not favorable to the use of these substances, one hypothesis to explain these results would be that the plant under stress, is induced to the production of hormones, which in the case of stress such as cutting, would be the production of auxins, which regulate the rooting and emission of shoots (Hartmann et al., 2011). By starting to add humic substances to the substrate, it signaled to the plant that it would not need the production of these hormones, but the concentrations were still low, not inducing buds. Adding higher doses of humic substances to the substrate consequently increased the auxin doses, thus leading to increased size and number of shoots (Geiss; Gutierrez; Bellini, 2009).

It was observed that for shoot height, number of leaves and number of shoots there was a decreasing behavior up to the 25 mg dm^{-3} dose of humic substances and from that dose there was a vegetative increment with linear response as it increases the dosages of humic substances. When analyzing the development with increasing concentrations of humic acid, the number of remaining leaves presented higher values at a concentration of approximately 40 mg dm^{-3} (Figure 2).
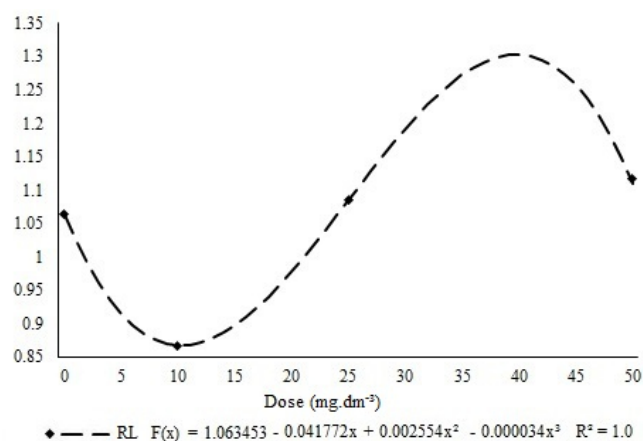
Figure 2: Number of remaining leaves (RL) as a function of the humic acid concentration.

Leaves are essential for the rooting of cuttings because the metabolites generated by photosynthesis, together with the auxins, are of vital importance for root formation (Van Overbeek et al., 1946). Ferreira et al. (2008) stated that sprouting before rooting can be harmful to plants, but if the opposite occurs–if roots are first formed followed by sprouting-it can indicate that the cutting will be able to form a new plant. The remaining pair of leaves is expected to assist in this balance between the shoot and the root until new leaves are formed.

The mean diameter of the roots was higher in the presence of humic acid. However, for root diameter, the finer roots with smaller diameters had a greater nutrient uptake efficiency; in this case fulvic acid was better.

The mean dry mass of the root system was higher when fulvic acid was used. The dry matter data are important for growth analysis because they can be used to compare several factors under study in different situations. The H+-ATPase enzyme activity was higher when humic acid was applied to the substrate. The enzyme H+-ATPase, which is also a proton pump, is active in the plasma membrane and is involved in the uptake of nutrients, creating a driving force for the ions to move across the membrane. By increasing its activity, nutrient use can also be increased, affecting not only the root system but also the development of the whole plant (Trevisan et al., 2010). The effects of HS on plant physiology include the promotion of root growth (Nardi et al., 2009).

The fresh and dry mass of the roots varied with increasing concentration, type of acid and the presence or absence of *Cyperus haspan* extract. When using humic acid without *Cyperus haspan* extract, both the fresh and dry mass showed a peak at the 10 mg dm⁻³ concentration (Figure 3A). When *Cyperus haspan* extract was added to the treatment, only the fresh mass was shown to be significant, increasing with increasing extract concentration (Figure 3B). When fulvic acid was used without the *Cyperus haspan* extract, the fresh mass showed two peaks (at 10 and 50 mg dm⁻³), while the dry mass varied little, with a peak at the 10 mg dm⁻³ concentration (Figure 4A). When using the *Cyperus haspan* extract in combination with fulvic acid, the fresh mass also had a peak at the 10 mg dm⁻³ concentration, and the dry mass increased after 40 mg dm⁻³ (Figure 4B). The *Cyperus haspan* extract had a stronger effect when the substrate was treated with humic acid rather than fulvic acid, which may be due to the combined effect of the auxins present in both the humic acid and extract. Morard et al. (2011) found satisfactory responses in cucumber, maize, pelargonium and wheat when analyzing the effect of humic substances on the fresh mass of roots and on other parts of the plants, and they observed a biostimulating effect from these substances.

According to Casimiro et al. (2003), the increase in the number of roots is linked to the action of auxins on the target cells, which resume cell differentiation activities. Therefore, it can be inferred that the concentration of auxins in the natural *Cyperus haspan* extract is not high enough to increase the number of roots.

When using humic acid, the H^+-ATPase activity increased linearly with increasing humic acid concentration. For fulvic acid, the increase followed a quadratic behavior and the relationship with increasing dose was not as strong, in addition to declining after a certain point (Figure 7). Promotion of the H^+-ATPase enzyme activity could also promote the development of the root system. H^+-ATPase is more commonly known as a proton pump and is primarily responsible for the uptake of nutrients by the roots, generating an electrochemical gradient that induces the transport of nutrients across the membrane of the root cells.
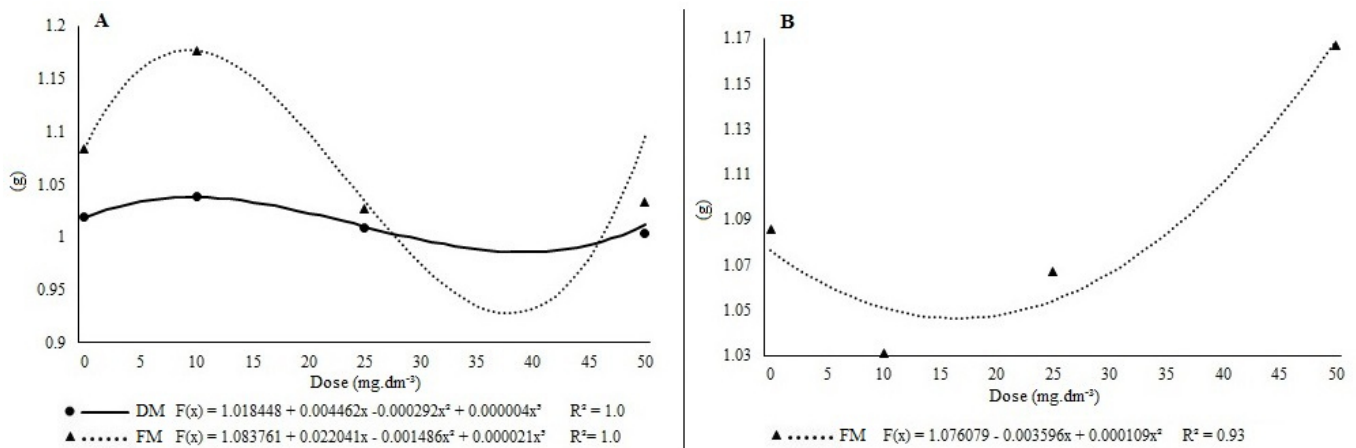

Figure 3: Dry mass (DM) and fresh mass (FM) of the root, expressed in grams, as a function of humic acid concentration in absence (A) and presence *Cyperus haspan* extract (B).
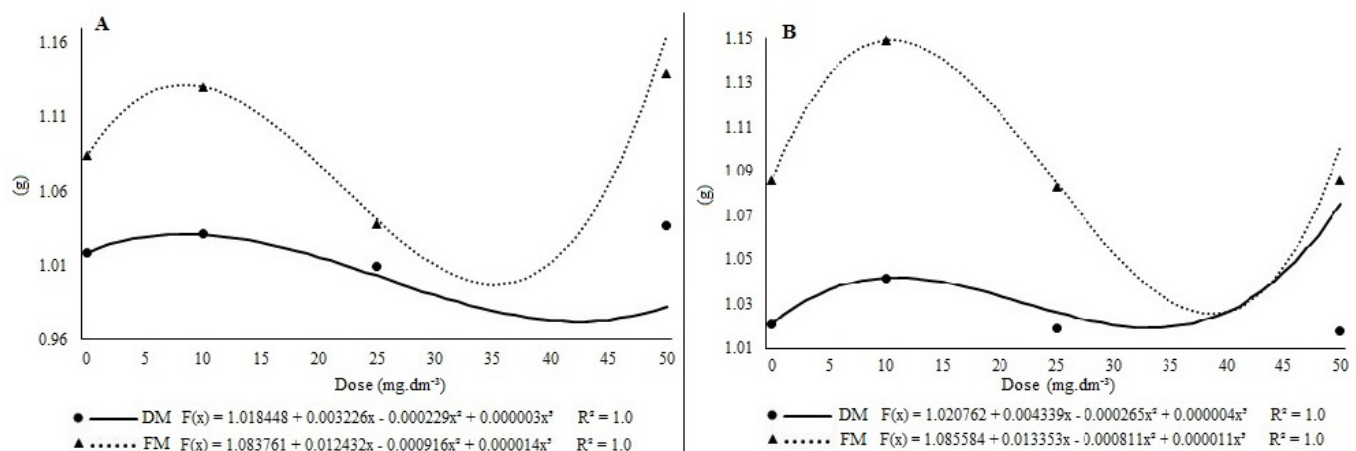
Figure 3A equations:
DM $F(x) = 1.018448 + 0.004462x - 0.000292x^2 + 0.000004x^3$ $R^2 = 1.0$
FM $F(x) = 1.083761 + 0.022041x - 0.001486x^2 + 0.000021x^3$ $R^2 = 1.0$

Figure 3B equations:
FM $F(x) = 1.076079 - 0.003596x + 0.000109x^2$ $R^2 = 0.93$

Figure 4: Dry mass (DM) and fresh mass (FM) of the root, expressed in grams, as a function of fulvic acid concentration in absence (A) and presence *C. haspan* extract (B).

Figure 4A equations:
DM $F(x) = 1.018448 + 0.003226x - 0.000229x^2 + 0.000003x^3$ $R^2 = 1.0$
FM $F(x) = 1.083761 + 0.012432x - 0.000916x^2 + 0.000014x^3$ $R^2 = 1.0$

Figure 4B equations:
DM $F(x) = 1.020762 + 0.004339x - 0.000265x^2 + 0.000004x^3$ $R^2 = 1.0$
FM $F(x) = 1.085584 + 0.013353x - 0.000811x^2 + 0.000011x^3$ $R^2 = 1.0$

In the case of Arabica coffee, the enzyme activity was increased but had no effect on the roots. Indicating that not necessarily the increase in root length or root mass will reflect better nutrient utilization, as happens when H^{+}-ATPase activity is increased, since the activation of this proton pump is decisive in the transport of nutrients through the membranes of the roots. cells (Palmgren, 2001). H^{+}-ATPase is the main ally to nutrient uptake and utilization, so by interacting with the biostimulant effects of humic substances, plants improve nutrient uptake with increasing enzyme (Canellas et al., 2009; Jindo et al., 2012; Pinton et al., 1999).

When using 10 mg dm^{-3} of humic substance, the fresh mass had higher mean values in the absence of *Cyperus haspan* extract, whereas at the 25 and 50 mg/dm³ concentrations the mean values were higher in the presence of the extract. The mean H+-ATPase enzyme activity was higher when the extract used at 10 and 25 mg dm^{-3}, whereas at 50 mg dm^{-3} the mean was higher in treatments without the extract (Table 1). This is explained by the difference in the auxin concentrations when the *Cyperus haspan* extract and the humic substances are mixed at different concentrations. In the case of the fresh mass, the lowest concentration of humic substance combined with the extract may not have reached an auxin concentration capable of promoting a positive effect, while the opposite occurred in the case of H+-ATPase, where the highest concentration combined with the extract may have led to auxin concentrations so high that they became toxic.

Table 1: Mean fresh mass (FM) and H+-ATPase enzyme activity at each significant acid concentration, in the presence (WE) or absence (WoE) *Cyperus haspan* extract.

	10 (mg dm^{-3})		25 (mg dm^{-3})		50 (mg dm^{-3})	
	FM (g)	H^{+} (U/mL)	FM (g)	H^{+} (U/mL)	FM (g)	H^{+} (U/mL)
WoE	1.153 a	0.995 b	1.032 b	1.063 b	1.087 b	1.393 a
WE	1.090 b	1.177 a	1.075 a	1.256 a	1.126 a	1.354 b

Means followed by the same letter in the column did not differ by the Scott-Knot test, p<0.05.

When analyzing the concentrations separately, the results were similar. When fulvic acid was used, the highest means were observed in the absence of the extract, except for the 50 mg dm^{-3} concentration. There are no previous reports on the effects of *Cyperus haspan* extract on H+-ATPase enzyme activity; however, in Arabica coffee it caused a slight increase in that activity. However, there are several reports showing increasing H+-ATPase activity when humic substances are used. In studies with coffee and maize species, the use of humic substances indicates the activation of the plasma membrane, more specifically H+-ATPase, to be a possible metabolic marker that enables humic acid to actively participate in biological reactions (Façanha et al., 2002).

The mean fresh and dry mass at the 10 mg/dm³ concentration were higher in the presence *Cyperus haspan* extract and when treated with fulvic acid. At 50 mg/dm³, the fresh mass had a higher mean when treated with humic acid in the presence of the extract, and when treated with fulvic acid in the absence of the extract. The mean dry mass of the roots treated with humic acid was higher in the presence of the extract. When evaluating the effect of *Cyperus haspan* extract on the rhizogenesis of *Solanum lycopersicum* leaves, Souza et al. (2012) obtained satisfactory fresh and dry mass values for rhizogenesis and considering the use of the extract to be a promising organic treatment.

There was a linear increase in the H+-ATPase enzyme activity for humic acid in the absence of *Cyperus haspan* extract, but when the extract was added to the treatment it caused a quadratic increase at lower concentrations of humic acid. Fulvic acid, in both the presence and absence of extract, showed a slight quadratic increase (Figure 5). Considering these results for the enzyme, it is evident that its activity is more strongly promoted in treatments with humic acid. The extract affected the results only slightly, but with a positive and significant effect. Increases in H^{+}-ATPase enzymes results in better efficiency of water use and nutrients, such as nitrogen (Azevedo, 2019).

H^{+}ATPase (HA WoE) $F(x) = 0.734335 + 0.017112x$ $R^2 = 0.94$
H^{+}ATPase (HA WE) $F(x) = 0.831439 + 0.043157x - 0.000537x^2$ $R^2 = 0.94$
H^{+}ATPase (FA WoE) $F(x) = 0.787613 + 0.021121x - 0.000285x^2$ $R^2 = 0.93$
H^{+}ATPase (FA WE). $F(x) = 0.802564 + 0.016363x - 0.000233x^2$ $R^2 = 0.84$
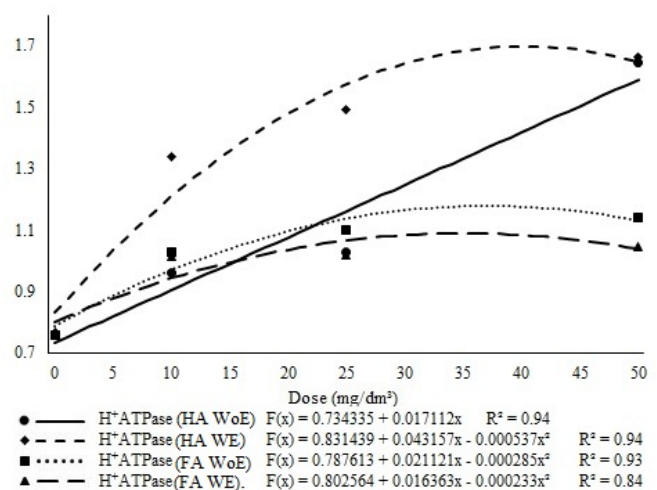
Figure 5: H+-ATPase enzyme activity (U/mL) as a function of humic acid concentrations in the absence *Cyperus haspan* extract (HA WoE), humic acid concentrations in the presence *Cyperus haspan* (HA WE), fulvic acid concentrations in the absence *Cyperus haspan* extract (FA WoE), fulvic acid concentrations in the presence *Cyperus haspan* extract (FA WE).

Dias et al. 2012 did not find *Cyperus haspan* extract to be a viable alternative for inducing rooting when analyzing its use in the rooting of Robusta coffee cuttings, even with

increased immersion times. In mofumbo, the extract was also not sufficient to promote the rooting of the cuttings (Oliveira et al., 2014), and the same effect was observed in golden dewdrop (Rezende; Zuffellato-Ribas; Koehler, 2013). However, for sugarcane the use of *Cyperus haspan* extract was very beneficial for rooting (Cruz Silva; Alves Neto; Viecelli, 2011).

Although *Cyperus haspa* has large quantities of IBA hormone in its leaves and roots, it also has many other constituents capable of producing negative allelopathic responses when in contact with some species (Andrade; Bittencourt; Vestena, 2009). That is one of the hypotheses for the fact that the extract did not have a strong effect on the parameters analyzed in this study.

The mean activities of catalase, superoxide dismutase and alcohol dehydrogenase were higher when in the presence *Cyperus haspan* extract compared to its absence. However, the mean esterase activity was higher in the absence of extract. In turn, in both the presence and absence of extract, humic acid led to statistically superior mean activities for catalase, superoxide dismutase and alcohol dehydrogenase, whereas for esterase, fulvic acid led to higher mean values in the cuttings, with and without *Cyperus haspan* extract.

These enzymes are important to help counteract the stress caused by reactive oxygen species, which can become very problematic in the process of seedling formation by vegetative propagation in coffee because they can cause lipid peroxidation in the plasma membrane, which can culminate in cell death and the death of the cutting. Among the reactive oxygen species, those that more strongly affect plants are the superoxide anion radical (O_2^-), the hydroxyl radical (HO^-) and hydrogen peroxide (H_2O_2), which are produced by the disruption of metabolic pathways and by other normally functioning pathways (Andrade; Bittencourt; Vestena, 2009).

In the case of antioxidant enzymes, an increase in value is not indicative of improvement, but rather indicates that there is some type of stress in the environment, which may be due to the treatments used. For esterase, the lowest stress was observed for the treatment with humic acid in the absence of the extract. At the 25 mg dm⁻³ concentration, catalase, superoxide dismutase and alcohol dehydrogenase showed that the cuttings suffered less stress when using fulvic acid regardless of the presence or absence of extract, whereas for the esterase the same was observed in the treatment with humic acid and *Cyperus haspan* extract. At the 50 mg dm⁻³ concentration, the four enzymes expressed the lowest stress for the treatment with fulvic acid in the presence of extract, except for esterase for which lower stress was observed in the absence of extract. This was due to the various compounds present in the extract and in the humic substances, which can have other effects. In general, the treatments with fulvic acid and in the presence of *Cyperus haspan* extract showed the lowest stress.

Humic acids, especially when used with growth-promoting bacteria, are able to significantly alter metabolic fingerprints. The effects of biostimulants such as these stimulate primary and secondary metabolism in non-leguminous plants. In addition, they can be used to genetically manipulate metabolic pathways to aid plant breeding programs (Canellas; Olivares; Canellas, 2019).

Humic acids are soluble in alkaline media, and fulvic acids are soluble in alkaline and acidic media so regardless of the substrate pH, fulvic acids will dissolve in the solution and be readily available for uptake by the plant. Fulvic acids have a low molecular weight, which facilitates their passage through the plasma membrane in the roots and thus can move within the plant and interact with or even influence its physiology and metabolism (Anjum et al. al., 2011). This means that foliar application rather than soil is a possible practice to improve the growth and nutrition of coffee seedlings, leading to improvements in leaf micronutrient accumulation (Justi; Morais; Silva, 2019).

Catalase, superoxide dismutase and alcohol dehydrogenase showed a decrease in activity with increasing humic acid concentrations in the treatments in absence *Cyperus haspan* extract until reaching a minimum value at approximately 30 mg dm⁻³. In contrast, esterase activity increased linearly with increasing humic acid concentration (Figure 6A). When using the *Cyperus haspan* extract in the treatment, there was almost no activity of the former three enzymes at the 10 mg dm⁻³ concentration, whereas the esterase activity decreased until the 25 mg dm⁻³ concentration, followed by a slight increase (Figure 6B). In the treatments with fulvic acid, both in the presence and absence of the extract the activity of catalase, superoxide dismutase and alcohol dehydrogenase were zero at approximately 20 mg dm⁻³. While the esterase activity oscillated, the lowest value was observed at 0 mg dm⁻³ in both situations (Figure 7). It was evident that the *Cyperus haspan* extract interacts with humic acid, leading to a decrease in the concentration of the acid. However, the interaction with fulvic acid was not as straightforward.

There are currently no studies of the effects *Cyperus haspan* extract on antioxidant enzymes, but it is known that aqueous and alcohol extracts of *Cyperus haspan* contain phenols that act directly on the indoleacetic acid (IAA) oxidase/peroxidase system of plants, regulating and inhibiting the oxidation of indoleacetic acid (Suzuki et al., 2012).

Reactive oxygen species are involved in various plant metabolic processes, including the regulation and development of plant growth and responses to biotic and abiotic stresses (Suzuki et al., 2012). The cutting process exerts a great stress on the cuttings, and humic substances have been shown to help mitigate the effects of this stress. To protect lipids from peroxidation, humic acids assist in the activation of antioxidant enzymes, helping to eliminate reactive oxygen species (García et al., 2014).
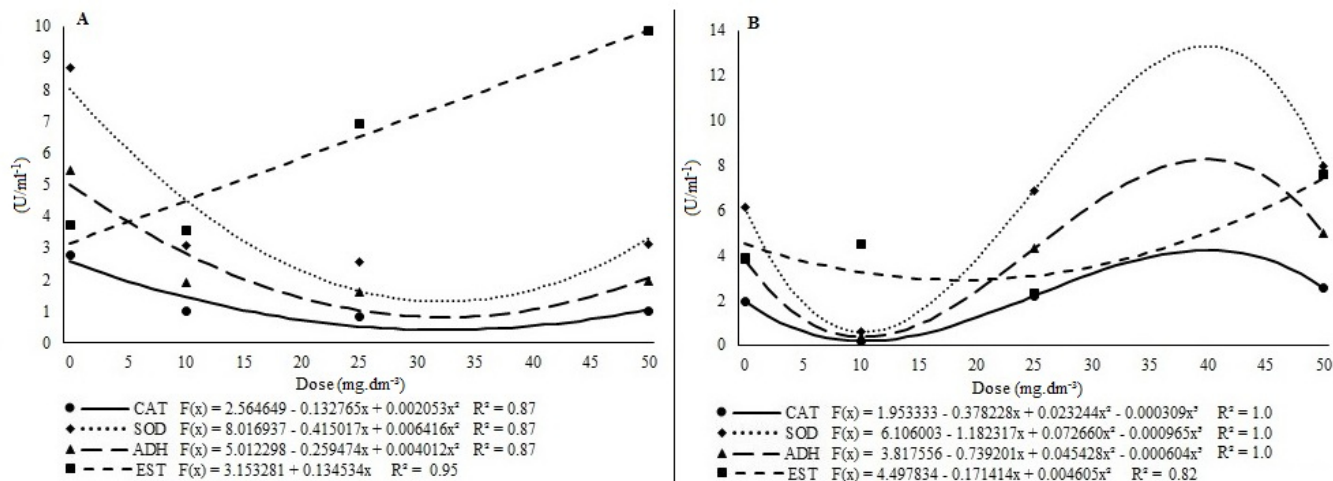
Figure 6: Activity of catalase (CAT), superoxide dismutase (SOD), alcohol dehydrogenase (ADH) and esterase (EST) according to the humic acid concentrations in absence *C. haspan*(A) and presence *Cyperus haspan* extract (B).
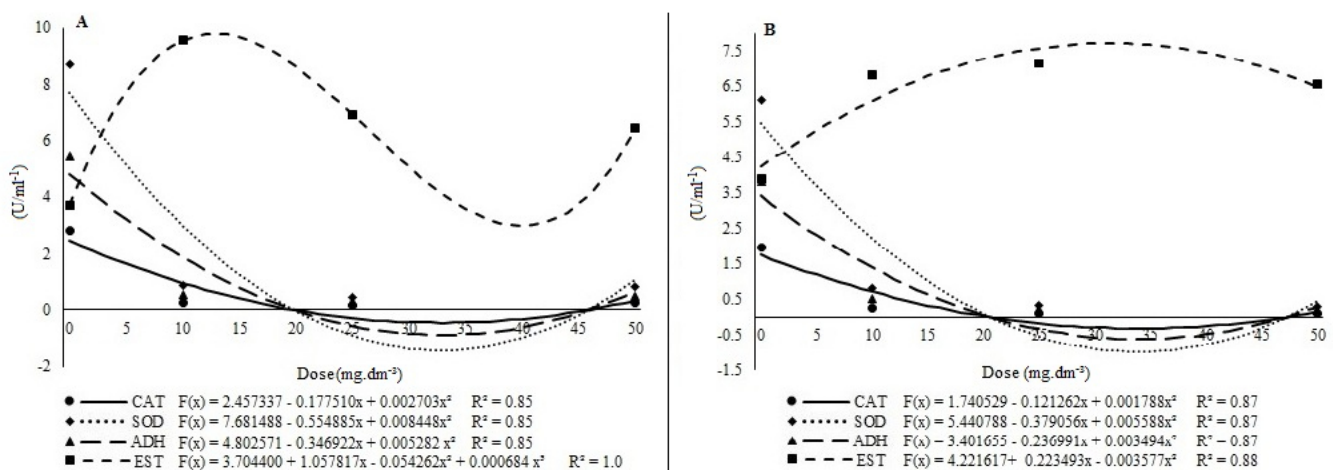

Figure 7: Activity of catalase (CAT), superoxide dismutase (SOD), alcohol dehydrogenase (ADH) and esterase (EST) according to the fulvic acid concentrations in absence *C. haspan* (A) and presence *C. haspan* extract (B).

4 CONCLUSIONS

There is a higher rooting rate of Arabica coffee cuttings when they are treated with humic acid at 10 mg dm^{-3} in the presence *Cyperus haspan* extract.

Further analysis and studies on the use of *Cyperus haspan* extract and humic substances in the Arabica coffee crop are needed to obtain more conclusive results.

5 ACKNOWLEDGEMENTS

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