



Use of *Cladosporium sp.* as a bioprotector of coffee quality in different post-harvest conditions

Felipe Aparecido de Paiva¹ , Bruno Manoel Rezende de Melo¹ , Sindynara Ferreira¹ ,
Emanuelle Morais de Oliveira¹ , Telma Miranda dos Santos¹ , Douglas Goulart Castro^{2*}

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ABSTRACT

The pre-harvest application of the bioprotective agent *Cladosporium cladosporioides* is an alternative to inhibit these fermentative processes that might occur in coffee beans that were either their storing bag or exposed to simulated rain, because a controlled amount of water was applied to the coffee. The objective of this study was to evaluate the effect of *Cladosporium cladosporioides* (Fresen) de Vries in the physical-chemical and sensory of the coffee beans. The study was conducted in an arabica coffee plantation, in the municipality of Inconfidentes, state of Minas Gerais, Brazil. In this study used four different doses of the bioprotector and three different pos-harvest conditions. The sour defective coffee beans, electrical conductivity, total titratable acidity, soluble solids, color and it was determined the general quality of coffee of both the left-in-bag and rainfall-exposed variants were positively influenced by the bioprotector. The bioprotector promoted an increase in the quality of the physical and chemical composition and sensory quality of rainfall and left-in-bag coffees compared to coffee beans without bioprotector. The bioprotector also maintained quality in rainfall and left-in-bag coffee when compared with the yard-grown variant. The sensory quality of the yard-grown coffee was not influenced by the bioprotector.

Keywords: *Coffea arabica* L.; biological agent; rain; bagged coffee; fermentation.

INTRODUCTION

The production of quality coffee beans aims at a growing demand of a public that look for sensory attributes found when consuming a beverage special, in addition to aspects related to food safety. However, there is a need to improve high quality coffee production. Post-harvest complications such as issues in transportation to the drying site due to low daily yield, as well as poor size management of the yard not allowing for the processing of all harvested yield, leads to a scenario in which coffee beans are left in their storing bags in the yard.

According to Angélico *et al.* (2011) coffee beans harvested and left bagged for drying can to present high levels

of infestation by yeasts and fungi of the genera *Aspergillus*, *Fusarium* and *Penicillium*, which lead to undesirable fermentation and loss of quality on the final beverage. Furthermore, these fungi can produce mycotoxins, making the coffee unsuitable for consumption (Silva *et al.*, 2008).

Another limiting factor for coffee quality in southern Minas Gerais is the occurrence of rainfall in the winter, coinciding with the harvest. This favors the development of fungi, bacteria and yeasts that cause undesirable fermentations in coffee. These microorganisms influence the quality and safety of the product (Barcelo & Barcelo, 2018; Gil-Serna *et al.*, 2011).

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¹ IFSULDEMINAS-Campus Inconfidentes, Departamento de Agricultura, Inconfidentes, MG, Brazil. felipepaiva_2506@hotmail.com; bruno.melo@ifsuldeminas.edu.br; sindynara.ferreira@ifsuldeminas.edu.br; emanuelle.oliveira@ifsuldeminas.edu.br; telma.miranda@ifsuldeminas.edu.br

² Universidade Federal de Uberlândia, Instituto de Ciências Agrárias, Uberlândia, MG, Brazil. douglasgoulartcastro@gmail.com

*Corresponding author: douglasgoulartcastro@gmail.com

To reduce these problems and prevent the use of fungicides, studies conducted with fungi of the genus *Cladosporium* sp. indicated that these microorganisms are very promising regarding their use as biological agents in several crops (Islam *et al.*, 2019), due to a metabolite called cladosporol, which has an inhibiting activity in the germination and development of other fungi (Nasini *et al.*, 2004) and the bacterium *Candidatus Liberibacter asiaticus* (Blacutt *et al.*, 2020).

In coffee, research has shown that the presence of *Cladosporium* sp. in coffee beans is related to the quality of the final beverage (De Pereira *et al.*, 2005). Chalfoun *et al.* (2007) identified that in the presence of *Cladosporium cladosporioides* (Fresen) de Vries there was no development of fungi harmful to coffee quality, thus denominating this organism as a “bioprotector”.

It was previously verified that the increase in the population of *Cladosporium* sp. coincided with the conversion of phenolic compounds into sugars, and thus the application of the bioprotective agent should begin as soon as the fruit is ripe (Chalfoun, 2010). Angélico *et al.* (2017) reported that the fungus *C. cladosporioides* (Fresen) de Vries is antagonistic towards the fungus *A. ochraceus* through antibiosis and hyperparasitism.

Despite the efficiency of *Cladosporium* sp. in maintaining coffee quality and antagonizing other fungi, there is no scientific information on its efficiency in coffee beans that undergone fermentation due to rainfall in the first days of the drying process or that were bagged for more than one day before spreading for drying. As such, there is relevancy of research on the efficiency of the bioprotective agent *Cladosporium* sp. in the post-harvest management of coffee.

Therefore, this study aims to evaluate the effect of *C. cladosporioides* (Fresen) de Vries on the physical, chemical and sensory quality of coffee in different post-harvest conditions.

MATERIAL AND METHODS

The study was conducted in the coffee sector of the Federal Institute of South of Minas Gerais - Campus Inconfidentes, in the state of Minas Gerais, Brazil, in the following geographical coordinates: Latitude 22°19'01" S, longitude 46°19'40" W, with altitude of 896 m.

The cultivar of coffee used was Topázio Amarelo (*Coffea arabica* L.), planted on the School Farm of the Institute. The harvested coffee had 48% of ripe fruits, 12% immature

fruits and 40% of dried fruits. It was not washed before drying process.

In this study was used a completely randomized design (CRD), composed of a factorial of 4 × 3, with: four different concentrations of the bioprotector [*Cladosporium cladosporioides* (Fresen) de Vries]: T1 = 0 ml/20 l of water (control), T2 = 200 ml/20 l of water, T3 = 400 ml/20 l of water, T4 = 600 ml/20 l of water); and three coffee conditions post-harvest conditions: rainy coffee, yard-grown and left-in-bag, amounting for 12 treatments and three replications. The bioprotectant had, respectively, a minimum viability of 8.5 × 10⁷ CFU (colony forming unit) with 85% germination and 1% spore count.

The coffee treatment that did not receive simulated rain and don't stayed in the coffee plantation for 48 hours in bags was designated as yard-grown coffee. Yard-grown coffee as well as the treatment with simulated rainfall were harvested and placed in bags of raffia palms, on the shade, with the upper part open and was transported to the yard at 4 pm of the same day. The yard-grown coffee was placed on the experimental plots and received the bioprotector soon thereafter.

For the treatment of rainfall coffee, a precipitation of 18 mm was simulated in each plot for a period of two consecutive days, without shifting the coffee beans, right after its allocation in the yard. A 1-mm sheet of water was poured every hour between 8 am and 4 pm. The bioprotector was applied soon after the end of the rain simulation.

The left-in-bag coffee stayed in the field under shade in the raffia bags for a period of 48 hours and was then transported to the yard. Then, the bioprotector was applied on the yard of drying.

The experimental plots had 1 m², and in each one 10 liters of coffee were placed. The bioprotector was diluted in water and applied with a back-strapped sprayer with flow of 10 liters/100 m² of yard. For application, a fan-spray nozzle 0.3 was used at an angle of 110°.

The experiment was carried out in a cement yard, and the drying process was performed with the coffee beans in a single layer (fruit to fruit) until the third day without moving. From this point on, the coffee layer was folded three times every two days, with eight shifts a day, reaching at the end of this period a height of 6 cm. By the time the coffee reached 28% moisture, the plots were heaped up at 3 pm and covered with raffia and canvas fabric until 9 am the next day, when it was again arranged in a uniform drying layer of 6 cm until the coffee beans reached 11 to 11.5%

moisture. After this procedure, the plots were placed in a kraft paper bag and stored in a box for 15 days; a three hundred grams of the samples were then peeled and felt for physical, chemical and sensory analysis.

After processing, coffee beans classification was carried out according to Normative Instruction (Brasil, 2003). The number of sour defective coffee beans was then determined.

The physical, chemical analyses were performed in the Soil and Bromatology Laboratory of the IFSULDEMINAS - Campus Inconfidentes. The variables evaluated were total titratable acidity (TTA) (AOAC, 1990) and soluble solids (SS) (AOAC, 1990). The color of the coffee was determined using a Konica Minolta colorimeter - model CM-2300 Iluminante D65, calibrated before readings. The color values were given in parameters of the L*, a* and b* scale, which are representatives of the color luminosity; L* = 0 represents black and L* = 100 represents white, while a* indicates the variance between red and green and b* the variation between yellow and blue. Electrical conductivity (EC) was also quantified (Prete & Abrahão, 1995).

Sensory analyses (SA) were carried out by three trained and qualified tasters of specialty coffees (Q-Graders), using the methodology proposed by the Specialty Coffee Association of America - SCA (2016).

It was conducted variance analyses as well as polynomial models for the effect of the bioprotector doses, for the specific characteristics within the treatments. The criterion for choosing the model was significance by the F test at a 5% probability of error, with the highest significant value for the coefficient of determination (R²). For the qualitative parameters that presented statistical differences, the Tukey test ($p \leq 0.05$) (1974) was carried out, using the Sisvar software (Ferreira, 2011).

RESULTS

From the results (Table 1), verified that there was significant interaction between post-harvest conditions and doses of *Cladosporium sp.* for the variables: TTA, SS, color b*, color L*, SA, number of defective sour coffee beans and electrical conductivity. This way there are a effect of dose of the product varied between for different post-harvest conditions. For color a* there were no differences between the isolated factors or the interaction.

The data for the TTA showed that in the absence of the bioprotector, yard-grown coffee obtained the lowest value, compared with the other treatments (Table 2). The highest TTA values found for both left in bag and rainfall coffee, in the absence of the bioprotector, can be attributed to the fermentative processes that occurred in the coffee beans (Lima Filho *et al.*, 2013).

According to the regression analysis, the mean values of TTA decreased with the increase of doses, with the values of 600 ml and 387.56 ml of bioprotector promoting the lowest values for rainfall and left in bag coffee, respectively. This was not significant for yard-grown coffee, given the non-occurrence of fermentation in this type of coffee (Figure 1).

For color a*, there were no differences in the treatments. However, the values tended to approach 0, indicative of green coloration, which is the product's desirable color (Table 3).

The regression analysis for bioprotector application indicated an improvement in color quality, with the doses of 310 ml and 390 ml presenting the lowest values of color b*, for the left-in-bag and rainfall coffees, while regression was not significant for the yard-grown coffee (Figure 2).

The application of the bioprotector promoted different results for the number of defective sour coffee beans between the rainfall and left-in-bag conditions when compared to the yard-grown; at dose 0 of the bioprotector, however, there were no differences between treatments (Table 4).

Table 1: Summary of variance analysis for total titratable acidity (TTA), soluble solids (SS), color a*, color b*, color L*, sensory analysis (SA), number of defective sour beans, and electrical conductivity (EC)

SV*	DF*	(p ≤ 0.05)							
		TTA	SS	Color L*	Color a*	Color b*	SA	Sour defective beans	EC
Conditions	2	0.000	0.316	0.000	0.083	0.048	0.000	0.000	0.000
Doses	3	0.000	0.179	0.067	0.126	0.000	0.000	0.000	0.000
Cond.*doses	6	0.000	0.013	0.000	0.229	0.018	0.000	0.000	0.000
CV %		1.72	4.97	8.3	30.07	4.22	0.53	3.22	1.72

*Source of variation. Degrees of freedom.

Table 2: Total Titratable acidity (TTA), soluble solids (SS) as a function of different post-harvest conditions and doses of *Cladosporium sp.*

Doses	Post-harvest conditions								
	(TTA)			SS			Color L*		
	Rainfall	Left-in-bag	Yard-grown	Rainfall	Left-in-bag	Yard-grown	Rainfall	Left-in-bag	Yard-grown
0	341.4b	349.9c	296.9a	26.4a	25.0b	27.3a	37.6aA	45.8bA	41.3bA
200	336.4c	242.1a	297.0b	25.7b	29.2a	29.0a	34.6aA	46.5bA	35.7aA
400	329.9c	250.6a	294.7b	28.3a	28.3a	26.6a	41.2aA	49.1bA	42.7aA
600	289.2b	257.4a	298.7c	28.0a	30.0a	26.3a	32.1aA	36.6aA	44.9bA

Means followed by the same lowercase letter in the rows and uppercase in the columns do not differ by the tukey test at 5% probability. *= Luminosity.

The results of the regression analysis (Figure 3) showed that there is a reduction in the number of sour defective coffee beans with the increase of doses, and doses of 270 ml and 600 ml providing the lowest number of this defect for the rainfall and left-in-bag conditions, respectively. For the yard-grown coffee, there was an increase in the sour defects up to the dose of 403 ml, and with dose 0, the smallest number of this defect was obtained.

DISCUSSION

With the application of the bioprotector, TTA values for left-in-bag coffee were lower than those of yard-grown and rainfall coffees at the doses of 200, 400 and 600 ml, showing the efficiency of the bioprotector against fermenting agents in the post-harvest conditions. For rainfall coffee, the TTA values were lower than the yard-grown only at the dose of 600 ml, showing that the microbiota present in the rainfall coffee are more difficult to control by the bioprotector, compared to the left-in-bag variant.

TTA does not correlate with sensory perceived acidity as different compounds are present in each of them vary during the different maturation stages of coffee beans. It may also be influenced by location of the crop, harvest processes, post-harvest management, and climatic conditions during the harvest and drying phase (De Siqueira & De Abreu, 2006).

The SS contents proved that the bioprotector maintained the same results of the left-in-bag coffee with the yard-grown coffee from the dose of 200 ml onwards. For the rainfall coffee, the soluble solids contents were higher than the left-in-bag coffee without the application of the bioprotector. With the application of the bioprotector, the soluble solids contents of the rainfall coffee were the same as the other treatments from the dose of 400 ml onwards (Table 2).

In the regression analysis, it was observed that there was an increase in soluble solids for the left-in-bag coffee, and the highest value was obtained with the dose of 420 ml

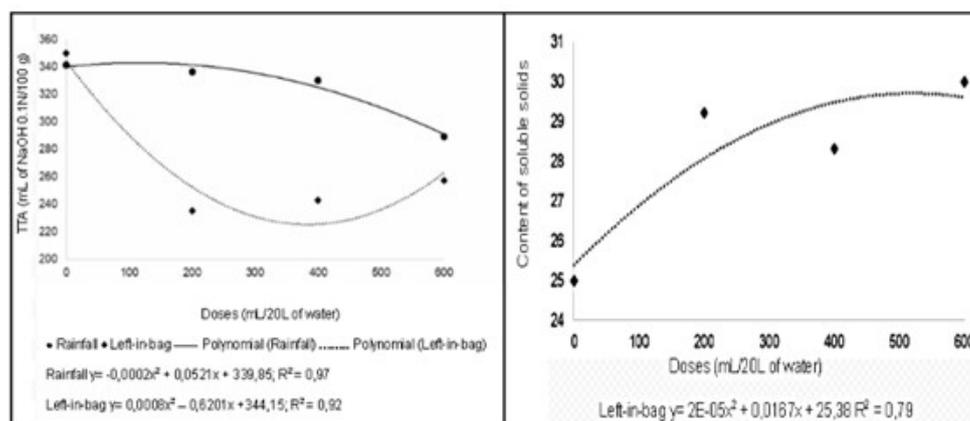
**Figure 1:** Total titratable acidity (TTA) and soluble solids (SS) contents as a function of bioprotector doses and post-harvest conditions.

Table 3: Color a*, b* and sensory analysis (SA) as a function of different post-harvest conditions and doses of *Cladosporium sp.*

Doses	Post-harvest conditions								
	Color a*			Color b*			SA		
	Rainfall	Left-in-bag	Yard-grown	Rainfall	Left-in-bag	Yard-grown	Rainfall	Left-in-bag	Yard-grown
0	1.9aA	1.1aA	1.9aA	18.7b	19.4c	17.7a	80.0b	77.2c	81.9a
200	1.7aA	0.3aA	0.4aA	17.1a	17.2a	17.1a	82.1a	81.8a	81.7a
400	1.2aA	0.1aA	-0.6aA	17.4a	17.3a	17.1a	81.3b	81.4b	82.1a
600	0.9aA	0.8aA	1.3aA	17.6a	17.8a	17.3a	81.2b	81.9a	82.2a

Means followed by the same lowercase letter in the rows do not differ by the tukey test at 5% probability. *= chromaticity coordinates

(Figure 1). Mendonça *et al.* (2005) stated that the content of soluble solids in greater quantity is desirable, both from the point of view of industrial income and for its contribution to ensuring the body of the final beverage.

For the rainfall and yard-grown coffees the regression test was not significant. The values found are in accordance with the results of the literature for arabica coffee, which are between 23.85% and 29.31% (Mendonça *et al.*, 2005).

The results obtained for the color L* of the rainfall coffee (Table 2) showed the lowest values of luminosity in any doses of the bioprotector, in relation to the left-in-bag coffee. The rainfall coffee presented lower luminosity at dose 0 and 600 ml of bioprotector in relation to the yard-grown coffee; so suggesting less oxidation of the coffee beans in these treatments. Changes in color indicate that oxidative processes occurred, causing biochemical transformations of enzymatic action that will negatively alter the flavor and aroma of the final beverage (Isquierdo *et al.*, 2011).

For the left-in-bag coffee, the luminosity was equal to

that of the rainfall coffee and lower than the yard-grown coffee in the dose of 600 ml, showing that the application of *Cladosporium* preserved the initial color of coffee beans subjected to adverse post-harvest management, which reflected in the quality (Table 2).

For the color variable b* the absence of the bioprotector the yard-grown coffee resulted in the lowest value, indicating that it tended towards bluish hues and that the fermentation processes in the left-in-bag and rainfall coffee negatively influenced color. According to De Siqueira & De Abreu (2006), color is an indicator of coffee quality, as coffee beans presenting a blue-to-green tonality tend to show better quality in the final beverage.

With application of the bioprotector, the values of color b* for the rainfall and left-in-bag coffees were equal to the yard-grown coffee, reducing the negative effects of the fermentation processes. Corrêa *et al.* (2002) reported that coffee beans color may be affected by inadequate management in the post-harvest process, such as fermentations. In this context, the lower coffee beans color value after applica-

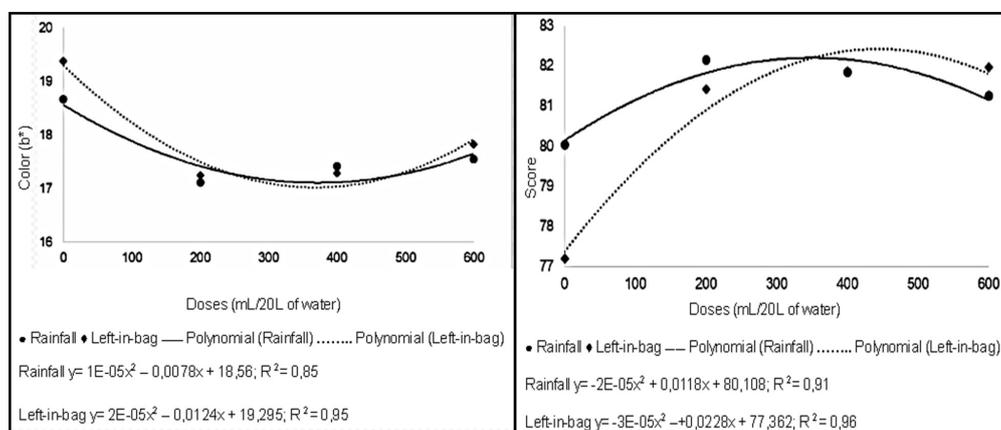


Figure 2: Color b* and sensory analysis score as a function of bioprotector dose and post-harvest conditions.

tion of *Cladosporium* sp. can be attributed to the control of fermentation processes by the bioprotector.

For the color variable b^* , the coffee beans tended to approach blue hues when the bioprotector was applied in both the rainfall and left-in-bag treatments; the yard-grown coffee beans, however, did not present significant difference, which allowed to infer that the bioprotector did not present effects due to absence of undesirable fermentations in the bluish coloration in this type of processing.

Sensory analyses showed us that both rainfall and left-in-bag coffee beans lost, on average, 4.7 and 1.9 points respectively when compared note of yard-grown coffee with with dose 0 of the bioprotector. These values indicate that left-in-bag coffee beans are more affected by these fermentative processes than their rainfall-exposed (Table 3). The data from the present study corroborate the studies by Angélico *et al.* (2011) on the influence of different bagging times after harvest on coffee quality. In that study, it was shown that from the first day of bagging, there were losses in quality due to fermentation.

The scores of sensory analysis showed that with the application of the bioprotector, both rainfall-exposed and left-in-bag coffees has similar quality to the yard-grown the dose of 200 ml. This proves the efficiency of the bioprotector as an antagonist to fungi that lead to undesirable fermentations and consequent loss of quality on the final beverage (Table 3). For the dose of 400 ml, there was no significant difference between rainfall-exposed and left-in-bag coffee; however, with the dose of 600 ml the left-in-bag was superior to the rainfall-exposed one.

According to Angélico *et al.* (2017), *Cladosporium cladosporioides* (Fresen) de Vries is a microorganism

capable of promoting the control of toxigenic fungi, present in coffee beans, thus promoting an improvement in the quality of the final product. Einloft *et al.* (2017) show that the fungi *Bacillus* spp. to be used as biocontrol agents to *Aspergillus westerdijkiae*, corroborating the results found.

In the regression analysis, there was an increase in the score of both the rainfall and left-in-bag coffee beans with the increase of bioprotector doses; the doses of 300 ml and 380 ml, respectively, were the ones that provided the highest score. For the rainfall-exposed coffee, the score in the appropriate dose of the bioprotector increased on average by 1.74 points; while for the left-in-bag coffee, there was an average increase of 4.33 points. This demonstrated the efficiency of *C. cladosporioides* in the bioprotection of coffee beans (Figure 2).

For the yard-grown coffee there was no influence of the bioprotector on the quality, most likely due to the existing fungal colony not being enough to compromise the beverage.

Results show that the conditions of rainfall-exposed coffee in doses of 200 and 400 ml and left-in-bag coffee at a dose of 600 ml enhanced the effect of the bioprotector in reducing defective sour coffee beans; while for yard-grown coffee there was a change in color similar to that of sour defective coffee beans regardless of the dose used (Table 4).

The results of the regression analysis (Figure 3) showed that there is a reduction in the number of sour defective coffee beans with the increase of doses, with doses of 270 ml and 600 ml providing the lowest number of this defect for the rainfall and left-in-bag conditions, respectively. For the yard-grown coffee there was an increase in the sour defects up to the dose of 403 ml,

Table 4: Number of defective beans, electrical conductivity (EC) and pH due to different post-harvest conditions and doses of *Cladosporium* sp.

Doses	Post-harvest conditions					
	Number of sour defective beans			EC		
	Rainfall	Left-in-bag	Yard-grown	Rainfall	Left-in-bag	Yard-grown
0	60.2 a	63.1 a	63.1 a	110.7 b	115.8 c	107.3 a
200	33.6 a	54.3 b	75.4 c	88.88 b	81.7 a	102.8 c
400	50.1 a	57.2 b	72.1 c	93.5 b	89.6 a	105.9 c
600	50.5 b	29.0 a	73.4 c	103.4 b	93.6 a	107.6 c

Means followed by the same lowercase letter in the rows do not differ by the tukey test at 5% probability.

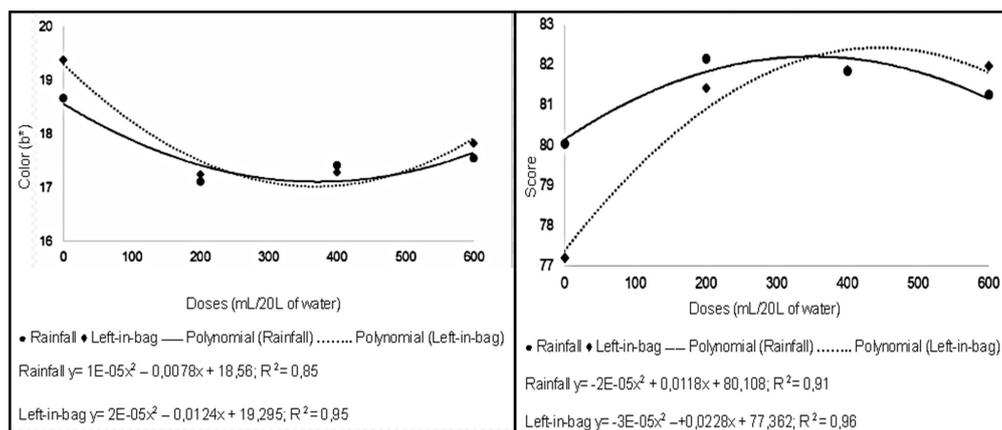


Figure 3: Number of defective beans and electrical conductivity as a function of bioprotector doses and post-harvest conditions.

with dose 0 the one that obtained the lowest number of this defect.

The application of this product when there is no fermentation (as is the case of yard-grown coffee) probably leads to color changes in the coffee beans that might resemble the “fox bean”, which can be mistaken for sour defective coffee beans. Therefore, this change in color is not related to the occurrence of undesirable fermentations. This hypothesis is justified since the yard-grown coffee maintained the quality in the sensory and chemical evaluations (Table 3 and Figure 2), regardless of the bioprotector dose; while for the rainfall and left-in-bag, the attributes were improved with the application of the product.

It is observed EC values were lower in the yard-grown coffee (Table 4) that in rainfall and left-in-bag coffees probably due to lower cell membrane degradation in the yard-grown coffee.

With the application of the bioprotector, the left-in-bag coffee presented the lowest values of EC, followed by rainfall coffee in any of the doses applied. The application of *Cladosporium sp.* promoted a synergistic effect, in a fermentation-favorable environment, in reducing the electrical conductivity and maintaining the integrity of the membranes of the coffee beans cells in relation to the yard-grown coffee beans that presented the worst results, regardless of dose. EC as a variable evaluates the integrity of the cell membrane system, and the test is able to detect losses of coffee quality in the initial stage, even if these changes in beverage quality may not be observed in the final product (Isquierdo *et al.*, 2011).

The results obtained show the efficiency of the bioprotector in reducing the damage caused by undesirable fermentations, which can compromise the quality physi-

co-chemical composition and sensory quality, of the product and reduce food safety of these types of coffee beans. In the same line of research Melo Pereira *et al.* (2016) show that the *P. fermentans* LPBYB13 have potential for of an efficient biological control of fungi in coffee beans.

CONCLUSIONS

The bioprotector based on *Cladosporium cladosporoides* promoted an increase in the quality of the physical, chemical and sensory quality of both rainfall and left-in-bag coffees.

The bioprotector also maintained quality sensory in rainfall and left-in-bag coffee when compared with the yard-grown. The application of bioprotector did not influence the sensory quality of coffee yard.

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