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Short Comunication

Zinc supplementation, production and quality of coffee beans[1]

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ABSTRACT

Besides its importance in the coffee tree nutrition, there is almost no information relating zinc nutrition and bean quality. This work evaluated the effect of zinc on the coffee yield and bean quality. The experiment was conducted with *Coffea arabica* L. in "Zona da Mata" region, Minas Gerais, Brazil. Twelve plots were established at random with 4 competitive plants each. Treatments included plants supplemented with zinc (eight plots) and control without zinc supplementation (four plots). Plants were subjected to two treatments: zinc supplementation and control. Yield, number of defective beans, beans attacked by berry borers, bean size, cup quality, beans zinc concentration, potassium leaching, electrical conductivity, color index, total tritable acidity, pH, chlorogenic acids contents and ferric-reducing antioxidant activity of beans were evaluated. Zinc positively affected quality of coffee beans, which presented lower percentage of medium and small beans, lower berry borer incidence, lower potassium leaching and electrical conductivity, higher contents of zinc and chlorogenic acids and higher antioxidant activity in comparison with control beans.

Key words: bean size, electrical conductivity, potassium leaching, chlorogenic acids, berry borer, antioxidant activity.

RESUMO

Efeito do zinco na produção e qualidade dos grãos de café

Apesar da importância do zinco na nutrição do cafeeiro, quase não há informações que relacionem o suprimento de zinco e a qualidade dos grãos. Este trabalho avaliou o efeito de zinco sobre a produção e a qualidade dos grãos de café. O experimento foi conduzido com *Coffea arabica* L. na Zona da Mata de Minas Gerais, Brasil. O delineamento utilizado foi inteiramente ao acaso, com dois tratamentos: suplementação com zinco (oito parcelas) e controle, sem zinco (quatro parcelas). Cada parcela útil constou de quatro plantas competitivas. Foram avaliados a produtividade, número de grãos defeituosos, grãos atacados por brocas, tamanho de grãos, qualidade da bebida, teor de zinco nos grãos, potássio lixiviado, condutividade elétrica, índice de coloração, acidez total titulável, pH, teores de ácidos clorogênicos e atividade antioxidante dos grãos de café. O zinco afetou positivamente a qualidade dos grãos de café, que apresentou menor percentagem de grãos de tamanhos médio e pequeno, menor incidência de broca, menor lixiviação de potássio e condutividade elétrica, maiores teores de zinco e ácidos clorogênicos e maior atividade antioxidante, em comparação com os grãos do controle.

Palavras-chave: tamanho do grão, condutividade elétrica, potássio lixiviado, ácidos clorogênicos, broca do café, atividade antioxidante.

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INTRODUCTION

The importance of zinc in coffee growth and production is widely known, particularly for crops growing in zinc poor clayey soils, as interactions occurring in the soil make zinc salt fertilization inefficient and cause the need for repeated leaf sprays. The problem is more serious in mountainous regions, such as "Zona da Mata" in Minas Gerais, where zinc deficiency is quite common because of difficulties in leaf spraying.

In a nutritional status assessment based on DRIS indices, Martinez *et al.* (2004) found that 57% of low productivity crops sampled in the region of Viçosa were zinc deficient. Zinc effect on crop productivity is well known mainly because its role in the synthesis of tryptophan, indolebutyric acid (IAA) and indolic compounds. Moreover, it also participates as component of alcohol dehydrogenase, carbonic anhydrase and superoxide dismutase, as well as in protein synthesis, carbohydrate metabolism and membrane integrity (Marschner, 2012).

In recent years, several studies have considered the chemical composition of defective coffee beans, green and ripe, relating the content of various compounds to the trade classification of green coffee and beverage quality, traditionally evaluated by "coffee cupping" (Prete, 1992; Pinto *et al.*, 2002; Franca *et al.*, 2005; Farah *et al.*, 2006). However, although nutrients directly affect primary and secondary metabolism of plants, there is a lack of studies relating mineral nutrition to quality of coffee beans, and there is almost no information available especially on zinc.

This study aimed to evaluate the zinc effect on coffee yield and quality characteristics of produced beans.

MATERIAL AND METHODS

The experiment was carried out in the experimental area of the Federal University of Viçosa, 20° 45' S: 42° 51' W, 581m altitude, Zona da Mata, Minas Gerais, Brazil, in November 2006. A commercial plantation with ten year-old plants of *Coffea arabica* L. cv. Catuaí Vermelho after the first cutting back was used for the trial. The soil of the area is classified as Red-Yellow Latosol and had 2 mg/kg zinc at the beginning of the experiment. The climate type is Cwa according to Köppen classification, 1221.4 mm average annual precipitation and 19.4°C average annual temperature.

Twelve plots were established at random with 4 competitive plants each. Treatments included plants supplemented with zinc (eight plots) and control without zinc supplementation (four plots).

All plots received lime and fertilizer according to Guimarães *et al.* (1999), as well as periodical hand-weeding. There was no need for disease and pest control during the experimental period.

Harvest was carried out by strip-picking berries onto laid cloth. Two kilograms of ripe berries harvested from the four central plants in the plot were used for quality assessment. Both the separate sample for quality assessment and the rest of the harvested beans were spread out in thin layers and dried in the sun to 11% moisture. After drying, the beans were hulled and yield calculated in ton ha^{-1} of processed coffee.

Classification was carried out by type, counting defective beans and calculating the number of equivalent defects, using a classification chart (Brasil, 2003). Bean size was evaluated by passing a 300-gram sample of processed coffee through a screen size sorting system. The screens usually have diameter ranging from 8 to 19/64". Then percentage of exportable beans was calculated, i.e., beans with diameter larger than 16/64". Exportable beans were separated into two size classes, retained in screen sizes 16 and 17 and retained in screen size above 17. Berry borer damage was calculated by counting damaged beans in a 300-gram sample of processed coffee. Cupping (Brasil, 2003) was used for samples from the 12 experimental plots assigning scores to different classes of beverage, as described in Table 1.

Zinc concentration in beans was determined by atomic absorption spectrophotometry, after nitric-perchloric digestion of dried (70 °C) and ground plant material.

Electrical conductivity and potassium leaching was assessed as proposed by Loeffler *et al.* (1988) with slight modifications described by Vieira (1994). Readings were taken after 5h of soaking 50 beans in 75 mL of distilled water using a conductivimeter to measure electrical conductivity (EC) and a flame photometer to determine potassium leaching. The amount of leached potassium was measured by the method proposed by Prete (1992), expressing in g/kg sample.

Color index was determined as described by Singleton (1966) and adapted for coffee by Carvalho *et al.* (1994).

Total titrable acidity (TA) was determined according to AOAC (1990), and results were expressed in mL of 1.0 N NaOH/100 grams of dry matter. The pH was measured directly on the filtrate obtained after shaking 2g of dried and grinded beans with 50 mL distilled water, using a potentiometer.

Table 1. Scores assigned to beverage classes by cupping coffee beans harvested from plants supplemented or not with zinc

Flavor	Score	Flavor	Score
Hard citric	100	Hard dirty	60
Hard fruity	100	Hard green fermented	60
Hard	80	Fermented	40
Hard astringent	80	Fermented sour	40
Hard fermented	60	Rio	20

Chlorogenic acid contents were determined by liquid chromatography coupled with mass spectrometry after extraction with 40% methanol and clarification with Carrez solution, as in Perrone *et al.* (2008), and results expressed in g/100 g. The antioxidant activity in extracts obtained for chlorogenic acid analysis used the FRAP method, which measures iron reducing power according to Moreira *et al.* (2005) and is expressed in equivalent µmol of Fe^{2+}/L^{-1}.

The qualitative assessments cupping and classification (type) took place at Incofex Armazéns Gerais Ltda., in Viçosa - MG. Zinc concentration in beans, K leaching (K LIX), electrical conductivity (EC), color index (CI), total tritable acidity (TTA) and pH, were determined in laboratories of the Federal University of Viçosa. Total content of chlorogenic acids (CGA) and antioxidant activity (FRAP) of beans were determined at the Laboratory of Biochemistry of Nutrition and Food Chemistry Institute of the Federal University of Rio de Janeiro.

Variance normality and homogeneity were tested by means of the Lilliefors and Cochran & Bartlett tests as indicated by Pimentel Gomes (2009). All data had normal distribution, and only the data of berry borer damage and equivalent defects didn't show homogeny variances. The homogeneity of the former was achieved transforming percentage of insect-damaged beans into $\sqrt{X+2}$, but for the last one, homogeneity couldn't be attained even when the data were submitted to transformation. All data were examined by analysis of variance and comparisons between treatments were made using the F test.

RESULTS AND DISCUSSION

Plants supplemented with zinc yielded 6.59 ton beans/ ha, whereas plants without supplement zinc produced 6.41 ton beans/ ha (Table 2, Figure 1A).

The difference in yield was not significant; however, there was effect of zinc supplementation on bean size. The average percentage of exportable beans was 90.7% and there was no difference between the treatments, but the plants supplemented with zinc had higher percentage of exportable large beans retained in screen sizes 17 and 18 (Table 2, Figure 1B). Guimarães *et al.* (1983) reported coffee yield increase from 1 to 1.6 $ton.ha^{-1}$ of processed beans with zinc sulfate foliar application. This increase in yield was followed by increase from 8 to 21 $mg.kg^{-1}$ of zinc concentration in leaves. Peck *et al*. (2008) treated two wheat varieties with six zinc doses, including foliar application in six different locations, and found that zinc supplementation increased grain yield in three locations and improved quality of grain protein in two of them.

Number of equivalent defects, considering green, shell, sour, black, broken, mal-formed and insect damaged beans was small in the classified sample, and in both treatments coffee was classified as type 2 (Table 2, Figure 1A). The percentage of borer-attacked beans was important, which, though small, was significantly lower in berries from plots that received zinc (0.65%) compared with those grown without zinc (1.85%) (Table 2, Figure 1A). According to Marschner (2012), nutrient availability may influence the selection of the host by the insect as it changes chemical composition, morphology and anatomy of the plant. It is known that the zinc acts in protein synthesis, and according to Mattson (1980) the insect's preference for particular plant may be caused by the concentration of proteins and amino acids in its composition. Moreover, phenolic compounds may have deterrent effect, determining insect non-preference. In this work, although the content and composition of amino acids of proteins have not been determined, zinc supplementation increased the chlorogenic acid content in the beans. It is then possible that this result could be attributed to the insect's non-preference for beans with higher content of phenolic compounds.

The sensory evaluation (cupping) transformed into scores resulted in non-significant difference between the treatments, although the average score of beverage prepared with beans from plants that received no zinc was 60 and beans of plants supplemented with zinc was 72.5 (Table 2, Figure 1B).

Zinc supplementation significantly increased the zinc concentration in beans from 5.1 to 7.1 mg/kg (Table 3, Figure 2A). In absolute terms the difference is small, but it may have been enough to cause relevant metabolic changes.

Amorim (1978) discussed that several conditions, including mechanical injury, affect the structure of cell membranes in coffee beans. When cell membranes collapse, they release cell contents, including enzymes such as polyphenol oxidase, proteases and lipases, altering the chemical composition of raw beans. During roasting, these beans will produce compounds that alter the aroma and taste of the infusion. Thus, potassium leaching and electrical conductivity, which is proportional to the amount of leached potassium, can be used to evaluate the collapse of membranes and consequently reduction in coffee quality. In this work, after 5 hours of soaking 50 beans in 75 mL water, beans from plants that had received zinc supplementation showed potassium leaching and electrical conductivity of 0.91 g kg^{-1} and 18.8 µS cm^{-1} g^{-1}, respectively. These values were significantly lower than 1.10 g kg^{-1} and 23.6 µS cm^{-1} g^{-1} obtained with beans from plants without zinc supplementation, respectively (Table 3, Figures 2A, B). This result reflects the role of zinc in maintaining the integrity of membranes. Amorim (1978), Prete (1992) and Lima *et al.* (2008) found an inverse correlation between potassium leaching and electrical conductivity and beverage quality.

Higher values of color index indicate coffees with more intense color, i.e., they still have not lost their characteristic color (Carvalho *et al.*, 1994). Such loss is caused, mainly, by oxidative reactions, with consequent bleaching of beans. Farah *et al*. (2006) observed, nevertheless, that poor quality coffees were the darkest ones. This effect was attributed to the higher percentage of defective beans in lower quality coffees and the indirect action of polyphenol oxidase on phenolic compounds of these samples. The main substrate for polyphenol oxidase would be the 5-caffeoylquinic acid (5-CQA), and then the ortho-quinones formed by the action of polyphenol oxidase on 5-CQA being responsible for the darkening. In this study there was no difference between treatments for color index, which was on average 0.95 D.O. 425 nm (Table 3, Figure 2B), consistent with the color index recorded for good quality coffee by Lima *et al.* (2008).

Coffee acidity results from non-volatile acids such as oxalic acid, malic, citric, tartaric and pyruvic, as well

Table 2. Summary of analysis of variance of yield (ton ha^{-1}), number of equivalent defects, insect damaged beans (%), beans retained in screen sizes below 17 (%), beans retained in screen sizes above 17 (%), and beverage quality and respective coefficients of variation (CV %) of coffee beans with and without zinc supplementation

Characteristic	Source of variation	D F	CV%	Mean squares
Yield (PROD)	Treatment	1	24.33	22.3377 ns
	Residue	10		700.4158
Equivalent defects (ED)	Treatment	1	51.17	2.2407 ns
	Residue	10		14.3614
Insect damage (ID)	Treatment	1	13.28	0.2852c
	Residue	10		0.0526
Exportable beans (Ex B%)	Treatment	1	4.73	6.0000 ns
	Residue	10		18.7000
Size of exportable beans (% B 16-17)	Treatment	1	32.72	58.7661 a
	Residue	10		14.1798
Size of exportable beans (% B > 17)	Treatment	1	4.26	58.7261 b
	Residue	10		14.1798
Beverage quality (BQ)	Treatment	1	36.88	416.6667 ns
	Residue	10		635.0000

a, b and c- F significant at 6.9%, 6.9% and 5% probability levels respectively.
ns, non-significant

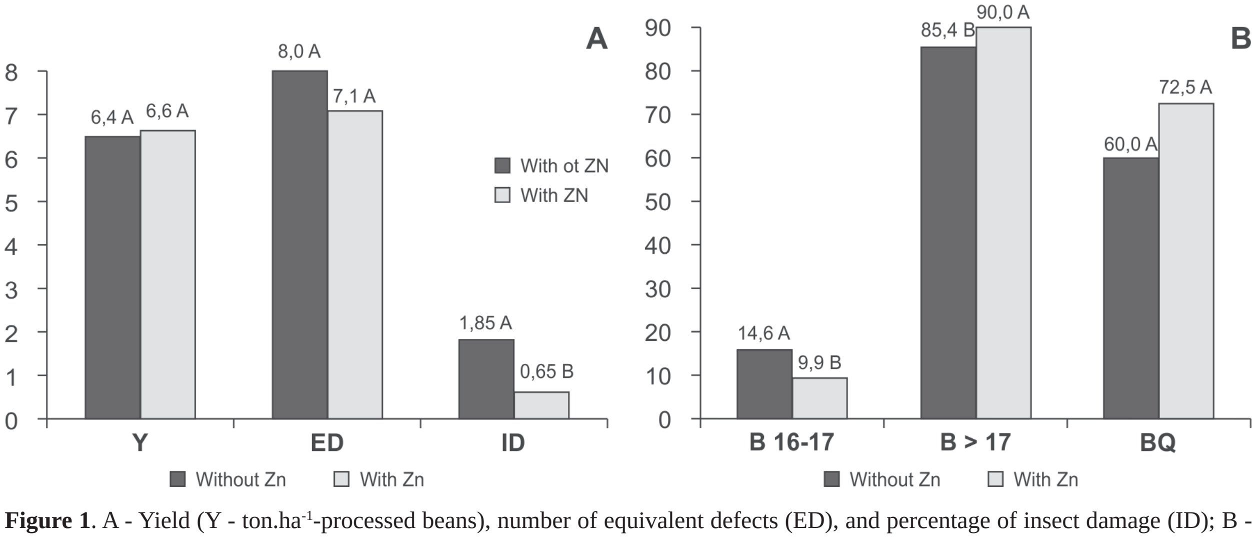

Figure 1. A - Yield (Y - $ton.ha^{-1}$-processed beans), number of equivalent defects (ED), and percentage of insect damage (ID); B - Percentage of exportable beans retained in screens 16 and 17 (B 16-17), percentage of exportable beans retained in screens above 17 (B > 17) and quality of beverage (BQ) in coffee beans produced with or without zinc supplementation.

as volatile, such as acetic, propionic, valeric and butyric, which are produced by internal pathways and undesirable fermentation. The resulting acidity of citric and malic acids has desirable effect on coffee quality, whereas the acids acetic, lactic, propionic and butyric result in undesirable effects. Franca *et al.* (2005) correlated low quality to acidity, probably originated from fermented beans. Pinto *et al.* (2002) studied the beverage standards for espresso preparation and found that poor quality coffee, riosh/rio taste, showed higher acidity than beverages that taste strictly soft and soft. Lima *et al.* (2008) differentiate what they called good acidity, a citric acidity typical of fine coffees, from other types of acidity. In this work, TTA and pH (Table 3, Figure 2C) were not affected by zinc supplementation and were on average 14.7 mL NaOH 100g^{-1} and 5.4 respectively. It is worth pointing out that during cupping, some bean samples from plants that received zinc received the names of hard citric and hard fruity, receiving the highest grades (Table 1).

Chlorogenic acids (CGA) are a group of phenolic compounds representing 6 to 12% of the dry mass of raw coffee. CGA are formed by esterification of quinic acid by a cinnamic acid. The main sub-groups are caffeoylquinic acids (CQA), dicaffeoylquinic (di-CQA) and feruloylquinic (FQA) (Farah *et al.*, 2006). Thermal degradation of CGA during coffee roasting results in the formation of pigmentation, flavor and astringency of the beverage. Some phenolic compounds are formed during roasting, contributing to bitterness.

There are a number of studies in the literature associating low-quality coffee with total concentration of CGA (Carvalho *et al.*, 1994; Menezes, 1990; Franca *et al.*, 2005), especially with 5-CQA. Farah *et al.* (2006) reported that CQA accounted for 83% of CGA in raw beans, as well as 3,4-di,CQA and to a lesser extent 3,5-di,CQA were correlated with good quality beverage, while others CQA, especially the 5-CQA, were negatively correlated with quality. In this study, the concentration of total CGA (4.22 g.100 g^{-1}) in beans from plants supplemented with zinc was significantly higher than in beans from non-supplemented plants (3.61 g.100 g^{-1}). Both treatments showed CGA concentrations consistent with those found in good quality coffee. Besides the CGA role in the development of coffee flavor during roasting, regardless of their effect on the sensory quality of the beverage, a greater concentration of these compounds is valuable from the nutraceutical point of view. According to Farah & Donangelo (2006), CGA have strong antioxidant activity, showing hypoglycemic, antiviral, hepatic- and immune-protection activities in humans. The antioxidant activity measured by FRAP was not significantly different between the treatments; supplemented or not with zinc (Table 3, Figure 2D), but showed the same trend of CGA concentration.

Table 3. Summary of analysis of variance of zinc content (mg.kg^{-1}), electrical conductivity (µS cm^{-1} g^{-1}), potassium leaching (mg kg^{-1}), color index (DO 425 nm), total titratable acidity (100 mL NaOH^{-1}), pH, size (%), concentration of chlorogenic acids (GCA - g.100g^{-1}), antioxidant capacity (FRAP - µmol Fe^{2+}. L^{-1}), and coefficients of variation (CV %) of coffee beans from treatments without and with zinc supplement

Characteristic	Source of variation	DF	CV%	Mean squares
Zinc content in beans (Bean Zn)	Treatment	1	26.27	11.1384 a
	Residue	10		2.9167
Electrical conductivity (EC)	Treatment	1	18.66	68.3687 b
	Residue	10		14.2837
K leaching (K Le)	Treatment	1	15.09	97922.43 c
	Residue	10		21651.99
Color index (CI)	Treatment	1	17.76	0.0001 ns
	Residue	10		0.0275
Total titratable acidity (TTA)	Treatment	1	11.34	3.0104 ns
	Residue	10		2.4906
pH	Treatment	1	2.93	0.0247 ns
	Residue	10		0.0255
Chlorogenic acids (CGA)	Treatment	1	11.13	0.9923d
	Residue	10		0.2000
Antioxidant capacity (FRAP)	Treatment	1	10.17	24.1049 ns
	Residue	10		15.1428

a - F significant at 7.9% probability level; b - F significant at 5.4% probability level; c - F significant at 5.9% probability level; d - F significant at 5% probability level; ns- non significant.

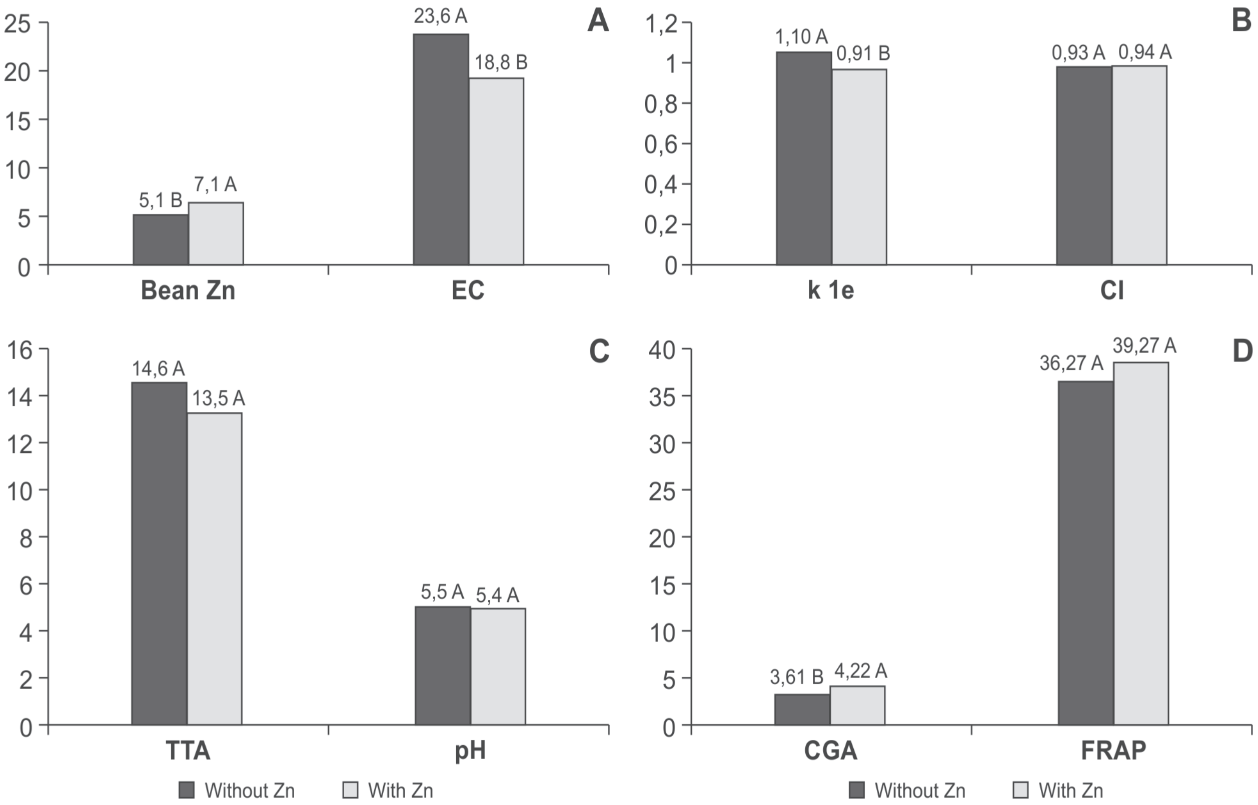

Figure 2. A - Zinc concentration (Bean Zn - mg $^{-1}$) and electrical conductivity (EC- µS cm^{-1} g^{-1}); B - K leaching (K le - g kg^{-1}) color index (CI - D.O. 425 nm); C - total tritable acidity (TTA- mL NaOH 100 g^{-1}) and pH of beans; D - total chlorogenic acids (CGA - g.100 g^{-1}) and antioxidant activity (FRAP - µmol Fe^{2+}. L^{-1} - F) in coffee beans produced with or without zinc supplementation.

The highest CGA concentration in beans from plants supplemented with zinc suggests that zinc is somehow involved in its synthesis, although in the literature there are only references on the participation of Cu, Fe, B and Mn in phenol synthesis (Marschner, 2012).

Our results showed that zinc supplementation affected positively coffee quality. Differences in chemical characteristics related to quality of beans from plants supplemented with zinc were not markedly reflected in cupping quality. However, it should be noted that although zinc concentration in the soil of the experimental area was low, 2 mg/dm^3 (Guimarães *et al.*, 1999), zinc foliar contents; from 10.8 mg.kg^{-1} for plants without additional zinc and 29.3 mg.kg^{-1} for plants that received zinc; are within the range of sufficiency proposed by Martinez *et al.* (2003) and do not indicate nutrient deficiency. We believe that under conditions of greater difference in zinc nutritional status of plants, differences in yield and bean quality would be more significant.

CONCLUSION

Zinc supply affected positively coffee bean quality, which is characterized by bean size, percentage of insect damage, zinc content in beans, electrical conductivity, potassium leaching and chlorogenic acid concentration.

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