

Zinc Fertilizers and Additives for Foliar Fertilization of Cocoa Seedlings

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Abstract

Foliar fertilization is an interesting strategy for nutrition with micronutrients in perennial plants; among the micronutrients, zinc (Zn) deficiency is the most frequent in cocoa trees (*Theobroma cacao* L.). The present study aimed to evaluate the efficiency of Zn sources through foliar application for the cocoa crop. The experiment was carried out in a randomized block design, with 10 treatments and four replicates. Treatments were: foliar fertilizations containing 1 g L⁻¹ of Zn using two inorganic sources (chloride and sulfate), in the presence or absence of additives (urea and sucrose); two organic sources (Zn-EDTA, and from chloride and sulfate); soil fertilization with 8 mg dm⁻³ of Zn, and a control (without addition of Zn). Foliar fertilizations with Zn were monthly applied for five months, and the experiment was conducted for 210 days. The results were subjected to analyses of variance and contrast. Zn fertilization, regardless of the form of application, increased Zn contents and accumulations in the leaves. Zn fertilization in the soil, at planting, led to a recovery rate by the plant similar to the mean value caused by foliar fertilizations. ZnCl₂ caused higher Zn contents and accumulations in the leaves and was more efficient than sulfate and EDTA; addition of urea to the ZnSO₄ solution increased Zn accumulation in the leaves, whereas addition of sucrose to the ZnCl₂ solution reduced Zn content in the leaves.

Keywords: *Theobroma cacao*, micronutrients, foliar spray, foliar nutrient absorption, adjuvants for foliar absorption

1. Introduction

Cocoa (*Theobroma cacao* L.) is a perennial crop of great economic importance, cultivated in tropical regions of the world to produce grains used mainly in chocolate manufacture (Santos, Almeida, Anher, Conceição, & Pirovani, 2014).

Brazil stands out as the sixth cocoa producer in the world, with area of approximately 680,000 ha and production of 210,000 t. Bahia is the state with largest planted area, 471,000 ha (CEPLAC, 2016), and 60% of the national production, but its yield is still low and raw material needs to be imported (Almeida & Valle, 2007).

In this context, the adoption of new technologies to produce cocoa seedlings on large scale and with quality (Sodré, Cora, Pereira, & Magalhães, 2007) has been a relevant foundation in the attempt to revitalize cocoa cultivation in Bahia.

Among agricultural techniques, mineral nutrition appears as one of the most important, leading to the increase of yield and quality of products (Weih, Asplund, & Berkvist, 2011). In agriculture, high yield can only be achieved when all factors related to production are at levels or conditions close to ideal. Soil fertility and plant nutrition

are among the easiest ones to manage and economically viable to be manipulated (Neto, Souza Júnior, Sodr e, & Baligar, 2015; Raji & Quaggio, 1991).

It is important to highlight that soil fertility, nutrition and fertilization are essential components to construct an efficient production system. Therefore, deficiency of one or more nutrients, including those required in lower amounts (micronutrients), will affect several physiological processes of the plant.

Zinc (Zn) is the micronutrient most frequently deficient in cocoa trees in Brazil (Chepote et al., 2013). In perennial crops, foliar fertilization with micronutrients has been a strategy used to supply them, especially when there are restrictions to the absorption by roots, such as: soils with low water availability, low root activity during the reproductive phase and for situations in which soil fertilization has lower efficiency (Wójcik, 2004; Faquin, 2005; Eichert & Fern andez, 2012; Fern andez, Sotiropoulos, & Brown, 2013).

For cocoa, the most used Zn fertilizer for foliar fertilization is $ZnSO_4$ (Malavolta, 1987; Chepote et al., 2013), but studies with other crops have demonstrated (Boaretto, Muraoka & Buoratto, 2003) or not (Teixeira, Bor em, Silva, & Kikuti, 2008) higher efficiency of Zn absorption when the inorganic source is $ZnCl_2$; other studies also indicate that the efficiency of Zn absorption by the leaves increases when KCl is added to the $ZnSO_4$ solution (Coutinho Neto et al., 2010).

Chelates, organometallic complexes, can be less adsorbed by the cuticle and cell wall than inorganic sources of Zn (Franco, Martinez, Zabini, & Fontes, 2005) and can be more mobile within the plant, but that does not guarantee that the chelated element has higher absorption in comparison to inorganic salts (Wójcik, 2004). The effect of chelates on leaf capacity to absorb nutrients can be related to some of their properties such as: complex's molecular weight, dissociation constant and complex's stability at solution pH (Reed, Lyons, J nior, & Mceachrn, 1988).

Addition of urea to solutions used in foliar fertilizations can favor nutrient absorption (Wójcik, 2004; Faquin, 2005) and even translocation (Aciksoz, Ozturk, Yazici, & Cakmak, 2014), and cocoa responds positively to foliar fertilizations with urea up to a concentration of about 25 g L^{-1} , when it is used alone (Souza J nior & Carmello, 2008). On the other hand, sucrose has been tested as an inductor of resistance to the witches' broom disease of the cocoa tree (Vieira, Silva, Damaceno, Santos Filho, & Valle, 2013) and also in foliar fertilizations (Lima, Cunha, Pinho & Guimar es, 2003; Ferreira et al., 2007).

Thus, this study aimed to evaluate the efficiency of Zn sources and additives for foliar application in cocoa seedlings.

2. Material and Methods

The experiment was conducted in a greenhouse at the State University of Santa Cruz (UESC), in the municipality of Ilh eus, Bahia state, Brazil.

Cocoa (*Theobroma cacao* L.) seedlings were produced by cuttings at the Cocoa Biofactory Institute (Instituto Biof brica de Cacau-IBC), cultivated in commercial substrate and coconut fiber at 1:1 volumetric proportion, in 288-dm^3 tubes, for a period of approximately 120 days. The experiment used the clone CCN51, which is widely known as highly productive and resistant to witches' broom.

Seedlings about 30 cm tall were transplanted to plastic pots containing 11 dm^3 of soil, classified as Yellow Latosol, with 220 g kg^{-1} of clay and the following chemical characteristics: 18 g kg^{-1} of organic matter; $105\text{ mmol}_c\text{ dm}^{-3}$ of CEC at pH 7.0; contents of Ca^{2+} , Mg^{2+} and Al^{3+} , extracted by $KCl\ 1.0\text{ mol L}^{-1}$, of 5.5, 4.0 and $35\text{ mmol}_c\text{ dm}^{-3}$, respectively; and contents of P, K, Cu, Fe, Mn and Zn, extracted by Mehlich-1, of 1.0, 20, 0.5, 60, 4.2 and 1.8 mg dm^{-3} , respectively.

The soil was previously sieved through a 5-mm mesh and corrected with calcium and magnesium carbonates (Analytical Reagents-AR), Ca: Mg ratio of 4:1, to increase base saturation to 80%. Fertilization at planting was performed with the nutrients P, N, K, S, Mn, Cu, B and Mo, at doses of 400, 169, 60, 26, 6, 2, 0.8 and 0.15 mg dm^{-3} , respectively, in the form of purified monoammonium phosphate (MAP) and the AR salts potassium sulfate, manganous chloride, copper sulfate, boric acid and ammonium molybdate. All fertilizers, except MAP, were applied through a nutrient solution in the entire soil volume.

The experimental design was randomized blocks, with two blocks and each one with two replicates, totaling 40 experimental plots. The experiment had 10 treatments, formed by a $(2 \times 3) + 2 + 2$ factorial scheme, which corresponded to two inorganic sources of Zn, zinc chloride ($ZnCl_2$) and zinc sulfate ($ZnSO_4 \cdot 7H_2O$), applied through the leaves at dose of 1 g L^{-1} of Zn, with and without the presence of two additives (urea at 10 g L^{-1} and sucrose at 3 g L^{-1}); plus four additional treatments: two treatments also with foliar application of Zn, but using

organic sources of Zn, from chloride and sulfate, and Zn complexed by EDTA (1 g L^{-1} of Zn + 6 g L^{-1} of EDTA); two control treatments, one with Zn fertilization in the soil at dose of 8 mg dm^{-3} of Zn (88 mg of Zn per pot), in the form of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, applied before transplanting the seedlings and in the entire soil volume; and an absolute control (without Zn fertilization). Zn + EDTA solutions were prepared and subjected to agitation for 24 h before utilization in the sprays.

Thus, the treatments used were: T1—without Zn fertilization; T2—fertilization with 8 mg dm^{-3} of Zn in the entire soil volume, before transplantation; T3 to T10—monthly foliar fertilizations with 1 g L^{-1} of Zn, with ZnCl_2 (T3 to T5) and with ZnSO_4 (T6 to T8), without additives (T3 and T6), plus 10 g L^{-1} of urea (T4 and T7) and 3 g L^{-1} of sucrose (T5 and T8), and Zn-EDTA (T9 and T10), in which Zn was chelated by ZnCl_2 and ZnSO_4 solutions, respectively.

Seedlings were irrigated with rainwater, collected from the greenhouse roof and stored in polyethylene tanks. Every two weeks, 30 days after transplantation, all treatments received soil fertilization with 20 mg dm^{-3} of N, 10 mg dm^{-3} of K and 10 mg dm^{-3} of P, with urea, potassium nitrate and purified MAP. Ninety days after transplantation, these doses were doubled.

Monthly, 30 days after transplantation, treatments with foliar fertilization were applied and the seedlings were sprayed with the respective solutions, trying to cover all leaf surface. During the application, the soil was covered to avoid the contact with the solution, and plant sides were protected with a cardboard barrier to avoid the contact of the solution with the adjacent plots.

Six foliar applications were performed; the first two and last four used 50 and 100 mL of solution for the four plots of each treatment, respectively, due to the increase in leaf area of the plants. These doses were equivalent to a total dose of 125 mg of Zn per plant along the experiment.

At 210 days of cultivation, plants were harvested and separated into leaves, stem and roots. Leaves were counted and leaf area (LA) was measured with a benchtop LI-3100C meter. Then, the leaves were washed in distilled water, followed by 30 mL L^{-1} HCl solution and, lastly, two additional washings with distilled water. This procedure was adopted to remove the Zn deposited on leaf surface and adsorbed to the cuticle.

After harvest, all plant parts were dried in oven at $65 \text{ }^\circ\text{C}$ for 72 hours and weighed to obtain leaf dry matter (LDM), stem dry matter (StDM), root dry matter (RDM), shoot dry matter (ShDM = LDM + StDM) and total dry matter (TDM = ShDM + RDM). The following parameters were calculated: shoot/root dry matter ratio (S/R = ShDM/RDM), mean leaf area per leaf (MLA = LA/number of leaves) and specific leaf area (SLA = LA/LDM)

The soil of each plot was homogenized, sampled and analyzed, and the Zn contents were extracted by Mehlich-1. Zn contents were also determined in the leaves, by atomic absorption spectrophotometry (EMBRAPA, 2009). Zn accumulation in the leaves was obtained by multiplying its leaf content by LDM.

The recovery rate (RR) of the applied fertilizer was calculated by the following equation: $RR = (\text{ZnA}_L - \text{ZnA}_{FC}) \times 100 / \text{ToDZn}$; where, ZnA_L : Zn accumulation in the leaves, ZnA_{FC} : mean Zn accumulation in the leaves in the absolute control, ToDZn: total dose of Zn, equal to 88 mg of Zn per plant, for the treatment with soil fertilization, and to 125 mg of Zn per plant, for the eight treatments which received foliar fertilizations.

Biometric variables, Zn contents in soil and leaves, and Zn accumulation in the leaves were subjected to analysis of variance, with follow-up test of treatment degrees of freedom by contrasts at 0.05 probability level by F test.

3. Results and Discussion

Mean values of biometric variables, Zn contents in soil (ZnC_S) and leaves (ZnC_L), Zn accumulation in the leaves (ZnA_L) and recovery rate of the applied fertilizer (RR), as well as their coefficients of variation (CV), are presented in Table 1. The contrasts analyzed are presented only for variables which showed significant differences (Table 2).

Table 1. Biometric variables¹ of cocoa seedlings (clone CCN 51) and zinc in soil and leaves² at 210 days of cultivation, as a function of treatments (T)³

T ³	H	D	LA	MLA	SLA	LDM	StDM	RDM	ShDM	TDM	S/R	ZnC_S	ZnC_L	ZnA_L	RR ⁴
	----- cm -----	----- cm -----	m ²	cm ²	cm ² g ⁻¹	----- g -----					mg dm ⁻³	mg kg ⁻¹	mg per plant	%	
1	98.5	2.4	1.79	153	163.4	105.6	58.4	83.9	164.1	247.9	2.0	1.5	32.1	3.3	-
2	105.0	2.2	2.00	184	183.1	109.0	60.3	92.3	169.4	261.6	1.9	3.5	130.2	14.2	12.4
3	84.9	2.5	1.71	168	169.0	101.1	51.8	78.3	152.9	231.1	2.0	2.5	270.4	28.1	19.9
4	100.0	2.6	1.84	150	166.7	110.9	62.9	93.1	173.8	266.9	1.9	2.8	224.1	24.9	17.3
5	94.0	2.5	1.81	133	160.3	108.1	63.5	84.4	171.6	256.0	2.1	2.6	193.9	21.1	14.2
6	93.4	2.5	1.76	156	171.0	102.7	63.7	91.2	166.3	257.5	1.9	2.2	134.0	13.8	8.4
7	105.0	2.3	2.15	149	187.0	116.3	60.6	84.2	176.9	261.1	2.1	2.5	192.6	22.8	15.7
8	103.1	2.5	2.04	161	175.6	115.1	61.5	86.2	176.6	262.8	2.1	2.6	144.2	16.5	10.6
9	98.5	2.4	2.13	146	195.5	109.4	66.4	78.8	175.8	254.7	2.4	2.3	127.2	13.8	8.5
10	95.3	2.6	1.73	156	174.3	99.7	58.5	74.9	158.2	233.0	2.1	2.8	111.2	11.3	6.4
CV ⁵	16.6	10.2	25.2	16.9	14.5	17.8	15.8	17.5	13.5	12.9	17.1	32.0	47.9	53.2	51.1

Note. 1: Biometric variables: height (H); diameter (D); leaf area per plant (LA); mean area per leaf (MLA); specific leaf area (SLA); masses leaf dry matter (LDM), stem dry matter (StDM), root dry matter (RDM), shoot dry matter (ShDM) and total dry matter (TDM) and shoot/root dry matter ratio (S/R).

2: Zinc contents in soil (ZnC_S) and in leaves (ZnC_L) and total content of zinc in leaves (ZnA_L).

3: Thus, the treatments used were: T1—without Zn fertilization; T2—fertilization with 8 mg dm⁻³ of Zn in the entire soil volume, before transplantation; T3 to T10—monthly foliar fertilizations with 1 g L⁻¹ of Zn, with ZnCl₂ (T3 to T5) and with ZnSO₄ (T6 to T8), without additives (T3 and T6), plus 10 g L⁻¹ of urea (T4 and T7) and 3 g L⁻¹ of sucrose (T5 and T8), and Zn-EDTA (T9 and T10), in which Zn was chelated by ZnCl₂ and ZnSO₄ solutions, respectively.

4: The recovery rate (RR) of the applied fertilizer.

5: Coefficients of variation (CV), %.

The biometric variables analyzed had lower experimental variability than Zn contents in soil and leaves, since the CV of the former oscillated between 10% and 25%, whereas the CV of the latter varied from 32 to 53%, and ZnA_L showed highest variability (Table 1).

There were significant effects for Zn content in the soil (ZnC_S) and especially for its content (ZnC_L) and accumulation (ZnA_L) in the leaves. In comparison to the control, Zn fertilizations significantly increased its contents in the soil and in the plant and, consequently, ZnA_L (Table 2, contrast 1), which had mean increment of 467% compared with the control (Table 1).

Both forms of fertilization resulted in similar mean contents and accumulations in the leaves (Table 2, contrast 2), indicating that the dose applied in the soil, 88 mg of Zn per plant, led to Zn contents and accumulations in the leaves similar to those caused by the dose applied through the leaves, 125 mg of Zn per plant, a fact confirmed by the fertilizer recovery rate (RR), which was equal to 12.4% for soil application and mean of 12.6% for the eight treatments which received foliar fertilization (Table 1). However, Zn fertilization at planting in the entire soil volume, compared with the mean of the eight treatments with foliar fertilization, significantly increased its content in the soil (Table 2, contrast 2). In other words, fertilization in the soil increases the residual effect of the fertilizer and, according to the results of Sartori et al. (2008), contributes to plant nutrition, especially for the organs born after foliar applications.

Table 2. Contrasts¹ between treatment means for the studied variables²

Contrast			MLA	ZnC_S	ZnC_L	ZnA_L
N ^o	Description	Treatments ³	cm ²	mg dm ⁻³	mg kg ⁻¹	mg per plant
1	Without vs with fertilization	T1 vs (T2 to T10)	ns	1.2**	137.6**	15.2**
2	Fertilizer type: soil vs leaf	T2 vs (T3 to T10)	-31.8**	-1.0**	ns	ns
3	Sulfate vs chloride	(T6 to T8) vs (T3 to T5)	ns	ns	72.5**	7.0**
4	EDTA vs chloride	T9 vs T3	ns	ns	143.3**	14.2**
5	EDTA vs sulfate	T10 vs T6	ns	ns	ns	ns
6	Without vs with urea for chloride	T3 vs T4	ns	ns	ns	ns
7	Without vs with urea for sulfate	T6 vs T7	ns	ns	ns	9.1*
8	Without vs with sucrose for chloride	T3 vs T5	-34.7*	ns	-76.7*	ns
9	Without vs with sucrose for sulfate	T6 vs T8	ns	ns	ns	ns

Note. 1: Only significant contrasts were calculated, which were calculated by the difference between the means of the second and the first group of treatments (** and * = significant at 1 and 5% by the F test, respectively; ns = not significant).

2: Mean area per leaf (MLA); zinc contents not only (ZnC_S) and in the leaves (ZnC_L) and total content of zinc in leaves (ZnA_L).

3: T1—without Zn fertilization; T2—fertilization with 8 mg dm⁻³ of Zn in the entire soil volume, before transplantation; T3 to T10—monthly foliar fertilizations with 1 g L⁻¹ of Zn, with ZnCl₂ (T3 to T5) and with ZnSO₄ (T6 to T8), without additives (T3 and T6), plus 10 g L⁻¹ of urea (T4 and T7) and 3 g L⁻¹ of sucrose (T5 and T8), and Zn-EDTA (T9 and T10), in which Zn was chelated by ZnCl₂ and ZnSO₄ solutions, respectively.

The sources of foliar Zn did not affect its content in the soil but significantly influenced its contents and accumulations in the leaves (Table 2). Fertilizations with ZnCl₂, in comparison to ZnSO₄, significantly increased the mean ZnC_L by 72.5 mg kg⁻¹ and mean ZnA_L by 7.0 mg per plant (Table 2, contrast 3). Subtracted from the contribution of root absorption by the control (treatment not fertilized with Zn), these values result in mean increments of 58% and 49% of ZnC_L and ZnA_L, respectively, *i.e.*, the mean RR of ZnCl₂ was approximately 50% higher than that of ZnSO₄, for foliar fertilization in cocoa trees (Table 1). These results are consistent with those observed by Boaretto, Muraoka & Buoratto (2003) for the coffee crop. However, Teixeira et al. (2008) found no significant difference in ZnC_L of common beans due to fertilizations with Zn chloride or sulfate.

ZnCl₂ was also a source superior to Zn-EDTA because it led to significant increments in mean ZnC_L and ZnA_L of 143.3 mg kg⁻¹ and 14.2 mg per plant, respectively (Table 2, contrast 4). Subtracted from the contribution of root absorption of the treatment not fertilized with Zn, these values represented mean increments of 150% and 135% in ZnC_L and ZnA_L, respectively, *i.e.*, the chelation of Zn from ZnCl₂ reduced its RR by 57% (Table 1). Lower efficiency of the source Zn-EDTA, compared with ZnCl₂, has also been observed in the coffee crop (Boaretto, Muraoka, & Buoratto, 2003).

Nonetheless, the sources ZnSO₄ and Zn-EDTA did not differ significantly with respect to ZnC_L and ZnA_L (Table 2, contrast 5), indicating a similar efficiency for foliar fertilization in cocoa trees and lower RR (Table 1). Franco et al. (2005), studying translocation and compartmentalization of Zn applied in coffee and common beans, under Zn sufficiency and deficiency, also observed similarities between the Zn contents effectively absorbed by the leaves when the sources were ZnSO₄ and Zn-EDTA, except for common bean plants under Zn sufficiency, in which foliar absorption of Zn was higher when the source was Zn-EDTA. Ojeda-Barrio et al. (2009) also found no significant difference in ZnC_L in pecan trees sprayed with ZnSO₄ and Zn chelates (EDTA and DTPA).

Although ZnSO₄ is a source of lower efficiency for foliar fertilization than ZnCl₂, the addition of 10 g L⁻¹ of urea significantly increased ZnA_L, by 9.1 mg per plant (Table 2, contrast 7), which represents an 86% increment in the total Zn accumulated in the leaves, indicating that urea is a good additive for foliar absorption of Zn by cocoa trees, when the source is ZnSO₄. Addition of urea to the ZnCl₂ solution did not have the same beneficial effect observed when the source was ZnSO₄, because it did not significantly affect ZnC_L and ZnA_L (Table 2, contrast 6), which may have occurred because the foliar absorption of Zn²⁺ is already originally facilitated by Cl⁻ (Table 2, contrasts 3 and 4).

Addition of 3 g L⁻¹ of sucrose to the 1.0 g L⁻¹ Zn solutions reduced ZnC_L when the source was ZnCl₂, but did not significantly affect ZnC_L when the source was ZnSO₄ (Table 2, contrasts 8 and 9, respectively).

Although fertilizations with Zn increase its absorption by plants, they did not result in increments of either growth or biomass since no significant effect was observed for almost all biometric variables (height, diameter, LA, SLA and dry matter of the different plant components), possibly due to the original Zn content in the soil, which guaranteed a minimum nutritional condition for plant growth, even for the absolute control, which had mean Zn_{C_L} of 32.1 mg kg⁻¹. Compared with sufficiency range for diagnostic leaves of adult cocoa trees, this value is within the ranges proposed by Rajj et al. (1997) and Salgado-García et al. (2006), but is below the ranges considered by Malavolta (1987) and Souza Júnior et al. (2012). Absence of response of growth or production variables to foliar fertilizations with Zn have also been observed for common beans (Teixeira et al., 2008) and silage corn (Coutinho Neto et al., 2010). However, increase in production (Zoz et al., 2012) and even in the nutritional value of the crops, due to foliar fertilizations with Zn, have been found when there is deficiency of the nutrient (Ghasemi et al., 2013).

The mean area per leaf (MLA) was the only biometric variable significantly affected by the treatments, being lower for foliar fertilization with Zn than for its application in the soil (Table 2, contrast 2), possibly due to the saline effect of the fertilizer solutions, which would also explain the reduction in MLA caused by the addition of sucrose in the ZnCl₂ solution (Table 2, contrast 8). Nonetheless, such restriction to leaf expansion was compensated by the increase in the number of leaves since the leaf area per plant (LA) was not significantly affected by the different solutions used in the foliar sprays.

4. Conclusions

- (1) Zn fertilization in the soil or leaves increased Zn contents and accumulations in the leaves of cocoa seedlings.
- (2) The recovery rate by the plant for Zn fertilization in the soil, at planting, was similar to that caused by foliar application.
- (3) For foliar Zn application, chloride was more efficient than sulfate and EDTA, and these latter sources were similarly efficient.
- (4) Addition of urea increased ZnSO₄ efficiency, but the addition of sucrose did not increase the efficiency of foliar fertilizations with either Zn chloride or sulfate.

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