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### Boron-zinc interaction in the absorption of micronutrients by cotton

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#### ABSTRACT

B-Zn interaction modifies the nutritional dynamics of copper (Cu), iron (Fe) and manganese (Mn) in cotton. This study aimed to evaluate the effect of B and Zn concentrations on the absorption of Cu, Fe and Mn in cotton plants cultivated in nutrient solution. The experimental design was completely randomized with three replicates, in a 4 x 5 factorial scheme, corresponding to four concentrations of B (0, 20, 40 and 80  $\mu\text{M L}^{-1}$ ) and five concentrations of Zn (0, 1, 2, 4 and 8  $\mu\text{M L}^{-1}$ ). At 115 days after emergence, the plants were collected, divided into roots, shoots and fruits, and subjected to chemical analysis. The results allowed to conclude that the Cu content and total Cu in the fruit, total Cu in the roots, Cu use efficiency, Fe content in the roots, Fe absorption efficiency, Mn content in the fruit and Mn absorption efficiency of cotton are influenced by the concentrations of B in the solution. The interaction between B and Zn affected total Fe in the roots, Fe content and total Fe in the fruit, Fe transport efficiency, total Mn in the shoots and Mn transport efficiency; in addition, Zn acts differently according to the supply of B and vice versa.

**Keywords:** *Gossypium hirsutum* L., micronutrients, nutritional efficiency

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## Resumo

A interação B e Zn modifica a dinâmica nutricional de Cu, Fe e Mn no algodoeiro. Objetivou-se com o presente trabalho avaliar o efeito das concentrações de B e Zn sobre a absorção de cobre, ferro e manganês pelo algodoeiro cultivado em solução nutritiva. O delineamento experimental utilizado foi o inteiramente casualizado com três repetições, em esquema fatorial 4x5, sendo quatro concentrações de boro (0, 20, 40 e 80  $\mu\text{M L}^{-1}$ ) e cinco concentrações de zinco (0, 1, 2, 4 e 8  $\mu\text{M L}^{-1}$ ). Aos 115 dias após emergência, as plantas foram coletadas, divididas em raiz, parte aérea

e frutos, e submetidas à análise química. Os resultados permitiram concluir que o teor e o conteúdo de Cu no fruto, conteúdo de Cu na raiz, eficiência de utilização de Cu, teor de Fe na raiz, eficiência de absorção de Fe, teor de Mn no fruto e a eficiência de absorção de Mn pelo algodoeiro são influenciados pelas concentrações de B na solução. A interação B e Zn afetou o conteúdo de Fe na raiz, teor e conteúdo de Fe no fruto, eficiência de transporte de Fe, conteúdo de Mn na parte aérea e eficiência de transporte de Mn, sendo que o Zn atua de maneira diferenciada em função do fornecimento de B e vice-versa.

**Palavras-chave:** *Gossypium hirsutum* L., micronutrientes, eficiência nutricional.

## Introduction

Cotton farming is one of the main activities of the Brazilian agribusiness. The increasing use of more productive varieties in the entire world, with great demand of nutrients, requires a better knowledge on the nutritional relationships in cotton (Rochester & Constable, 2015). In this context, better knowledge on the nutritional dynamics is important for an efficient fertilization program.

*Gossypium hirsutum* L. – species of greatest use for cotton production in Brazil – is responsive to the application of micronutrients, especially in areas of low natural fertility. Among the micronutrients, boron (B) and zinc (Zn) are the ones that most limit the yield of crops (Araújo & Camacho, 2012; Araújo et al., 2013). B is directly related to the metabolism of the ribonucleic acid (RNA), to different functions of the plasmatic membrane and is a constituent of the structure of the cell wall and pectic substances associated with it, especially the middle lamella (Wimmer & Eichert, 2013). Zn is required in many active sites of different proteins, such as carbonic anhydrase and superoxide dismutase. The deficiency of this nutrient reduces the photosynthetic rate

through the modification of the activity of enzymes involved in the process of carbon fixation (Broadley et al., 2007; Assunção et al., 2013).

B-Zn interaction influences different metabolic processes of the plant, interfering with the mineral composition through the stimulation or inhibition of the absorption of other nutrients (Baxter, 2009). These relationships are highly variable and may occur inside the cells or in the rhizosphere (Morgan & Connolly, 2013). The B-Zn interactions in cotton are correlated (Araújo & Camacho, 2012) and the reduction in B absorption as a function of Zn is common. Besides cotton (Araújo & Camacho, 2012), this result has already been observed for lemon (Rajaie et al., 2009), olive tree (Jasrotia et al., 2014) and wheat (Nasim et al., 2015). Thus, it is evident the effect of the B-Zn interaction on the absorption of these nutrients. However, there is still limited information on the effect of B-Zn interaction on the dynamics of absorption of other micronutrients, such as Cu, Fe and Mn. Many nutritional relationships have been observed between micronutrients (Baxter, 2009; Milner et al., 2013; Aibara & Miwa, 2014). These nutritional interactions demonstrate to be

complex, with initial modification at a molecular level, resulting in variation of the absorption and mineral composition of the plants. Thus, the observed nutritional interaction depends on the availability of nutrients and on the analyzed plant species (Araújo & Camacho, 2012; Araújo et al., 2013). Thus, it is evident the need for studies that address the classic nutritional interaction between B and Zn, evaluating the absorption of Cu, Fe and Mn. The knowledge about the interactions on micronutrients helps the efficient use of fertilizers, consequently reducing production costs. The hypothesis of this study is that the combinations of B and Zn in nutrient solution modify the content, total and the efficiency of absorption, use and transport of Cu, Fe and Mn in cotton. Given the above, the present study aimed to evaluate the effect of B and Zn concentrations on the absorption of Cu, Fe and Mn in cotton cultivated in nutrient solution.

### Material and methods

The experiment was conducted in an agricultural greenhouse of the Plant Production Sector of the State University of Mato Grosso do Sul (UEMS), in Aquidauana-MS, at the geographic coordinates of 20°28'S, 55°48'W and altitude of 174 m. The experimental design was completely randomized with three replicates, in a 4 x 5 factorial scheme, corresponding to four concentrations of B (0, 20, 40 and 80  $\mu\text{M L}^{-1}$ ), applied in the form of boric acid, and five concentrations of Zn (0, 1, 2, 4 and 8  $\mu\text{M L}^{-1}$ ), applied in the form of zinc sulfate.

The experimental units consisted of plastic pots with capacity for 3 L, filled with washed and sterilized quartz sand. Cotton seeds, cv. FiberMax 910, were placed to germinate on trays with moistened sand. Five days after emergence, when cotyledon leaves appeared, three seedlings were transplanted to each experimental unit, where they received nutrient solution for growth, complete and diluted at 1/5 (Epstein & Bloom, 2006). At 28 days after

emergence, thinning was performed, leaving only one plant in each experimental unit and the application of nutrient solution started according to each treatment, with irrigations three times a day using deionized water. In the solutions of treatments with absence of nutrient, the concentrations were identical to those of the complete solution, except for the absent nutrient.

The nutrient solution showed the following composition: 6.0 mL of 1 mol  $\text{L}^{-1}$   $\text{KNO}_3$ ; 4.0 mL of 1 mol  $\text{L}^{-1}$   $\text{Ca}(\text{NO}_3)_4 \cdot \text{H}_2\text{O}$ ; 2.0 mL of 1 mol  $\text{L}^{-1}$   $\text{NH}_4\text{H}_2\text{PO}_4$ ; 1.0 mL of 1 mol  $\text{L}^{-1}$   $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 1.0 mL of 0.2 mol  $\text{L}^{-1}$  Fe-EDTA; 1.0 mL of 0.05 mol  $\text{L}^{-1}$  KCl; 1.0 mL of 0.02 mol  $\text{L}^{-1}$   $\text{H}_3\text{BO}_3$ ; 1.0 mL of 0.002 mol  $\text{L}^{-1}$   $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ; 1.0 mL of 0.002 mol  $\text{L}^{-1}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; 1.0 mL of 0.0005 mol  $\text{L}^{-1}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 1.0 mL of 0.0005 mol  $\text{L}^{-1}$   $\text{H}_2\text{MoO}_4$  (85%  $\text{MoO}_3$ ).

At 115 days after emergence, the plants were collected and divided into roots, shoots (stem and leaves) and fruits (boll). All the collected plant material was washed in detergent solution at 3 mL  $\text{L}^{-1}$ , running water, 0.1 mol  $\text{L}^{-1}$  HCl solution and deionized water, respectively. The samples were placed in paper bags and then dried in forced-air oven at 65 °C for 72 h. After drying, the plant material was ground in a Wiley-type mill. The samples were subjected to nitric-perchloric digestion for the determination of Cu, Fe and Mn contents in the different plant parts (Malavolta et al., 1997).

The determination of Cu, Fe and Mn was used to calculate the absorption efficiency ( $\text{mg g}^{-1}$ ) = [total content of nutrient in the plant (mg)/root dry matter (g)] (Swiader et al., 1994); transport efficiency (%) = [(content of nutrient in the shoots (mg)/ total content of nutrient in the plant (mg)) x 100] (Li et al., 1991); and use efficiency ( $\text{g}^2 \text{mg}$ ) = [(total dry matter produced (g))<sup>2</sup>/ total content of nutrients in the plant (mg)] (Siddiqi & Glass, 1981).

The results received statistical treatment, with analysis of response surface using the statistical package SAS, adopting a 0.05 significance level. Initially, analysis of variance

was performed and, according to the significance of the F test, the study of polynomial regression was conducted (for cases with significant interaction), through the RSREG procedure.

## Results and Discussion

The interaction between the treatments of B and Zn influenced the Fe content in the boll, total Fe in the roots and in the boll, total Mn in the shoots and transport efficiency of Fe and Mn (Table 1). There was significant response ( $p < 0.05$ ) of B concentrations for Cu content in the boll, total Cu in the roots and in the boll, Fe content and total Fe in the roots, Mn content in the boll, besides Cu use efficiency and the efficiencies of absorption of Fe and Mn (Table 1).

Zn doses in the solution did not affect individually any of the studied variables. Various studies indicate antagonistic relationship between Zn and cationic micronutrients, such as Cu, Fe and Mn. This interaction occurs due to the competition of the cations for the absorption sites (Baxter, 2009; Broadley et al., 2007; Assunção et al., 2013). This study did not find antagonistic relationship between Zn and other cationic micronutrients (Fe, Mn and Cu), disagreeing with the results reported by Rajaie et al. (2009), Aref (2012) and Lima Neto & Natale (2014) in other plant species. Hence, only B doses and the combined doses of B and Zn influenced the absorption of Cu, Fe and Mn in cotton (Table 1).

Cu content and total Cu in the cotton fruit were influenced by the B concentrations in the solution. The increase in B concentrations in the solution until  $40 \mu\text{M L}^{-1}$  promoted reduction in the Cu content and total Cu in cotton bolls (Figure 1A, 1B). B doses higher than  $40 \mu\text{M L}^{-1}$  increased Cu content and total Cu in the bolls. Aref (2012) observed increment in the concentration of Cu in maize leaves with the increase in B concentrations in the cultivation

substrate. Ahmed et al (2011) observed a positive effect of B doses on Cu content and total Cu in different parts of cotton plants. However, the total Cu in cotton roots linearly decreases with the increment in B concentrations in the solution (Figure 1C), agreeing with the results found by. Esringu et al. (2002) observed reduction of Cu concentration in strawberry roots with the increase in the supply of B, reporting an antagonistic relationship between the nutrients.

Cu use efficiency for the production of dry matter was positively influenced by the supply of B in the solution, so that the increase in B concentrations promoted linear increment in the Cu use efficiency of cotton plants (Figure 1D). B is related to various biochemical processes in the plants; thus, its concentrations in the cultivation substrate may directly or indirectly affect the use of Cu by the plant (Malavolta, 2006; Wimmer & Eichert, 2013).

B concentrations in the solution promoted significant increment in Fe content and total Fe in cotton roots (Figure 2A, 2B). Similar results were obtained by Esringu et al. (2012) in strawberry, in which the Fe content in the roots increased with the concentration of  $10 \mu\text{M L}^{-1}$  of B, decreasing at the concentration of  $20 \mu\text{M L}^{-1}$  of B. These results suggest that B has certain affinity for Fe and that there may be a synergetic relationship between the nutrients. Rajaie et al. (2009) also observed significant increase in the concentration of Fe with the increment in B levels in *Citrus Aurantifolia*.

Fe content and total Fe in the cotton fruit were significantly affected by the interaction between B and Zn in the solution. The increase in B concentrations in the solution promoted reduction in Fe contents and total Fe in the fruit with the increment in Zn concentrations (Figure 2C, 2D). Ahmed et al. (2011) reported that the Fe content, in different parts of the cotton crop, significantly increased with the increment in the applied levels of B. The increase in Fe concentration in the fruit

**Table 1.** Summary of the analysis of variance: coefficient of variation and p-value for the effect of B, Zn and the B-Zn interaction for content, total and efficiency of absorption, transport and use of Cu, Fe and Mn.

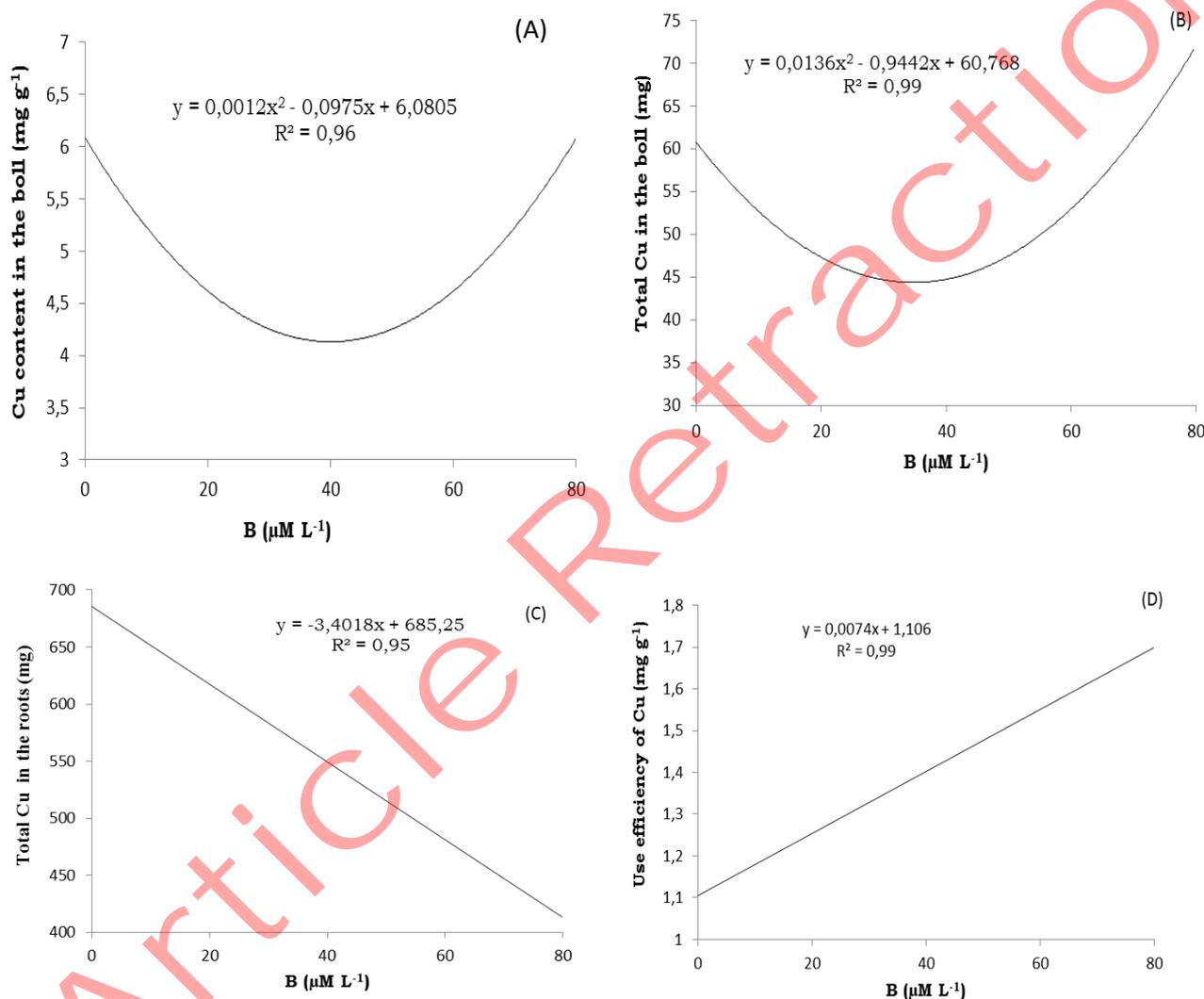
Parâmetros	CV (%)	p-valor		
		B	Zn	B x Zn
<b>Copper (Cu)</b>				
Cu content in the shoots (g kg <sup>-1</sup> )	67,29	0,846	0,065	0,532
Cu content in the roots (g kg <sup>-1</sup> )	37,53	0,110	0,216	0,184
Cu content in the boll (g kg <sup>-1</sup> )	26,09	0,000*	0,468	0,119
Total Cu in the shoots (mg)	66,09	0,805	0,065	0,488
Total Cu in the roots (mg)	44,49	0,028*	0,476	0,053
Total Cu in the boll (mg)	32,30	0,000*	0,555	0,129
Absorption efficiency of Cu(mg g <sup>-1</sup> )	33,21	0,197	0,105	0,387
Transport efficiency of Cu (%)	42,92	0,560	0,159	0,173
Use efficiency of Cu (mg g <sup>-1</sup> )	32,17	0,013*	0,339	0,205
<b>Iron (Fe)</b>				
Fe content in the shoots (g kg <sup>-1</sup> )	28,92	0,209	0,364	0,173
Fe content in the roots (g kg <sup>-1</sup> )	32,57	0,003*	0,878	0,121
Fe content in the boll (g kg <sup>-1</sup> )	32,66	0,000*	0,078	0,000*
Total Fe in the shoots (mg)	31,16	0,220	0,531	0,207
Total Fe in the roots (mg)	32,06	0,000*	0,646	0,005*
Total Fe in the boll (mg)	41,74	0,000*	0,346	0,000*
Absorption efficiency of Fe(mg g <sup>-1</sup> )	27,72	0,005*	0,800	0,253
Transport efficiency of Fe (%)	37,44	0,000*	0,830	0,009*
Use efficiency of Fe (mg g <sup>-1</sup> )	31,32	0,054	0,578	0,186
<b>Manganese (Mn)</b>				
Mn content in the shoots (g kg <sup>-1</sup> )	14,73	0,060	0,103	0,053
Mn content in the roots (g kg <sup>-1</sup> )	28,83	0,057	0,491	0,469
Mn content in the boll (g kg <sup>-1</sup> )	19,53	0,000*	0,653	0,218
Total Mn in the shoots (mg)	18,69	0,000*	0,264	0,003*
Total Mn in the roots (mg)	36,62	0,059	0,867	0,055
Total Mn in the boll (mg)	24,89	0,063	0,089	0,456
Absorption efficiency of Mn (mg g <sup>-1</sup> ) <sup>1)</sup>	17,72	0,000*	0,131	0,592
Transport efficiency of Mn (%)	21,8	0,104	0,777	0,035*
Use efficiency of Mn (mg g <sup>-1</sup> )	19,6	0,051	0,116	0,506

B: Boron; Zn: Zinc; CV: Coefficient of variation. \*Significant by F test at 0.05 probability level

with the increasing levels of B has also been reported in guava (Salvador et al., 2003) and in *Citrus Aurantifoli* (Rajaie et al. 2009), disagreeing with the results obtained in the present study. Little information is available about the influence of B on the absorption of other micronutrients, and the results vary according to the analyzed plant species.

Fe absorption efficiency positively responded to the supply of B in the solution, so that the increase in B concentrations promoted

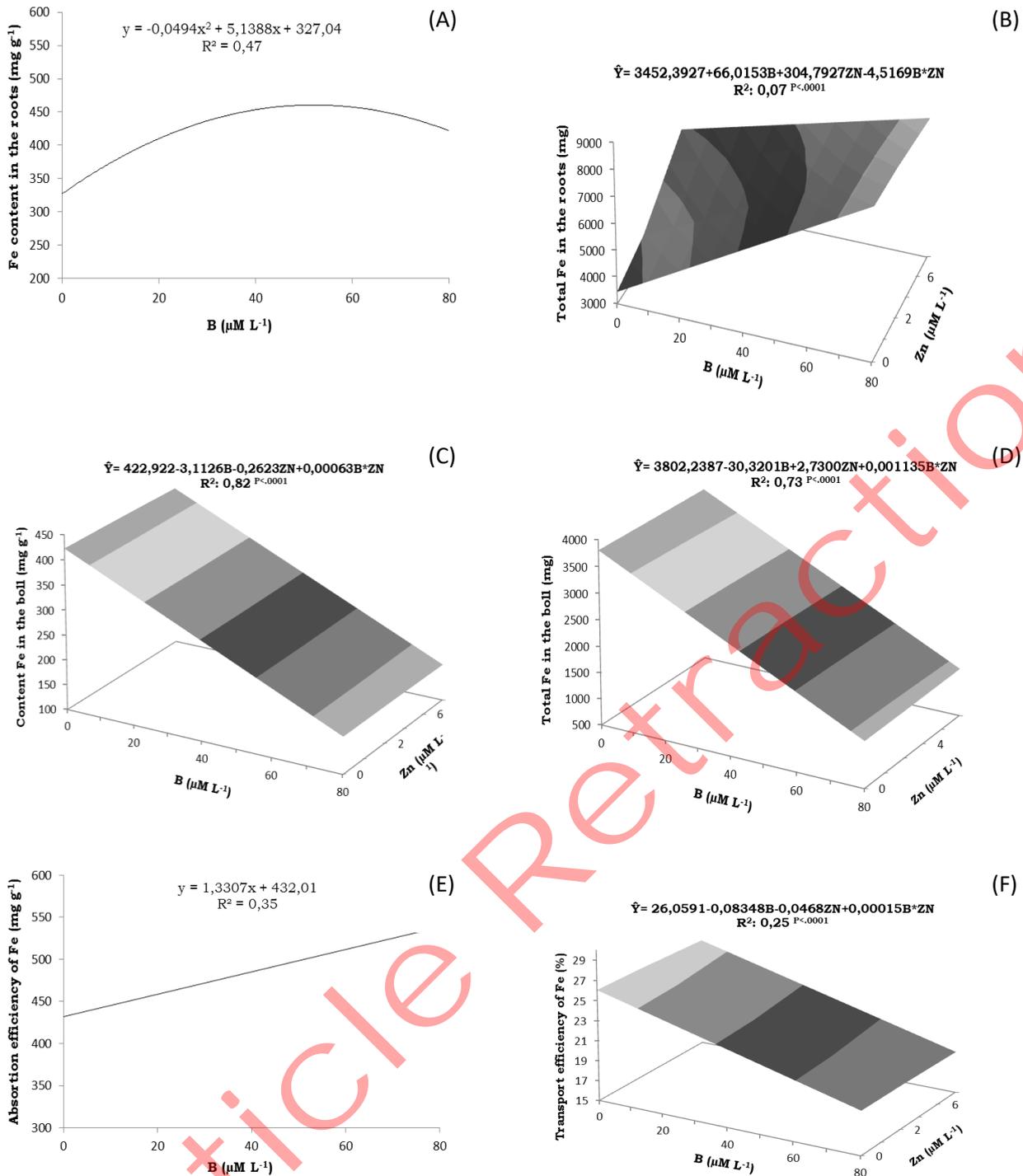
increment in the Fe absorption efficiency of cotton (Figure 3E). Fe transport efficiency was affected by the interaction between B and Zn in the solution, demonstrating an antagonistic relationship between B and Zn at the highest concentrations of B, and a synergetic relationship at the highest concentrations of Zn (Figure 3F). It is possible to observe an increment of 25% in the transport of Fe by the roots with the increase in Zn concentrations at 0  $\mu\text{M L}^{-1}$  of B.



**Figure 1.** Content of Cu in the boll (A), total Cu in the boll (B), content of Cu in the roots (C) and Cu absorption efficiency (D) of cotton in response to different B concentrations in the nutrient solution

The Mn content in the fruit decreased with the increment in B concentrations, until 40  $\mu\text{M L}^{-1}$  of B, with an increase at the highest concentrations of the nutrient (80  $\mu\text{M L}^{-1}$ ), the same behavior observed for Cu content and

total Cu in the cotton fruit (Figure 3A). Aref (2012), working with maize plants, observed that the content of Mn was affected by B fertilization, with a reduction in leaf Mn content under these conditions. On the other hand,



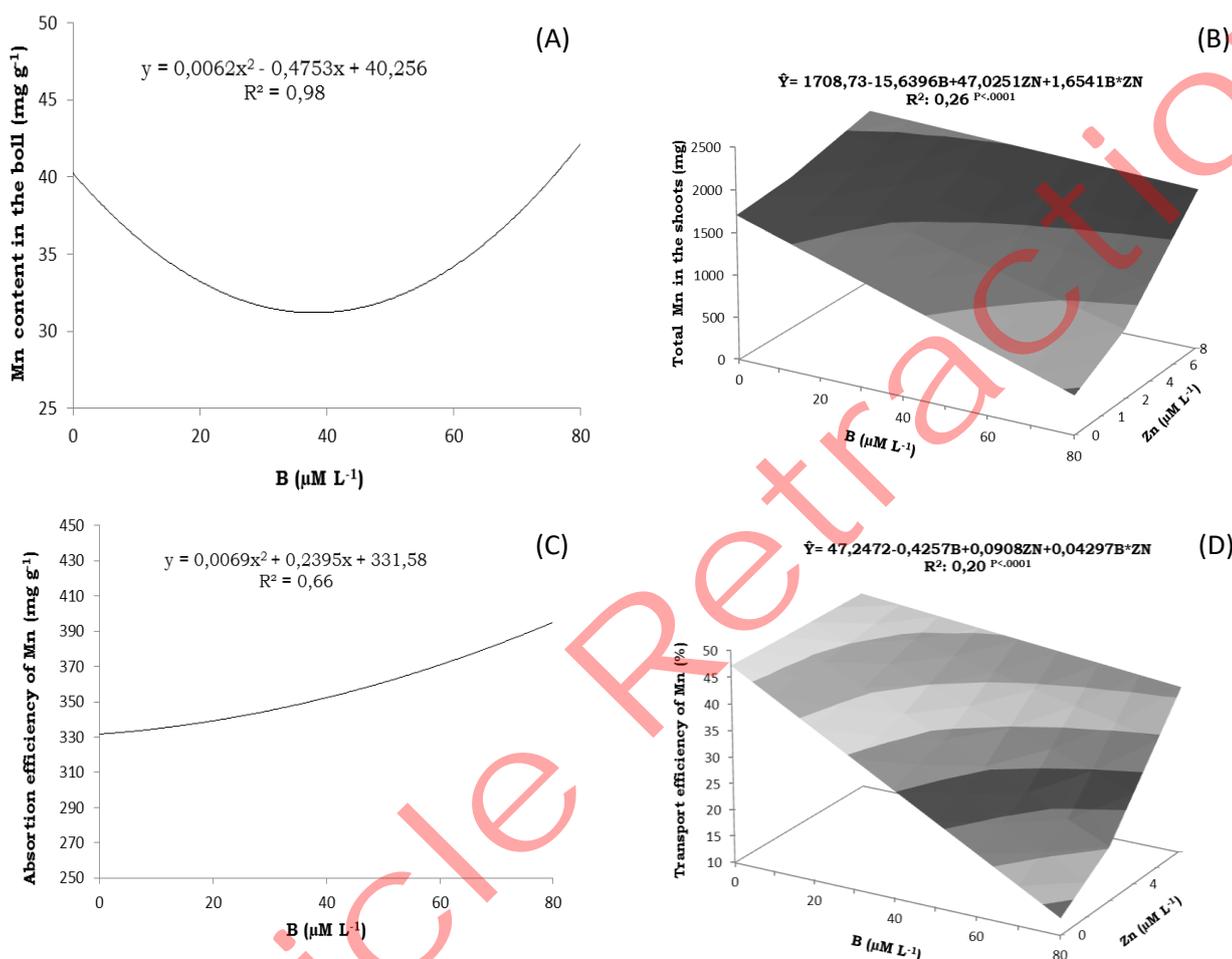
**Figure 2.** Content of Fe in the roots (A), total Fe in the roots (B), content of Fe in the boll (C), total Fe in the boll (D) and efficiency of absorption (E) and transport (F) of Fe of cotton in response to different B concentrations in the nutrient solution.

Esringu et al. (2012) observed increment in leaf Mn content with the increase in B concentrations, until  $10 \mu\text{M L}^{-1}$ , with reduction at the highest B concentration ( $30 \mu\text{M L}^{-1}$ ). In contrast, the data of the present study indicate that Mn positively responded to B, only at the highest concentration of the nutrient.

The total Mn in the shoots and Mn transport efficiency were negatively influenced by the increase in B concentration at  $0 \mu\text{M L}^{-1}$  of Zn and positively influenced at  $8 \mu\text{M L}^{-1}$  of Zn (Figure 3B, 3D). It is possible to observe an increment of 49% in the transport of Mn by the roots with the increase in Zn concentrations at

0  $\mu\text{M L}^{-1}$  of B. This reduction in Mn content and total Mn in cotton roots and shoots in response to the increase in B concentrations may be due to the effect of dilution or the antagonistic relationship between B and Mn. The source of B can influence, as a regulator or inhibitor, the accumulation and use of other nutrients (Aref, 2012). Mn absorption efficiency linearly

increased with the increment in B concentrations (Figure 3C). B is involved in physiological processes that control the absorption and transport of Cu, Fe, Mn and Zn (Malavolta, 2006; Wimmer & Eichert, 2013). Dursun et al. (2009) also observed increase in Cu, Fe and Mn contents in leaves of tomato and pepper as a function of B doses.



**Figure 3.** Content of Mn in the boll (A), total Mn in the shoots (B) and efficiency of absorption (C) and transport (D) of Mn of cotton in response to different B and Zn concentrations in the nutrient solution.

## Conclusions

Cu content and total Cu in the fruit, total Cu in the roots, Cu use efficiency, Fe content in the roots, Fe absorption efficiency, Mn content in the fruit and Mn absorption efficiency of cotton are influenced by the concentrations of B in the solution.

The B-Zn interaction affected total Fe in the roots, Fe content and total Fe in the fruit, Fe

transport efficiency, total Mn in the shoots and Mn transport efficiency; in addition, Zn acts differently according to the supply of B and vice versa.

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