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Effect of zinc and boron interaction on growth and mineral composition of lemon seedlings in a calcareous soil

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Abstract

The impact of different concentration of zinc (Zn) and boron (B) on growth and mineral composition of lemon seedlings (*Citrus Aurantifolia* L.) was studied under greenhouse conditions. We used five concentration levels of B (0, 2.5, 5, 10 and 20 μg g^{-1} soil) and three of Zn (0, 5 and 10 μg g^{-1} soil). Fresh and dry plant weights of the control treatment were strongly decreased with B levels higher than 5 μg g^{-1} of soil. In above mentioned B levels, lemon seedlings displayed slight to severe B toxicity symptoms. Zn treatments reduced B accumulation and the associated inhibitory effect on plant growth. Increased B level in soils enhanced the concentration of B in plant shoots to a greater extent in the absence of applied Zn. The effectiveness of Zn for the reduction of B accumulation and toxicity decreased as the level of applied B was increased. In a comparison of Zn-deficient and Zn-treated soils, plant root and shoot analysis for B indicated an increased transport of B in the absence of Zn. The best plant production was achieved when 2.5 and 10 μg g^{-1} soil of B and Zn were applied simultaneously. This combination was associated with the highest uptake of Zn, nitrogen (N), phosphorous (P), potassium (K), iron (Fe), manganese (Mn) and copper (Cu), suggesting that the combination resulted to a suitable condition in which plants had a well-balanced nutritional status. While B application increased nutrients accumulation in plant shoot, the role of Zn was a dilution effect or antagonistic relationship. The results of this study indicated that supplemental Zn has potential and practical importance in the control of B absorption and toxicity in soil where plants are grown under Zn deficiency and B toxicity.

Keywords: Zinc; Boron; lemon seedlings; mineral composition; calcareous soils

Introduction

Although boron (B) is an essential plant micronutrient, it is also phytotoxic if present in excess amounts in growth medium. Reisenauer et al. (1973) noted that the range between deficient and toxic levels of B is narrow for most plants, and that the ratio of adequate to toxic concentration is narrower than for any other nutrient element. Levels of B above the optimum range cause significant changes in the activity of numerous enzymes and consequently, the metabolism of higher plants (Shkolnik, 1974). Furthermore, B toxicity also resulted in increases in membrane permeability, and possible role of membrane integrity and structure in tolerance mechanism of B toxicity reported by Karabal et al. (2003). B toxicity is a common problem of many plants grown on soils with high B concentration. Nable et al. (1997) reported that soils containing more than 5 to 8 mg L^{-1} of hot water soluble B is considered to probably cause B toxicity. Natural soils derived from marine evaporates contain a great concentration of B. In addition, irrigation water and industrial sources of B may also play an important role in increased B level in cultivable soils (Nable et al., 1997). Moreover, poor drainage especially in saline soils may be responsible for the increased concentration of B in the soil solution (Goldberg, 1997; Grieve and Poss, 2000; Malasha et al., 2008). Soil pH is one of the most important factors that affects B uptake by plants. Many investigators (Bartlett and Picarellin, 1973; Peterson and Newman, 1976; Gupta and Macleod, 1981) have found that increasing soil pH by liming to above pH 6.5 reduces B concentration in many plants. Despite the fact, B toxicity is a serious problem in some calcareous soils of arid and semiarid regions where soils are usually alkaline in nature but irrigated with water high in B.

Among micronutrients, zinc (Zn) deficiency is one of the widespread nutritional disorders in many plants. Zn availability is inversely related to soil pH and its deficiency in variety of plant species is frequently noted on calcareous soils with pH>8.0 (Swietlik, 1989; Ma and Lidsay, 1990; Srinivasara et al., 2008). Studies have revealed that, B toxicity and Zn deficiency are interestingly related with each other. Swietlik and Laduke (1991) observed that increasing Zn concentration in grapefruit (*Citrus paradisi*) leaf decreased B toxicity. In a subsequent study, Swietlik (1995) reported that B toxicity symptoms were severe in Zn-deficient sour orange (*Citrus aurantium*) seedlings, but extremely mild in Zn-sufficient plants. This study suggests that Zn-deficient seedlings were more sensitive to B toxicity than Zn-sufficient ones. In nutrient culture, Graham et al. (1987) reported that low Zn treatment did not affect plant growth, but enhanced B concentration to a toxic level in barley (*Hordeum vulgare*). Similarly, Zn deficiency enhanced B concentration in wheat (*Triticum aestivum*) grown on Zn deficient soils (Singh et al, 1990). Sinha et al. (2000) noted a synergistic interaction between Zn and B in mustard (*Brassica nigra*) when both the nutrients were either in low or excess supply. Hosseini et al. (2007) reported that high levels of B decreased plant height and dry matter production of corn (*Zea mays* L.). There was a significant B and Zn interaction on plant growth and tissue nutrient concentration which were rate dependent. In general, the effect was antagonistic in nature on nutrient concentration and synergistic on plant growth.

In certain highly calcareous soils of Southern Iran, high concentration of soluble B and low level of available Zn may occur simultaneously, leading to a complex nutritional disorder. Although large proportion of these soils is under cultivation of citrus plants, restricted published information could be found in respect to the effect of Zn and B interaction on the performance of citrus species. Since lemon seedling is used as main plant or dominant citrus rootstock in gardens of the region, the aim of this study was to investigate the interactive effect of applied Zn and B on the growth and chemical composition of lemon seedlings (*Citrus Aurantifolia* L.) grown in a calcareous soil.

Materials and methods

Soil materials

A bulk sample was collected from surface horizon (0-20 cm) of a calcareous soil that had been homogenized by plowing. The sampling site was located in Experiment Station of Azad University, Jahrom, Iran, which is under cultivation of agronomic crops. The soil was air-dried and passed through a 2-mm sieve. Particle-size distribution determined by hydrometer method (Gee and Bauder, 1986), soil pH and Ec_e were measured in saturated paste and saturated extract respectively, calcium carbonate equivalent (CCE) was determined by neutralization with HCl (Loeppert and Suarez, 1996), Cation exchange capacity (CEC) and organic matter (OM) were determined by replacing cations with NaOAc (Summer and Miller, 1996) and Walkley-Black method (Nelson and Sommers, 1996), respectively. Available Zn, Fe, Mn, and Cu were determined by DTPA extraction (Lindsay and Norvell, 1978), phosphorus by sodium bicarbonate extraction (Olsen et al., 1954). Soil available B was extracted by hot water (Berger and Truog, 1939) and measured by azomethine-H colorimetric method (Bingham, 1982). The characteristics of the soil materials are presented in Table 1.

Table 1. Selected chemical and physical properties of the soil.

Property	Quantiy
Sand (%)	30
Silt (%)	46
Clay (%)	24
OM[a] (%)	0.7
Ec (dS m^{-1})	0.9
CCE[b] (%)	51.5
pH	7.56
CEC[c] ($cmol_{(+)}$ kg^{-1})	10.1
Hot water-soluble B(mg kg^{-1})	0.2
$NaHCO_3$-available P (mg kg^{-1})	5.5
Soluble P in $NaHCO_3$ (mg kg^{-1})	14.3
Soluble Fe in DTPA(mg kg^{-1})	2.2
Soluble Mn in DTPA(mg kg^{-1})	4.2
Soluble Zn in DTPA(mg kg^{-1})	0.56
Soluble Cu in DTPA(mg kg^{-1})	0.78

[a] Organic matter , [b] Calcium carbonate equivalent, [c] Cation exchange capacity

Seedling culture and growth

Five hundred lemon seeds (*Citrus Aurantifolia* L.) obtained from Citrus Research Station of Jahrom, Iran, were sown in the calcareous soil in plant nursery of Azad University, Jahrom, Iran. On the basis of soil analysis, recommended chemical fertilizers were added to soil. Seedlings were allowed to grow for about one year under controlled greenhouse conditions (15 h photoperiod and 25 ± 2 ^oC). During the period plants were irrigated once every 3 days with distilled water. When seedlings were approximately one year old, uniform ones (4 mm diameter and 30 cm height) were selected and one seedling was transplanted to each plastic pots (with dimensions of 20 cm diameter and 30 cm height) containing 6 kg of the described soil. Soil of pots were fertilized with 50 µg nitrogen (N) g^{-1} soil as $(NH4)_2$ SO_4, 20 µg phosphorous (P) g^{-1} soil as monopotassium phosphate (KH_2PO_4), 5, 5, and 2.5 µg g^{-1} soil of iron (Fe) as ethylenediamine di-hydroxyl acetic acid (Fe-EDDHA), manganese (Mn) as manganese sulfate (MnSO4.H2O), and copper (Cu) as copper sulfate (CuSO4.5H2O) before transplantation. All nutrients were added in solution form and the soil in each pot was thoroughly mixed and poured back into the plastic pots.

Treatment design

Soil treatments with Zn and B started immediately after seedlings establishment. Treatments consisted of five concentration levels of B (0, 2.5, 5, 10 and 20 µg B g-1 soil as boric acid) and three of Zn [(0, 5 and 10 µg Zn g-1 soil as zinc sulfate (ZnSO4.7H2O)]. All Zn and B treatments were added in solution form on the basis of soil dry weight. The statistical design was completely randomized factorial with four replicates. After application of Zn and B treatments, the experiment lasted for five months in the above mentioned greenhouse conditions. Two months after treatments utilization 50 µg N g-1 soil as (NH4)2 SO4 was added to the pots as side dressing. During the experiment period pots were daily irrigated to field capacity with distilled water. At the end of the experiment, shoots were harvested from the above ground and roots were separated from the soil. Shoots fresh weight were measured by weighing and plant samples were rinsed with distilled water and oven dried at 70○C for 48 hours.

Plant analytical procedure

After weighing of shoots to interpret dry matter production, all samples were ground to pass a 40-mesh sieve, dry-ashed at 500°C, dissolved in 2 N of hydrochloric acid (HCl) and made to 100 ml volume with hot distilled water. In shoot extracts B determination was made by the azomethine-H colorimetric method (Bingham, 1982). Total N was measured by micro-Kjeldhal method, P by spectrophotometer, K by flame photometer, and Zn, Fe, Mn and Cu by atomic absorption spectrophotometer. In root extracts only the concentration of B and Zn was measured by the above mentioned methods.

Statistical analysis

The data were subjected to statistical analysis using MSTATC computer software. Duncan's multiple-ranged test was also performed to identify the homogenous sets of data. All statements reported in this study are at the $P < 0.05$ levels.

Results and discussion

Plant growth and B toxicity symptoms

Application of Zn and B to soil significantly affected plants fresh and dry weight (Table 2). In all levels of B application, Zn supply up to 10 µg g^{-1} soil improved fresh and dry matter production. While application of 2.5 and 5 µg B g^{-1} soil had no/negligible effect on plant growth, higher B concentrations decreased plant fresh and dry weight significantly (Table 2). Although plant available B in a specific soil is controlled by the soil's chemical and physical properties, such as pH, soil texture, clay mineralogy, organic matter, etc (Goldberg, 1997), soil containing more than 5 to 8 mg L^{-1} of hot water soluble B is considered to probably cause B toxicity (Nable et al., 1997). In the present study it can be concluded that B concentrations of 10 and 20 µg B g^{-1} soil have reached soil available B to toxic level.

Table 2. Effects of B and Zn application on fresh and dry weight of lemon seedlings.

Applied Zn (µg g^{-1})	Applied B (µg g^{-1})					
	0	2.5	5	10	20	Mean
			Fresh weight (g pot^{-1})			
0	21.5 b	20.7 bc	19.0 cd	15.1 e	6.02 f	16.5 C
5	21.7 b	22.5 b	21.9 b	15.8 e	5.87 f	17.5 B
10	26.0 a	27.3 a	22.2 b	18.4 d	6.36 f	20.0 A
Mean	23.0 A	23.5 A	21.0 B	16.4 C	6.08 D	
			Dry weight (g pot^{-1})			
0	5.71 c	5.65 c	5.01 d	4.05 e	1.65 f	4.41 C
5	5.72 c	6.01 c	5.66 c	4.11 e	1.74 f	4.65 B
10	6.88 b	7.61 a	6.07 c	4.84 d	1.85 f	5.45 A
Mean	6.10 B	6.42 A	5.58 C	4.33 D	1.75 E	

For B and Zn main effects, means with similar capital letters and for their interaction, means with similar small letters have no significant difference according to Duncan's multiple range test at $P<5\%$.

Soil fertilization with Zn alleviated the suppressing effect of B on plant growth (Table 2). For example, in Zn-untreated plants, application of 10 µg B g^{-1} of soil reduced plant dry weight by 29.0%, whereas at the same B rate and 10 µg Zn g^{-1} of soil this reduction was 15.8% (Table 2). Decrease in B toxicity by Zn application was also observed in barley (Graham et al., 1987), wheat (Singh et al., 1990), grapefruit (Swietlik and Laduke, 1991), sour orange (sweitlik, 1995), mustard (Sinha et al., 2000) and corn (Hosseini et al., 2007). Increase in the level of applied B decreased the effectiveness of Zn on the alleviation of B toxicity. For instance, at B rate of 2.5 µg g^{-1} soil, increase in Zn supply from 0 to 10 µg Zn g^{-1} soil increased plant dry weight by 34.7%, whereas at 20 µg B g^{-1} and similar Zn applications this increase was 12.1% (Table 2). The best plant biomass production was achieved when 2.5 and 10 µg g^{-1} soil of B and Zn were applied simultaneously (Table 2).

Irrespective of Zn treatment, no B toxicity symptoms were observed in B rate of 2.5 µg g^{-1} soil. Symptoms in the form of tip chlorosis of old leaves, followed by marginal and interveinal chlorosis were slightly developed in 5 and severely in 10 and 20 µg B g^{-1} soil. The severity of leaf injury due to B toxicity was more pronounced in plants that received no additional Zn supply. These symptoms were similar to those reported in citrus plants by Oertli (1960), Sweitlik (1995), Papadakis et al (2004a) and Papadakis et al (2004b). According to these researchers, usually these chlorotic/necrotic patches have greatly elevated B concentration compared with surrounding leaf tissue and reflect a passive uptake of B which is associated with B accumulation at the end of transpiration stream. Furthermore, such symptoms indicate that B is immobile in the studied citrus species. According to Brown and Hu (1996) leaf burn is not the only visible symptom of B toxicity in all plant species. In plant species that B is phloem mobile; B remobilizes and accumulates in other parts than in the leaves. In such plant species toxicity symptoms are fruit disorders (gummy nuts and internal necrosis) and bark necrosis resulting from the death of the cambial tissues or stem die back.

B and Zn concentration in plant shoot and root

B and Zn concentrations in plant shoot and root as affected by B and Zn application are shown in Table 3. Increasing additions of B to the soil increased the concentration of B in plant shoot and root. Concentration of B in plant shoots was enhanced in the absence than in the present of applied Zn (Table 3). In the present study, the diminishing effect of applied Zn on shoot B concentration became less pronounced as the level of applied B was increased. For instance if B rates of 2.5, 5, 10 and 20 µg g^{-1} soil were applied, increasing Zn supply from 0 to 10 µg g^{-1} soil decreased shoot B concentration by 47.0, 34.3, 30.1 and 25.2% respectively (Table 3).

Table 3. Effects of B and Zn application on B and Zn concentrations in shoot and root of lemon seedlings.

Applied Zn	Applied B (µg g^{-1})					
(µg g^{-1})	0	2.5	5	10	20	Mean
			Shoot B concentration (µg g^{-1})			
0	23.9 j	117 h	201 g	332 d	699 a	275 A
5	21.8 j	85.0 i	187 g	282 e	574 b	230 B
10	21.7 j	62.0 i	132 h	232 f	523 c	194 C
Mean	22.5 E	87.8 D	174 C	282 B	599 A	
			Root B concentration (µg g^{-1})			
0	4.92 h	21.8 g	38.3 f	76.5 e	136 c	55.6 C
5	5.11 h	21.4 g	40.0 f	80.6 de	155 b	60.4 B
10	4.79 h	25.1 g	45.4 f	87.5 d	163 a	65.1 A
Mean	4.94 E	22.8 D	41.3 C	81.5 B	151.4 A	
			Shoot Zn concentration (µg g^{-1})			
0	28.3 h	33.0 gh	33.1 gh	37.8 fg	68.0 c	40.0 C
5	39.5 f	46.8 e	47.5 e	53.5 d	77.4 b	52.9 B
10	65.7 c	68.8 c	70.4 c	79.6 b	94.8 a	75.9 A
Mean	44.5 D	49.5 C	50.3 C	57.0 B	80.1 A	
			Root Zn concentration (µg g^{-1})			
0	22.0 gh	20.7 h	23.2 fgh	23.6 fgh	31.1 d	24.1 C
5	29.0 de	28.2 de	25.4 efg	26.8 ef	48.3 b	31.5 B
10	43.0 c	45.0 bc	43.3 c	45.6 bc	54.7 a	46.3 A
Mean	31.3 B	31.3 B	30.7 B	32.0 B	44.7 A	

For B and Zn main effects, means with similar capital letters and for their interaction, means with similar small letters have no significant difference according to Duncan's multiple range test at P<5%

Data presented in Table 2 and 3 show slight reduction in plant growth when the concentration of B in lemon shoot was less than 200 µg g^{-1} dry weight. According to the data reported by Chapman (1968) in citrus plants, acute to slight B deficiency occurs when B concentration is in the range of 15-25 µg g^{-1} leaf dry matter. Normal range is reported to be 30-100 µg g^{-1}, while slight to moderate, pronounced and severe toxicity are considered to be associated with concentrations of 100-300, 200-500 and 500-2400 µg g^{-1} respectively.

The best plant production was achieved when 2.5 and 10 μg g^{-1} soil of B and Zn were applied simultaneously. In these treatments the concentration of B and Zn in plant shoot were 62.0 and 68.8 μg g^{-1} dry weight respectively (Table 2 and 3).

Unlike the suppressing effect of Zn on concentration of B in lemon shoot, Zn supply increased the concentration of B in plant root (Table 3). This suggests that higher B concentration in shoots of Zn-deficient plants might be due to the increased B transport from root to shoot, implying that Zn provides a protective mechanism against excessive B uptake. As stated by Swietlik (1995), roots of Zn-sufficient plants appear to be more efficient in restricting B accumulation in the above ground parts, although the mechanism of this restriction is not known. Graham et al. (1987) reported that Zn deficiency caused an accumulation of B in barley to a toxic level. They suggested that Zn performs a protective role at the external root surface and/or root cell membranes which decreases B absorption by plant.

In the present study higher B concentrations were found in plant shoot than in the root (Table 3). These data are in agreement with those reported for citrus and other plant species, where B concentration was highest in the leaves and low in the roots, fruits and other storage and meristematic tissues (Lovatt and Bates, 1984; Bellaloui and Brown, 1998; Papadakis et al., 2004a; Papadakis et al., 2004b). The results suggest that B is transported to the leaves via transpiration stream, and that the remobilization of B from leaves to other organs is limited.

The concentration of Zn in plant shoot and root increased with both Zn and B application (Table 3). Since Zn uptake decreased when B treatment was higher than 2.5 μg g^{-1} soil, the enhancement in shoot Zn concentration can be attributed to an accumulation effect due to reduced plant dry matter production resulting from B toxicity. While similar results were reported by Gunes et al. (1999) in tomato, Hosseini et al. (2007) and Ohki (1975) observed that shoot Zn concentration did not follow a definite trend with B addition in corn and cotton respectively. The highest Zn uptake occurred when 2.5 and 10 μg g^{-1} soil of B and Zn were applied simultaneously. This observation indicates that when Zn is in an adequate supply and B concentration is not too high to cause toxicity a synergistic effect may be present between these two elements. Almost similar results were observed in oilseed rape by Grewal et al. (1998), showing that sufficient B application increases Zn uptake when Zn supply is adequate.

Mineral composition of lemon seedlings

Nitrogen (N), phosphorous (P) and potassium (K) concentrations in shoot of lemon seedlings as affected by B and Zn application are shown in Table 4. Increments in B addition significantly increased the concentration of N, P and K in lemon shoot (Table 4). These observations are in agreement with those reported in corn (Hosseini et al., 2007), groundnut (Patel and Golakiya, 1986) and lentil (Singh and Singh, 1983). Although B addition increased the concentration of N, P and K in lemon shoot, the total uptake of these elements did not follow a definite trend. For example, irrespective of Zn supply, the highest N uptake occurred in B rate of 2.5 μg g^{-1} soil and it decreased as the level applied B was further increased (total uptakes are not shown but can be calculated from the data reported in Tables 2 and 4). P uptake was not significantly affected in B levels of 2.5, 5 and 10 μg g^{-1}soil, but it showed a significant decrease in 20 μg B g^{-1} soil.

Table 4. Effects of B and Zn application on total nitrogen, P and K concentrations in shoot of lemon seedlings.

Applied Zn (μg g^{-1})	Applied B (μg g^{-1})					
	0	2.5	5	10	20	Mean
			N concentration (%)			
0	1.83 g	2.31 de	2.09 efg	2.51 cd	3.61 a	2.47 A
5	1.87 fg	2.07 efg	1.90 fg	2.61 c	3.32 b	2.36 B
10	1.55 h	1.88 fg	1.88 fg	2.14 ef	3.10 b	2.11 C
Mean	1.75 D	2.09 C	1.96 C	2.42 B	3.34 A	
			P concentration (g kg^{-1})			
0	1.75 h	1.99 ef	1.97 fg	2.38 bc	2.73 a	2.16 A
5	1.78 gh	1.60 hi	1.73 h	2.18 de	2.56 ab	1.97 B
10	1.39 j	1.33 j	1.42 ij	1.78 gh	2.23 cd	1.63 C
Mean	1.64 C	1.64 C	1.71 C	2.12 B	2.50 A	
			K concentration (%)			
0	2.23 gh	2.59 ef	3.90 b	3.37 c	4.27 a	3.27 A
5	2.26 gh	2.34 fgh	3.16 cd	2.86 de	3.93 b	2.91 B
10	2.02 h	2.48 fg	3.10 cd	3.16 cd	3.74 b	2.90 C
Mean	2.17 E	2.47 D	3.39 B	3.13 C	3.98 A	

For B and Zn main effects, means with similar capital letters and for their interaction, means with similar small letters have no significant difference according to Duncan's multiple range test at P<5%

The total uptake of K increased as the level of applied B changed from 0 to 5 μg B g^{-1} soil, but decreased in higher B concentrations. Judging on these data, it can be concluded that in high B treatments, increase in concentration of N, P and K was an accumulation effect resulting from the reduction of plant growth, rather than the enhanced uptake of these elements. In the case of K, increased plant uptake of K in B rates of 2.5 and 5 μg g^{-1} soil may be a synergistic relationship between these two nutrients. Kumar et al. (1981) reported that B addition elevated K concentration in rice shoot. Also such mutual synergistic relationship was reported by Hosseini et al. (2007) in corn. In spite of the observed differences, the highest uptake of N, P and K was achieved when 2.5 and 10 μg g^{-1} of B and Zn were applied simultaneously. This suggests that the combination has created a suitable condition in which plants had a well balanced nutritional status and optimum yield. Patel and Golakiya (1986) reported that B application, up to 2 μg g^{-1} soil, registered the highest pod yield in groundnut and significantly increased the uptake of N, P, K, iron (Fe), copper (Cu) and B.

On the other hand, Zn application decreased the concentration of N, P and K in plant shoot (Table 4). Judging on plant uptakes, the effect of Zn on N and K concentration was a dilution effect, whereas in the case of P it might be an antagonistic relationship. As suggested by Welch et al. (1982), Zn is necessary for root cell membrane consistence and in this function, Zn prevents excessive P uptake and its transport from roots to shoots. Similarly, Sofaya (1976) observed that Zn deficient corn plants had higher P concentrations than Zn sufficient ones.

The effect of B and Zn application on shoot concentrations of Fe, manganese (Mn) and Cu are shown in Table 5. There was a significant increase in shoot concentration of these elements with increasing B levels. As it was discussed for N, P and K, the effect of B on concentration of Fe, Mn and Cu was an accumulation effect rather than increased uptake resulted from B. Zn application decreased the concentration of Fe, Mn and Cu in plant shoot. Since there was no significant decrease in plant uptake of Fe, Mn and Cu with Zn addition, reduction in the shoot concentration of these nutrients may be due to dilution effect or antagonistic relationship between Zn and other micronutrients. Swietlik and Zhang (1994) observed that in nutrient solution, increase in Zn concentration from 5 to 37 µM increased the growth of sour orange seedlings and as a result the leaf P, K, Ca, Mg, Fe, Mn and Cu concentrations fell quiet rapidly at first and then decreased gradually or leveled off. Loneragan and Webb (1993) reported that antagonistic relationship between Zn and other cationic micronutrients (Fe, Mn and Cu) appears as a result of competition at the absorption sites of plant root. Swietlik (1995) reported that B and particularly Zn treatments modified the concentration of P, K, Mg, Fe, Mn and Cu in the leaves and roots of sour orange seedlings, but these changes were too small to explain the observed growth responses.

Table 5. Effects of B and Zn application on Fe, Mn, and Cu concentrations in shoot of lemon seedlings.

Applied Zn (mg kg^{-1})	Applied B (mg kg^{-1})					
	0	2.5	5	10	20	Mean
			Fe concentration (µg g^{-1})			
0	136 def	138 def	149 cd	164 c	283 a	174 A
5	143 de	124 fg	143 de	167 c	278 a	171 A
10	116 g	115 g	125 efg	150 cd	241 b	149 B
Mean	132 CD	126 D	139 C	160 B	267 A	
			Mn concentration (µg g^{-1})			
0	70.1 ef	80.0 bc	78.2 cd	87.2 b	97.5 a	82.6 A
5	63.1 fg	71.8 de	76.3 cde	82.9 bc	79.0 cd	74.6 B
10	54.1 h	52.6 h	59.9 gh	68.7 ef	78.6 cd	62.8 C
Mean	62.4 D	68.1 C	71.5 C	79.6 B	85.0 A	
			Cu concentration (µg g^{-1})			
0	11.7 de	11.7 de	13.5 c	13.4 c	17.3 a	13.5 A
5	11.1 ef	11.5 de	10.2 fg	12.7 cd	15.1 b	12.1 B
10	9.18 g	9.20 g	9.03 g	10.0 fg	13.3 c	10.2 C
Mean	10.6 C	10.8 C	10.9 C	12.0 B	15.2 A	

For B and Zn main effects, means with similar capital letters and for their interaction, means with similar small letters have no significant difference according to Duncan's multiple range test at P<5%

The highest plant uptake of Fe, Mn and Cu occurred when 2.5 and 10 µg g^{-1} soil of B and Zn were applied simultaneously. As it was already discussed it indicates that the combination has created a well-balanced nutritional status for plant growth. Patel and Golakiya (1986) reported that B application up to 2 µg g^{-1} soil registered the highest pod yield in groundnut and significantly increased the uptake of N, P, K, Fe, Cu and B.

Conclusions

The observation that B toxicity in Zn-deficient lemon seedlings could be alleviated with Zn applications is of potential and practical importance as B toxicity and Zn deficiency are simultaneously encountered in some soils of arid and semiarid regions. It is recommended that B and Zn interaction be evaluated under field conditions. Furthermore, B toxicity is the result of B accumulation in plant leaves which as the main photosynthetic organs play a key role in the development of toxicity symptoms and growth reduction. It seems useful to study the effect of Zn on the distribution of B in plant material, where in the role of Zn on the alleviation of B toxicity could be related to the distribution of B in various plant and leaf parts.

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