Concentration of zinc and boron in corn leaf as affected by zinc sulfate and boric acid fertilizers in a deficient soil

Farshid Aref
Department of Soil Science, Science and Research Branch, Islamic Azad University, Fars, Iran
farshidaref@iauf.ac.ir, farshidaref@yahoo.com

Abstract: Zinc (Zn) and boron (B) deficiency is one of the most widespread micro nutritional disorders in crops, and occurs predominantly in calcareous soils of arid and semiarid regions. A field experiment with maize plant grown on Zn and B deficient soil was carried out in a calcareous soil at Fars Province, Iran. This work aimed to study the interaction effect of Zn and B on the concentration of Zn and B in the maize leaf during 2008. Treatments included five levels of Zn (0, 8, 16 and 24 kg ha^{-1} Zn added to the soil and Zn foliar spray at 0.5 weight percent of zinc sulfate) and four levels of B (0, 3, and 6 kg ha^{-1} B added to the soil and B foliar spray at 0.3 weight percent of boric acid) in a completely randomized block design. The findings showed that application of Zn, B, and Zn−B interaction on the B concentration in the leaf was insignificant. Zinc spraying increased Zn concentration in the leaf but adding Zn to the soil had no significant effect on it. Presence of B prevented from the increase in Zn concentration in the leaf; that is, an antagonism was seen between the Zn and B. Boron application decreased leaf Zn content and this increase was not influenced by the amount of Zn. Zinc use at no presence B in the soil (zero B and B spraying levels) increased leaf Zn content.
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1. Introduction

Plant analysis is an important component of soil fertility and plant nutrition research. Zinc an essential element for the normal growth and metabolism of plants played very important role in enzyme activation and was also involved in the biosynthesis of some enzymes and growth hormones (Ranja and Das, 2003). Zinc deficiency is very common in many agricultural crops especially on high-pH soils. Zinc is one of the essential elements for plants, animals and humans, but it is deficient (less than 1 mg kg^{-1} DTPA-extractable Zn) in most calcareous soils and consequently in plant, animal and human diets (Cakmak 2006). The amount of extractable Zn varied markedly depending on the soils and extractants used. 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 Lindsay, 1990; Srinivasara et al., 2008). Total Zn concentration is in sufficient level in many agricultural areas, but available Zn concentration is in deficient level because of different soil and climatic conditions. Soil pH, lime content, organic matter amount, clay type and amount and amount of applied P fertilizer affect the available Zn concentration in soil (Adiloglu, et al., 2006).

Boron is an essential micronutrient for plants, but the range between deficient and toxic B concentration is smaller than for any other nutrient element (Goldberg, 1997). Soils may contains 0.5−2 mg kg^{-1} of available B, but this represents only a small part of the total since only 0.5−2.5% of the total B in the soil is available to plants. There is also a very narrow range between B deficiency and toxicity as more than 5 mg kg^{-1} available B can be toxic to many agronomic crops (Kelling, 2010). 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). Boron uptake by plants is controlled by the B level in soil solution rather than the total B content in soil. 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. The range of B concentrations in the soil solution, in which plants suffer neither toxicities nor deficiencies, is very narrow (0.3−1 mg kg^{-1}). Soil factors affecting availability of B to plants are: pH, texture, moisture, temperature, organic matter and clay mineralogy (Goldberg, 1997). Soils low in organic matter are deficient in B more often than soils with high organic matter content. When the surface sol dried out, plants are unable to fee in the zone where most of the available B in present. This can lead to B deficiency. When rain or irrigation moistens the soil, the plants can again fee from the surface soil and the B deficiency often disappears (Kelling, 2010). Soil pH is one of the most important factors that affects B uptake by plants. Many investigators (Peterson and Newman, 1976; Gupta and Macleod, 1981, Kelling, 2010) have found that increasing soil pH by liming to above pH 6.5 reduces B concentration in many plants. B deficiency causes various changes in properties such as membrane integrity and permeability, auxin metabolism, sugar transport, lignifications in the cell wall, carbohydrate metabolism and transport, respiration (Loomis and Durst, 1992), and reduced fertility (Marschner, 1995).

Analysis of plant tissue gives a good indication of the B nutrition of plants. Excessive amounts of B can cause problems with some crops; B application in a fertilizer program should be stopped if the B concentration in the plant tissue is in the sufficient or high range. Kelling (2010) reported that, because B levels in the plant change with age, it is important to indicate the stage of development at sampling. Relative levels of B in mg kg^{-1} for corn in sample of whole plant when plant height is 15−38 cm are: < 4 deficient, 4−6.4 low, 6.5−40 sufficient, 40.1−55 high, and > 55 excessive. Also, B levels in ear leaf of corn at tassel to silking stage are: < 2 deficient, 2−5 low, 5.1−40 sufficient, 40.1−55 high, and > 55 excessive (Kelling, 2010). Deficiency or excess of B not only affects the relative values of individual elements, but it also affects the balance among certain nutrient elements within plants, causing either an increase or decrease of dry matter production (Tariq and Mott, 2007).

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. Shaaban et al. (2004) reported that increasing rates of B and Zn were applied to maize plant in calcareous soil under greenhouse conditions, Egypt. Boron concentration of plant increased with increasing B application. On the other hand, Zn concentration of plant decreased with this application. Zinc concentration of maize increased with Zn application. Whitehead (1987) found that the accumulations of zinc (Zn^{+2}) varied in different plant species. Frey et al. (2000) measured Zn concentration in shoot which was higher and reached a maximum value of 83 mM·kg^{-1} dry mass whereas total concentration of Zn in roots were lower up to 13 mM·kg^{-1}. Concentration of Zn in dry matter of corn (mg kg^{-1}) in lower leaves at tasseling stage was: 9−9.3 showing deficiency symptoms, and 31.1−36.6 intermediate (Barker and Pilbeam, 2007); in leaves at 6th node from base at silking stage was 15−24 showing deficiency symptoms, 25–100 intermediate, and 101–150 high (Deleers et al., 1985); and in ear leaf at silking stage was: < 10 showing deficiency symptoms, 20−70 Intermediate, 71−100 high, and > 100 showing toxicity symptoms.

The objective of this research was to measure concentration and uptake of Zn and B in corn leaves as affected by Zn and B application and interactions between Zn and B in plant.

2. Materials and Methods

A field experiment was conducted at the farm of F. Aref in Abadeh Tashk, Fars province of Iran, on the corn (*Zea mays* L.), cultivar "Single Cross 401" during 2008 cropping season. The experiment site located 200 km northeast of Shiraz on latitude 29° 43' 10" N and longitude 53° 51' 56" E and 1580 m altitude. Composite surface soil samples were collected from surface horizon (0− 30 cm) of the soil before the experiment was initiated, air-dried, passed through a 2-mm sieve and analyzed for the following properties. Selected soil chemical and physical characteristics for the soil are presented in Table 1. Particle-size distribution determined by hydrometer method (Gee and Bauder, 1986), soil pH and ECe were measured at a 1:2.5 soil/water ratio and saturated extract, respectively, organic matter (OM) content by the Walkley-Black method (Walkley, 1947). Soil available K was determined by 1 M NH_4OAc extraction and K assessment in the extract by flame photometer (Thomas, 1982). Soil P available was measured by Olsen method. Available Fe, Zn, Mn and Cu in the soil were first extracted by DTPA and then were read by atomic absorption. Soil available B was extracted by hot water and measured by Azomethine-H colorimetric method (Bingham, 1982). This soil had a loam texture, pH of 8, 0.56 % organic matter, 9 mg kg^{-1} available P, 205 mg kg^{-1} available K, DTPA extractable Fe, Mn, Zn and Cu concentration were 6.2, 9.9, 0.63 and 1.4 mg kg^{-1} and available B with hot water extractable was 0.87 mg kg^{-1}.

This experiment consisted of 20 treatments and 3 replications in the form of completely randomized block design and factorial that combinations of five levels Zn (0, 8, 16 and 24 kg ha^{-1} Zn added to the soil and Zn foliar spray at 0.5 weight percent of zinc sulfate) and four levels of B (0, 3, and 6 kg ha^{-1} B added to the soil, and B foliar spray at 0.3 weight percent of boric acid). Nitrogen, P and K used at 160, 90 and 80 kg ha^{-1} according to the recommendation, from sources of urea (with 46% N), triple super phosphate (with 46% P_2O_5) and potassium sulfate (with 50% K_2O), respectively, were added to all treatments.

Half of the urea was used when planting and the remainder two times: At vegetative growth and when the corn ears were formed. Potassium and P used before planting. Zinc and B, from zinc sulfate and boric acid sources, respectively, were used by two methods: adding to the soil and spraying. Addition to the soil was made at the time of plantation and the sprayings were made at 0.5% zinc sulfate and 0.3% boric acid two times: one at vegetative growth stage and the other after corn ears formation. The Zn and B were both applied to the leaves with uniform coverage at a volume solution of 2500 L ha^{-1} using a knapsack sprayer. Each experimental plot was 8 m length and 3 m width, had 5 beds and 4 rows, equally spaced, and seeds 20 cm apart on the rows. At silking stage, leaf samples were taken from the second and third leaves from the top of plant. The leaves were dried in a forced air oven at 70°C for 48 h. Total elements were analyzed after digestion of dry and milled plant material with HCl 2 N (Wolf, 1982). The total micronutrient concentrations: Fe, Mn, Zn, and Cu were analyzed by atomic-absorption spectrophotometry (Hocking et al, 1977). All micronutrient concentrations were expressed in mg kg^{-1} DW. To measure B concentration in leaf tissues, the Azomethine-H method was followed and the mixture was read by spectrophotometry (Wolf, 1974). The concentration of B was expressed as mg kg^{-1} DW.

Standard analysis of variance techniques were used to assess the significance of treatment means. Each variable was subjected to ANOVA using the Statistical Analysis System (SAS version 8.2, SAS Institute, 2001) for each soil. Treatment (fraction) means were separated by Duncun's multiple range test ($P < 0.05$ level). Multiple regression analyses (stepwise procedure) were conducted to evaluate the relationships between concentration of Zn and B in leaf with other factors.

3. Results and Discussion

3.1. Soil analysis

Physicochemical characteristics of soil taken before the experiment was initiated in the May 2008 are presented in Table 1. While table 1 indicates the soil available K was high but available P was lower than the critical level suggested in scientific sources (Karimian and Yasrebi, 1995). Karimian and Ghanbari (1990) have reported the critical P level by the Olsen method in calcareous soils as 18 mg kg^{-1}. The soil Zn and B content was lower than the critical level. High soil pH and $CaCO_3$ content induce B deficiency in the surveyed area. Similar results were found by Borax (1996) and Rashid et al. (1997). In soil, the B concentration of <0.65 mg kg^{-1} and >3.5 mg kg^{-1} are deficient and toxic levels for cotton crop, respectively (Annonymous, 1981). For many crops, a DTPA-extractable Zn level of 0.5−0.8 mg kg^{-1} has been regarded as a soil critical level below which crop production would be limited by Zn deficiency (Martins and Lindsay, 1990). The soil Mn, Cu and Fe content was above the critical level. Sims and Johnson (1991) have reported the critical levels of Fe, Zn, Mn and Cu by the DTPA extraction method and B by the hot water in the soil method to be 2.5−5, 0.2−2, 1−5, 0.1−2.5 and 0.1−2 mg kg^{-1}, respectively. Chen et al., (2001) reported that Fe deficiency is likely to be observed if the Fe concentration in the soil is less than 4−5 mg kg Fe, extracted by DTPA-Calcium chloride, with a pH of 7.3. Elgbla et al., (1986) showed that critical levels of Fe, Mn and Cu in the calcareous soils were 3.8, 1.2 and 0.7 mg kg^{-1}, respectively.

Table 1. Soil physical and chemical analysis

Properties	Values
Depth of soil (cm)	0−30
Soil texture	Loam
pH	8
EC (ds m^{-1})	1.2
Organic matter (%)	0.56
Nutrients (mg kg^{-1})	
P	9
K	205
Fe	6.2
Mn	9.9
Zn	0.63
Cu	1.4
B	0.87

3.2. The leaf B concentration

Application of different levels of Zn, B and the Zn-B interaction showed no significant effect on the leaf B concentration (mg kg^{-1}) at the 5% level (Table 2). Aref (2011) studied the effect of Zn and B on the leaf Zn and B concentrations and reported that Due to a Zn and B antagonism, high amounts of Zn in the soils, prevented from increase of leaf B content by B application; also Zn application prevented from B use affecting B concentration in the leaf. The lowest and the highest leaf B content were 25 and 39.33 mg kg^{-1} DW but showed no significant difference relative to the control. The B concentration in leaf was higher than other research. So that, Mills and Jones (1996) reported that sufficiency range of B in ear leaf of maize at silking stage was 5−25 mg kg^{-1}; Of course, compared to results of other researchers was sufficient (Kelling, 2010).

3.3. The leaf Zn concentration

The main effect of Zn on the Zn concentration in the leaf was significant at 5% level, but the effect of different B levels on the Zn concentration in the leaf was insignificant at 5% level (Table 3). Zinc application to the soil had no significant difference from the zero Zn level, but Zn spraying increased Zn concentration in the leaf relative to the no Zn level. Foliar spray increased leaf Zn concentration from 32.42 to 39.67 mg kg^{-1}, showing a 22.4% increase as compared with the no Zn level. In fact, most calcareous soils of Iran prevent absorption of Zn; in this soils Zn are soon rendered insoluble.

On calcareous soils where Zn and B deficiency are common, applications of Zn and B compounds to the soil have not been very successful because the Zn and B are soon rendered insoluble. Barker and Pilbeam (2007) reported that applications of nutrients by foliar spray are effective in areas of California and Arizona where soil applications of micronutrients are ineffective because elements such as Zn, Mn, and Cu are fixed in forms that are not readily available to certain crops. Foliar applications, besides resulting in higher Zn accumulation in plants, could be used to advantage if a farmer omitted the addition of Zn in the NPK bulk fertilizer or if Zn deficiency was suspected. Foliar spray applications in the early growth stages resulted in greater absorption of Zn than did those applied at later stages of growth (Gupta and Cutcliffe, 1978). This result indicates that Zn uptake, at least by corn, is more efficient through leaves than through the soil–root systems. Soils deficient in Zn frequently are low in organic matter, are sandy and/or have an alkaline pH (pH greater than 7.0) (Follett and Westfall, 2010). Foliar spray of Zn is the most common because it is the most widely deficient micronutrient. Treatment can also be quite effective if the correct material and methods of application are used (Christensen, 1982). Neutral Zn (52% Zn) and Zn oxide (75% Zn) are the most economical and effective on a recommended label basis (Christensen et al., 1978 and Christensen, 1982). There is no advantage in using chelated Zn products in sprays. They were originally intended for soil application, are more expensive, and less effective than neutral. Therefore in calcareous soils such as this region foliar application of Zn is effective than soil application.

The lowest and the highest mean Zn concentration in the leaf, 31.17 and 39.67 mg kg^{-1}, were seen at 16 kg ha^{-1} Zn and Zn spraying levels, respectively. Concentration of Zn in this research was sufficiency range according to the results of Mills and Jones (1996), they stated that sufficiency range for Zn in ear leaf of maize at silking (mg Zn per kg dry matter of plant tissue) was 25 – 100.

The effect of Zn-B interaction on leaf Zn concentration showed B application in any Zn levels had no significant effect on the Zn concentration in the leaf. The use of Zn foliar spray at B spraying level, increased leaf Zn concentration from 30.67 to 44.33 mg kg^{-1} which showed a 44.5 percent increase relative to the no Zn use at this B level, but showed no significant difference relative to the control with a leaf Zn content of 35 mg kg^{-1} (Table 3). At other B levels, Zn use had no significant effect on the leaf Zn content. Also, any treatments showed no significant effect on the leaf Zn concentration relative to the control (no Zn and no B use).

Table 2. The effect of Zn and B on B concentration in the leaf (mg kg^{-1})*

B (kg ha^{-1})	Zn (kg ha^{-1}) 0	8	16	24	Zn foliar spray	Mean of B levels
0	31.67 ab	30.67 ab	31.33 ab	30.67 ab	29.67 ab	30.80 a
3	31.67 ab	35.33 ab	34.33 ab	33 ab	33 ab	33.47 a
6	39.33 a	32 ab	30.67 ab	39 ab	32.67 ab	34.73 a
B foliar spray	32.67 ab	32.33 ab	32.67 ab	25 b	37 ab	31.93 a
Mean of Zn levels	33.83 ab	32.58 a	32.25 a	31.92 a	33.08 a	

The same letters are not significantly different in each row or in each column (p<0.05) by Duncan's test

Table 3. The effect of Zn and B on Zn concentration in the leaf (mg kg^{-1})*

B (kg ha^{-1})	Zn (kg ha^{-1}) 0	8	16	24	Zn foliar spray	Mean of B levels
0	35 abc	27.33 c	29.67 bc	32 bc	37.67 abc	32.33 a
3	31.67 bc	34 bc	32.67 bc	37.67 abc	37.33 abc	34.67 a
6	32.33 bc	35.67 abc	32.67 bc	36.33 abc	39.33 ab	35.27 a
B foliar spray	30.67 bc	33 bc	29.67 bc	36.33 abc	44.33 a	34.8 a
Mean of Zn levels	32.42 b	32.5 b	31.17 b	32.58 ab	39.67 a	

The same letters are not significantly different in each row or in each column (p<0.05) by Duncan's test

3.4. The correlation between the concentration of Zn and B in the leaf with other variables

The correlation coefficients (r) between different variables by the Pearson method and the relevant equations were obtained by the step by step method using the SPSS software. One can use each of the following equations depending on what are the variables measured and r and r^2, but the last equation derived, is the most complete equation containing dependent and independent variables and we must measure more variables to derive that equation. The symbols * and ** in equations and correlation coefficients (r or r^2), are significance at 5% ($\alpha = 0.05$) and 1% ($\alpha = 0.01$) levels.

3.4.1. The leaf B concentration

BL = 2.63 + 14.3 NL, $r = 0.62^{**}$

BL and NL are leaf B content (mg kg^{-1}) and leaf N content (%).There was a positive correlation between leaf B concentration and leaf N content ($r = 0.62^{**}$), K content (r = 0.36), Fe content ($r = 0.61^{**}$), Mn content (r = 0.42) and Cu content (r = 0.43), ear length (r = 0.38). ear diameter (r = 0.34), and number of grains along the ear (r = 0.37).

3.4.2. The leaf Zn concentration

The leaf Zn content showed a positive correlation with leaf N content (r = 0.30), K content (r = 0.36), Fe content (r = 0.36), Mn content ($r = 0.45^{*}$), and Cu content ($r = 0.84^{**}$), and ear weight (r = 0.33), and a negative correlation with leaf P content (r = - 0.36) and plant height (r = - 0.33). The equations of which were:

1) ZnL = 9.711 + 3.418 CuL, $r = 0.84^{**}$

2) ZnL = 39.765 + 3.439 CuL – 0.141 H, $r^2 = 0.82^{**}$

ZnL, CuL and H denote leaf Zn content (mg kg^{-1}), leaf Cu content (mg kg^{-1}) and plant height (cm).

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