

## Zinc and Boron Content by Maize Leaves from Soil and Foliar Application of Zinc Sulfate and Boric Acid in Zinc and Boron Deficient Soils

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**Abstract:** Among many growth factors Zn and B were recognized as two of main limiting factors of maize crop growth and yielding. To identify the effect of Zn and B fertilizers on concentration of Zn and B in the corn leaves two field experiments were conducted in a calcareous soil at Fars Province of Iran during 2009 to 2010. Treatments included five levels of Zn (0, 8, 16 and 24 kg ha<sup>-1</sup> Zn added to the soils and Zn foliar spray with a 0.5 percent concentration) and four levels of B (0, 3 and 6 kg ha<sup>-1</sup> B and B foliar spray with a 0.3 percent concentration) in a completely randomized block design. Results of this study clearly show that there was an antagonism between the Zn and B. No B content in the soil (zero and B spraying levels) helped increasing leaf Zn content, but presence of B in the soils prevented from increase of leaf Zn content. The presence of B in the soil, assisted to decreasing of B concentration in the leaf by Zn application; but B spraying helped increasing leaf B content by Zn use. 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.

**Key words:** Deficiency • Interaction • Concentration • Fertilizer • Nutrition

### INTRODUCTION

Plant analysis can help predict Zn and B needs when soil tests and deficiency symptoms are not conclusive. Zinc (Zn) deficiency in soils has been reported worldwide, particularly in calcareous soils of arid and semiarid regions. The regions with Zn-deficient soils are also the regions where Zn deficiency in human beings is widespread, for example in India, Pakistan, China, Iran and Turkey [1, 2]. In a global soil survey study, Sillanpaa [3] found that 50% of the soil samples collected in 25 countries were Zn deficient. Nearly 50% of the cereal grown areas in the world have soils with low plant availability of Zn [4, 5]. Zinc deficiency is a particularly widespread micronutrient deficiency in wheat, leading to severe depressions in wheat production and nutritional quality of grains [6, 7, 5]. In Iran, Zn deficiency is the most widespread micronutrient deficiency in soils and plants [8]. Aref [9] stated that concentrations of Zn in Iran soils were among the very lowest recorded. Micronutrient deficiency has been reported widely in plant crops grown in different countries. Zinc deficient soils are widespread in Mediterranean countries [10, 11]. Some crops (maize, rice) are especially susceptible to Zn deficiency in most

countries where they are grown. Several studies have reported the significant reduction in growth and yield of different plant species in soils deficient in Fe, Mn, Zn, Cu, B and other micronutrients [12, 13, 11]. Therefore, correcting micronutrient deficiency through appropriate approaches is necessary to achieve higher crop yields.

Most research on soil and foliar application of Zn focused on alleviating its deficiencies, particularly on wheat and corn cultivated in semiarid or arid regions of the world [14, 15]. Maize was recognized by farmers for a long time as a crop of high response to Zn supply [16]. Maize is a crop plant characterized by a high potential of biomass and grain yield, especially in comparison with other cereal crops. The basic aspect of a balanced agriculture is a balanced fertilization which should take into consideration all nutritive components indispensable for a correct growth and development of plants. The insurance of the optimal level of plant growth including the availability of nutritive agents guarantees the realization of plant yield-creating potential. Maize grain yield potential (GYP) is twice as high as compared to other cereal crops [17]. However, even if quantitative requirements for nutrients are almost the same, actual harvested yields are low [16].

As well documented by plant physiologists, Zn exerts a great influence on basic plant life processes, such as (i) nitrogen metabolism - uptake of nitrogen and protein quality; (ii) photosynthesis - chlorophyll synthesis, carbon anhydrase activity; (iii) resistance to abiotic and biotic stresses - protection against oxidative damage [1, 14, 15]. In most crops, the typical leaf Zn concentration required for adequate growth approximates 15-20 mg Zn kg<sup>-1</sup> DW [18]. Toxicity symptoms usually become visible at leaf Zn > 300 mg kg<sup>-1</sup> DW, although some crops show toxicity symptoms at leaf Zn < 100 mg kg<sup>-1</sup> [19, 18] and toxicity thresholds can be highly variable even within the same species. For example, leaf Zn associated with a 50% yield reduction in radish ranged from 36 to 1013 mg kg<sup>-1</sup> DW [20].

There are a number of soil and environmental factors that affect B uptake by plants. Knowing these will improve our assessment of B deficiency and toxicity under different conditions. When B is released from soil minerals, or is mineralized from organic matter, or is added to soils by means of irrigation or fertilization, part of it remains in the soil solution while part of it is adsorbed by the particles of soil [21]. They do not usually determine the solubility of B in the soil solution, which is controlled mainly by B adsorption reactions. An equilibrium exists between the soil solution and adsorbed B [22]. In general, soils derived from igneous rocks and those in tropical and temperate regions of the world, have much lower B content than soils derived from sedimentary rocks and those in arid or semi-arid regions [23]. Soil reaction is one of the most important factors affecting the availability of B in soils. When the soil solution has a high pH, the B it contains becomes less available to plants. Therefore, applying lime to acid soils can sometimes result in B deficiency symptoms in plants. The level of soluble B in soils has a close correlation with the pH of the soil solution [24].

Among all major microelements, B is the one that is most often found deficient in soil, which in turn leads to a deficit of this element in cereal crops [25]. Boron adsorption was greater on soils having higher calcium carbonate content [24]. The critical level of available soil B is likely to be highly variable. It is influenced by soil characteristics such as pH, moisture and texture, above ground conditions of light, humidity and temperature and is also highly dependent of genotypes [26]. A deficiency of B, shown by a positive response to B application, has been reported in more than 80 countries and for 132 crops over the last 60 years [26].

The strongest evidence that organic matter affects the availability of soil B is derived from studies that show a positive correlation between levels of soil organic matter and the amount of hot water- soluble B [27]. The association between B and soil organic matter is said to be caused by the assimilation of B by soil microbes [28]. Although the B present in soil organic matter is not immediately available to plants, it seems to be a major source of available B when it is released through mineralization [28].

There are serious problems with the use of foliar analysis for diagnosing B toxicity. In species that accumulate B in their leaves, these tissues normally contain about 40 mg to 100 mg of B kg<sup>-1</sup> dry wt. However, the leaves can contain 250 mg kg<sup>-1</sup> dry wt when B in the soil approaches toxic levels. What is of particular concern is that there is an unacceptably wide range of critical values for B toxicity, sometimes even for the same species. In wheat and barley, for example, critical values range from 10 to 130 mg B kg<sup>-1</sup> dry weight [29, 30]. Kelling [31] reported that, because B levels in the plant change with age, it is important to indicate the stage of development at sampling. The critical B concentration for early vegetative growth in wheat is reported to be about 1 mg B kg<sup>-1</sup> DW [32]. In contrast grain set failure in wheat has been found associated with less than 2-4 mg B kg<sup>-1</sup> DW in the ear [33] and 3-7 mg B kg<sup>-1</sup> in the flag leaf at boot stage [34]. Wheat plants with >4 mg B kg<sup>-1</sup> in the ear [33] or >7mg B kg<sup>-1</sup> in the flag leaf at boot stage are unlikely to be affected by B deficiency [34].

The objective of the study was to evaluate the concentration and interactions of Zn and B in corn leaves as affected by Zinc sulfate and Boric acid application.

## MATERIALS AND METHODS

These experiments were carried out in a calcareous soil during two consecutive years 2009 and 2010 at agricultural farm of Aref in Abadeh Tashk, Fars province of Iran on the corn (*Zea mays* L.), cultivar "Single Cross 401". The experimental site is located at latitude 29° 43' 44" N and longitude 53° 52' 07" E and 1580 m altitude. Site of study has cold winter and warm summer (Semiarid) [9]. Composite surface soil samples were collected from surface horizon (0- 30 cm) of the soil before the experiment was initiated, air-dried, crushed to pass through a 2 mm mesh sieve and analyzed for the following properties.

Table 1: Soil physical and chemical analysis

Properties	Values
Depth of soil (cm)	0 -30
Soil texture	Loam
pH	8.2
EC (ds m <sup>-1</sup> )	2.41
Organic matter (%)	0.59
Nutrients (mg kg <sup>-1</sup> )	
P	12.1
K	229
Fe	1.65
Mn	8.14
Zn	0.32
Cu	0.62
B	0.78

Selected soil chemical and physical characteristics for the soil are presented in Table 1. Soil texture was determined by hydrometer method [35]. Soil pH and ECe were measured at a 1:2.5 soil/water ratio and saturated extract, respectively, organic matter (OM) content was determined by Walkley and Black procedure as recommended by Jackson, (1958). Soil available K was determined by 1 M NH<sub>4</sub>OAc extraction and K assessment in the extract by flame photometer [36]. Soil available P 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 [37].

Treatments consisted of five concentration levels of Zn (0, 8, 16 and 24 kg Zn ha<sup>-1</sup> added to the soil and Zn foliar spray with a 0.5 percent concentration as zinc sulfate) and four levels of B (0, 3 and 6 kg B ha<sup>-1</sup> added to the soil and B foliar spray with a 0.3 percent concentration as boric acid). The statistical design was completely randomized factorial with three replicates. Nitrogen, P, K used at 180, 70 and 75 kg ha<sup>-1</sup> according to the recommendation, from sources of urea (with 46% N), triple super phosphate (with 46% P<sub>2</sub>O<sub>5</sub>) and potassium sulfate (with 50% K<sub>2</sub>O), respectively, were added to all treatments (plots). Half of the urea was used when planting and the remainder at two different 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. 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 [38]. The Zn was analyzed by atomic-absorption spectrophotometry [39]. To measure B concentration in leaf tissues, the azomethine-Hp method was followed [40]. The concentrations of Zn and B were expressed as mg kg<sup>-1</sup> 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 [41]. Treatments (fraction) means were separated by Duncan's multiple range test.

## RESULTS AND DISCUSSION

### Physical and Chemical Characteristics of Soils:

Agrochemical soil characteristics of the experimental field were determined at the beginning of the growing season. Soil chemical and physical properties are presented in Table 1. Soil tests are an important tool to collect information on the Zn status of soils. The soil of the field was loam, low in organic matter (0.59%) and alkaline in reaction (pH 8.2), high in available K (229 mg kg<sup>-1</sup>), low in available P (12.1 mg kg<sup>-1</sup>). These result supported by other researchers [42], so that Karimian and Ghanbari [42] have reported the critical P level by the Olsen method in calcareous soils as 18 mg kg<sup>-1</sup>. When soil test levels are below the critical value crop yield and/or quality may be restricted by nutrient availability in the soil. Potassium critical level depends on crop, soil extracting solution and cropping system [9].

DTPA extractable Fe, Zn, Cu and Mn concentration were 1.65, 0.32, 0.62 and 8.14 mg kg<sup>-1</sup> and available B with hot water extractable was 0.78 mg kg<sup>-1</sup>. The soil Zn and B content were lower than the critical level. Measurement of DTPA-extractable Zn is the most widely used soil test method to determine Zn status of the soils. Generally, soils containing less than 0.5 mg kg<sup>-1</sup> DTPA-extractable Zn are classified as potentially Zn deficient. Increases in wheat grain yield (more than 20%) as a result of Zn fertilization have been obtained on soils containing less than 0.25 mg kg<sup>-1</sup> DTPA-extractable Zn [43]. In low pH soils, Zn concentrations may even be approaching levels that are toxic to soil microorganisms. Most plants require 0.5 to 1 ppm as the critical soil Zn level when using

DTPA soil extracts [44]. The critical soil levels for occurrence of Zn deficiency are between 0.6 and 2.0 mg Zn kg<sup>-1</sup> depending on the method of extraction used [45]. Calcareous soils (pH > 7) with moderate to high organic matter content (>1.5% organic C) are likely to be Zn deficient due to high HCO<sub>3</sub><sup>-</sup> in the soil solution [45]. For many crops, a DTPA-extractable Zn level of 0.5-0.8 mg kg<sup>-1</sup> has been regarded as a soil critical level below which crop production would be limited by Zn deficiency [46].

Kao and Juang [47] listed the content of hot-water-soluble B in representative soils from sugarcane growing areas. They showed that calcareous sand and shale alluvial soils have a B content, ranging from 0.63 to 1.12 mg kg<sup>-1</sup> (an average of 0.9 mg kg<sup>-1</sup>). The B content of mixed alluvial soils was 0.5 mg kg<sup>-1</sup> on average. Based on the growth response of young sugarcane to applied B, Kao and Juang [47] concluded that the critical level of B in the soil was 0.44 mg kg<sup>-1</sup>.

The soil Fe and Cu were low but available Mn was above the critical level according to the results of researchers. So that, Johnson and Fixen [48] and Soltanpour [49] demonstrated that the critical levels of Fe, Zn, Cu and Mn by the DTPA extraction method and B by the hot water in the soil method to be 5.0, 1.5, 0.5, 1.0 and 1.0, respectively. Results of different researches in Iran show that critical levels of Fe, Zn, Mn and Cu with DTPA-extractable and B with hot water are 4-4.5, 0.75-2, 3.6-4.6, 0.87-1.1 and 0.65 mg kg<sup>-1</sup> soil, respectively [50, 51].

**Zinc Concentration in the Leaf:** Zinc application to the soil had no significant effect on leaf Zn content relative to the no Zn level but Zn spraying increased Zn concentration in the leaf from 32.8 to 45.2 mg kg<sup>-1</sup>, showing a 38% increase relative to the no Zn level (Table 2). Because the soil was calcareous with soil pH of 8.2. Soil reaction is one of the most important factors affecting the availability of Zn in soils [9]. When the soil solution has a high pH, the Zn it contains becomes less available to plants [9]. Plant grown on calcareous soils, frequently suffers from Zn deficiency. Higher calcium carbonate content of soil and its subsequent effect on the soil pH greatly affect Zn availability. Soil carbonate, on the other hand, appeared to be strongly associated with poor ability of the trees to take up Zn [52]. Prior research on several crop plants has shown that the bicarbonate concentration of the soil solution is strongly correlated with the occurrence of both Zn and Fe deficiency [52]. Dissolved bicarbonate has two effects on plant uptake of trace metals. The first effect involves pH buffering at

Table 2: The effect of Zn and B on leaf Zn concentration (mg kg<sup>-1</sup>)\*

B (kg ha <sup>-1</sup> )	Zn (kg ha <sup>-1</sup> )					Mean
	0	8	16	24	Foliar Spray	
0	31.0c	38.0bc	33.8c	36.0bc	48.0a	42.4a
3	35.7bc	34.8c	36.3bc	44.3ab	44.7ab	39.2a
6	31.7c	33.8c	34.5c	30.5c	36.7abc	34.0b
Foliar Spray	32.8c	32.8c	35.2c	36.8bc	48.3a	37.2ab
Mean	32.8b	34.9b	35.0b	37.0b	45.2a	

\*Means with same letters lack a significant difference at 5% level by Duncan's test

the root surface, which limits the effectiveness of the normal root response to trace metal deficiency. As a specific response to Fe deficiency, most plants release hydrogen ions (acid) into the soil around the roots to help dissolve trace metals [52]. The second effect of bicarbonate is related to impaired translocation of trace metals in the xylem. Normally Zn and Fe are complexed with citrate as soon as they are taken up by the roots. This complex holds the metal ions in a soluble form so that they can cross cell walls and move from the roots to the leaves. When bicarbonate is taken up by the roots, the pH of the xylem is increased, which causes citrate to preferentially form complexes with calcium rather than Fe or Zn. Visual evidence for this poor translocation can be seen in the leaves of trace metal deficient plants, which typically have green veins, but yellow tissue between the veins (Fe), or mottle leaf (Zn). In this case, the metals are not translocated out of the xylem vessels in the leaf veins into the leaf parenchyma tissue [52].

It was recently documented that Zn foliar application is a simple way for making quick correction of plant nutritional status, as reported for wheat [53] and maize [54]. In calcareous soils, micronutrients such as Zn and B rapidly convert to immobile forms and generally several foliar applications are required to prevent deficiencies throughout the growing season. Until recently Zn fertilizer recommendations have been based on foliar diagnosis but, because this system of measurement can only be carried out once the crop is well grown, the information is often obtained too late to provide advice for fertilizing the current crop [9]. Wilhelm *et al.* [55] and Savithri *et al.* [56] stated that there are three main methods of applying micronutrients to crops: soil fertilization, foliar sprays and seed treatment. Foliar applications of micronutrient sprays are effective towards both goals, but this method is too costly to be widely practiced by resource-poor farmers in some regions because of the amount of fertilizer, equipment and labor required for repeated spraying. Potarzycki and Grzebisz [57] reported that

the maize crop responded significantly to Zn foliar application and the optimal rate of Zn foliar spray for achieving significant grain yield response was in the range from 1.0 to 1.5 kg Zn ha<sup>-1</sup>. Results showed that foliar application of Zn and Fe (alone or together) has significant effect (at 0.01 probability) on wheat yield, grain-Zn and Fe concentration [58].

The lowest and the highest mean Zn concentration in the leaf, 32.8 and 45.2 mg kg<sup>-1</sup>, were seen at no Zn and Zn spraying levels, respectively. Leaf analysis of corn plants can be very useful when evaluating the adequacy of nutrients required for corn production. This process is helpful when determining the sufficiency of the fertilizer program used by the producer. In this research Zn concentration in the leaf was intermediate relative to the results of other researchers [59]. According to the other researches concentration of Zn in dry matter of corn (mg kg<sup>-1</sup>) in lower leaves at tasseling stage was: 9-9.3 showing deficiency symptoms and 31.1-36.6 intermediate [59]; in leaves at 6th node from base at silking stage was 15-24 showing deficiency symptoms, 25-100 intermediate and 101-150 high [60]; and in ear leaf at silking stage was: < 10 showing deficiency symptoms, 20-70 Intermediate, 71-100 high and > 100 showing toxicity symptoms. Depending on the Zn level, Zn deficiency status of plants can be classified as follows: less than 10 mg kg<sup>-1</sup> definite Zn deficiency; between 10 and 15 mg kg<sup>-1</sup> likely to be Zn deficient; between 15 and 20 mg kg<sup>-1</sup> likely to be Zn-deficient; more than 20 mg kg<sup>-1</sup> Zn-sufficient. The ratios of P: Zn and Fe: Zn in the shoot at tillering to pod initiation stage are good indicators of Zn deficiency, while leaf Zn concentration is a less reliable indicator of Zn deficiency, except in extreme cases. Leaf Zn concentration below 15 mg kg<sup>-1</sup> is regarded as Zn-deficient. Critical concentrations of Zn in different plant tissues of cereals [45]. In wheat, despite presence of some genotypic variation, the critical Zn concentrations of leaves or whole shoot at the vegetative growth stage are generally around 15-17 mg kg<sup>-1</sup>. In Zn deficient locations, wheat grain Zn concentrations are found to be below 15-20 mg kg<sup>-1</sup>. Zinc deficiency symptoms on plants appear earlier and more severe under water deficient soil conditions [61].

Application of B to the soil and spraying had no significant effect on the leaf Zn content relative to the zero B level; but application of a high amount of B (6 kg ha<sup>-1</sup>) decreased leaf Zn content relative to the 3 kg ha<sup>-1</sup> B level. Probably due to a Zn and B antagonism, application of high B decreased leaf Zn content. These results also confirm the findings of Vitti [62] who stated that the presence of B reduces the absorption of Zn.

Table 3: The effect of Zn and B on leaf B concentration (mg kg<sup>-1</sup>)\*

B (kg ha <sup>-1</sup> )	Zn (kg ha <sup>-1</sup> )					Mean
	0	8	16	24	Foliar Spray	
0	37.0bcde	32.0e	32.6de	30.6e	37.7bcde	34b
3	46ab	43.1abcd	35.5bcde	36.5bcde	32.5e	38.7a
6	52.2a	31.3e	31.5e	44.1abc	37.7bcde	39.3a
Foliar Spray	34.1cde	44.7ab	35.8bcde	35.8bcde	45.0ab	39.1a
Mean	42.3a	37.8ab	33.9b	36.8b	38.2ab	

\*Means with same letters lack a significant difference at 5% level by Duncan's test

Rezaei and Malakouti [8] reported that B toxicity decreased with application of ZnSO<sub>4</sub>.

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, but Zn use at all levels of B, showed significant effect on the leaf Zn content. Zinc use in spray form in cases where B was not applied directly to the soil (zero and B spraying levels) increased leaf Zn content but in cases where it was applied directly to the soil (3 and 6 kg ha<sup>-1</sup> B), it had no significant effect on leaf Zn content. Zinc spraying at no B level increased leaf Zn content from 31 to 48 mg kg<sup>-1</sup> (55 percent increase relative to the no Zn use at this B level. Also, Zn spraying use at B spraying level increased leaf Zn content from 33 to 48.3 mg kg<sup>-1</sup> (47% increase relative to the no Zn use at this B level), but Zn application to the soil at this B level had no significant effect on the leaf Zn content. Therefore, no B content in the soil (zero and B spraying level) helped increasing leaf Zn content, but presence of B prevented from increase of leaf Zn content. In fact, there was an antagonism between the Zn and B in the soils.

The highest leaf Zn content, 48.3 mg kg<sup>-1</sup>, was obtained by joint use Zn and B spraying, showing a 56 percent increase as compared with the control with a leaf Zn content of 31 mg kg<sup>-1</sup>. The lowest leaf Zn content, 30.5 mg kg<sup>-1</sup>, was seen by joint use of 6 kg ha<sup>-1</sup> B and 24 kg ha<sup>-1</sup> Zn, but had no significant difference from the control.

**Boron Concentration in the Leaf:** The main effect of Zn on the concentration of B in the leaf was significant at 1% level (Table 3). The highest mean B concentration in the leaf, 42.3 mg kg<sup>-1</sup>, was seen at no Zn level. The use of 16 and 24 kg ha<sup>-1</sup> Zn, decreased leaf B content from 42.3 at no Zn level to 33.9 and 36.8 mg kg<sup>-1</sup>, respectively (20 and 13 percent decreased relative to no Zn level).

Therefore, there was an antagonism between the Zn and B. Graham *et al.* [63] reported that Zn deficiency enhanced accumulation of B in barley (*Hordeum vulgare* L.) up to a toxic level. In nutrient culture, Graham *et al.* [63] 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 [64]. Eraslan *et al.* [65] concluded that B uptake of plants increases in Zn deficient soils, therefore B toxicity develops and plant growth is adversely affected by this soil situation. Rajaie *et al.* [66] reported 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. Studies have revealed that, B toxicity and Zn deficiency are interestingly related with each other. Swietlik and Laduke [67] observed that increasing Zn concentration in grapefruit (*Citrus paradisi*) leaf decreased B toxicity.

The least mean B content, 33.9 mg kg<sup>-1</sup>, was seen at 24 kg ha<sup>-1</sup> Zn level. Zinc spraying had no significant effect on the leaf B content relative to the no Zn level. Also, there was no significant difference between the Zn spraying and applying Zn to the soil. Therefore, unlike leaf Zn content, Zn spraying was not more effective than Zn soil application on the leaf B content.

The main effect of B on the leaf B content was significant at 5% level. The lowest mean B concentration in the leaf, 34 mg kg<sup>-1</sup>, was seen at zero B level. Boron application to the soil and spraying increased leaf B content relative to the no B level. Application of 3 and 6 kg ha<sup>-1</sup> B significantly increased leaf B content from 34 mg kg<sup>-1</sup> at no B level to 38.7 and 39.3 mg kg<sup>-1</sup>, respectively (14 and 16 percent increase relative to the no B level). Also, B spraying increased B concentration in the leaf to 39.1 mg kg<sup>-1</sup>, but there was no significant difference between the B spraying and B application to the soil. The highest mean B concentration in the leaf, 39.3 mg kg<sup>-1</sup>, was seen at high B level (6 kg ha<sup>-1</sup>). Therefore, B concentration in the leaf increased with increasing level of B. The same results were obtained for various plants [68, 69, 70].

The effect of Zn-B interaction on leaf B concentration was significant at 1% level (Table 3). Boron use at no Zn level showed greatest impact. So that, the use of 6 kg ha<sup>-1</sup> B at zero Zn level increased leaf B content from 37 to 52.5 mg kg<sup>-1</sup> (41 percent increase), but other B amounts had no significant effect on the leaf B content at this Zn

level. In fact, B use at no presence Zn increased leaf B content. Graham *et al.* [63] found that B uptake by barley was lower if Zn was applied than if it was not. Further studies showed that low levels of Zn and high levels of phosphorus both increased the rate of B accumulation. Therefore, applying Zn may reduce B accumulation and lessen the risk of toxicity in plants.

With increasing of Zn amount, the higher rate of B was needed for increasing B concentration in the leaf. So that, at 8 kg ha<sup>-1</sup> Zn level, the use of 3 kg ha<sup>-1</sup> B increased leaf B content from 32 to 43 mg kg<sup>-1</sup>; but at 24 kg ha<sup>-1</sup> Zn level, only application of 6 kg ha<sup>-1</sup> B increased leaf B content from 30.6 to 44.1 mg kg<sup>-1</sup>. Therefore, large amounts of Zn, prevented from increase of leaf B content by B application; due to a Zn and B antagonism, Zn application prevented from B use affecting B concentration in the leaf.

The use of Zn at no B level, had no significant effect on the leaf B content, but with increasing B level (3 and 6 kg ha<sup>-1</sup>), Zn use decreased leaf B content. At 3 kg ha<sup>-1</sup> B level, Zn application to the soil had no significant effect on the leaf B content but B spraying decreased leaf B content from 46 to 32.5 mg kg<sup>-1</sup>. At 6 kg ha<sup>-1</sup> B level, the use of 8 and 16 kg ha<sup>-1</sup> Zn decreased leaf B content from 52.2 to 31.3 and 31.5 mg kg<sup>-1</sup>, respectively. Also, Zn spraying at 6 kg ha<sup>-1</sup> B level, decreased leaf B content from 52.2 to 37.7 mg kg<sup>-1</sup>, but there was no significant difference between Zn spraying and applying Zn to the soil. At B spraying level, Application of 8 kg ha<sup>-1</sup> and foliar spray of Zn increased leaf B concentration from 34.1 to 44.7 and 45 mg kg<sup>-1</sup>, respectively. Therefore, the presence of B in the soil, assisted to decreasing of B concentration in the leaf by Zn application; but B spraying helped increasing leaf B content by Zn use. Ho [21] reported that a foliar spray of 0.3% boric acid solution was also effective. However, a considerable amount of B accumulated in the soil when spraying took place annually. For this reason, it is recommended that a foliar spray should not be applied every year.

The highest B concentration in the leaf, 52.5 mg kg<sup>-1</sup>, was obtained by joint use of 6 kg ha<sup>-1</sup> B, showing a 41 percent increase as compared with the control with a leaf B concentration of 37 mg kg<sup>-1</sup>. No treatments, except the treatment with the highest leaf B concentration (application of 6 kg ha<sup>-1</sup> B) had no significant difference on the B concentration in the leaf from the control. According to the other researchers, in this research B concentration in the leaf was sufficient to high [29, 30]. Relative levels of B in mg kg<sup>-1</sup> 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 [37].

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## REFERENCES

- Alloway, B., 2004. Zinc in soils and crop nutrition. Areas of the World with Zinc Deficiency Problems. Available at: <http://www.zinc-crops.org/Crops/Alloway-all.php>.
- Hotz, C. and K.H. Brown, 2004. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr. Bull. 25: 94-204. J.N.A. Lott and E. Spitzer, 1980. X-ray analysis studies of elements stored in protein body globoid crystals of Triticum grains. Plant Physiol., 66: 494-499.
- Sillanpaa, M., 1990. Micronutrients assessment at the country level: An international study. FAO Soils Bulletin 63. Food and Agriculture Organization of the United Nations, Rome.
- Cakmak, I., 2002. Plant nutrition research: Priorities to meet human needs for food in sustainable ways. Plant Soil, 247: 3-24.
- Graham, R.D. and R.M. Welch, 1996. Breeding for staple-food crops with high micronutrient density: Working Papers on Agricultural Strategies for Micronutrients, No. 3. International Food Policy Institute, Washington DC.
- Cakmak, I., A. Yilmaz, M. Kalayci, H. Ekiz, B. Torun, B. Erenoglu and H.J. Braun, 1996. Zinc deficiency as a critical problem in wheat production in Central Anatolia. Plant and Soil, 180: 165-172.
- Graham, R.D., J.S. Ascher and S.C. Hynes, 1992. Selecting zinc efficient cereal genotypes for soils of low zinc status. Plant Soil, 146: 241-250.
- Rezaei, H. and M.J. Malakouti, 2001. Critical levels of Iron, zinc and boron for cotton in Varamin region. J. Agric. Sci. and Technol., 3(2): 147-153.
- Aref, F., 2010. Residual Available Copper and Boron in Soil as Affected by Zinc sulfate and Boric acid in a Zinc and Boron Deficient Soil. Journal of American Sci., 6(11): 977-984.
- Cakmak, I., R. Derici, B. Torun, I. Tolay, H.J. Braun and R. Schlegel, 1997. Role of rye chromosomes in improvement of zinc efficiency in wheat and triticale, Plant Soil, 196: 249-253.
- Khoshgoftarmanesh A.H., H.R. Sharifi, H.M. Mirzapour and R. Schulin, 2007. Plant genotype and Zn fertilization effects on nutritional quality of wheat grain produced in saline soils. 9<sup>th</sup> International Conference of the Biochemistry of Trace Elements (ICOBTE), July 2007, Beijing, China.
- Cakmak, I., B. Torun, B. Erenoglu, L. Oztuerk, H. Marschner, M. Kalayci, H. Ekiz and A. Yilmaz, 1998. Morphological and physiological differences in cereals in response to zinc deficiency. Euphytica, 100: 349-357.
- Kalayci, M., B. Torun, S. Eker, M. Aydin, L. Ozturk and I. Cakmak, 1999. Grain yield, zinc efficiency and zinc concentration of wheat cultivars grown in a zinc-deficient calcareous soil in field and greenhouse. Field Crops Res., 63: 87-98.
- Alloway, B.J., 2004. Zinc in soils and crop nutrition. IZA Publications. International Zinc Association, Brussels, pp: 1-116.
- Cakmak, I., 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant and Soil, 302: 1-17.
- Benton, J.J., 2003. Agronomic Handbook. CRC Press, Boca Raton, pp: 17-227.
- Tollenaar, M. and E. Lee, 2002. Yield potential, yield stability and stress tolerance in maize. Field Crops Res., 75: 161-169.
- Marschner, H., 1995. Mineral Nutrition of Higher Plants, 2<sup>nd</sup> ed., Academic Press, London.
- Chaney, R.L., 1983. Plant uptake of inorganic waste constituents. In: J.F. Parr, P.B. Marsh and J.M. Kla, eds. Land treatment of hazardous wastes. Park Ridge, NJ, USA: Noyes Data Corporation, pp: 50-76.
- Davies, B.E., 1993. Radish as an indicator plant for derelict land - uptake of zinc at toxic concentrations. Communications in Soil Science and Plant Analysis, 24: 1883-1895.
- Ho, S.B., 1999. Boron deficiency of crops in Taiwan. Food and Fertilizer Technology Center (FFTC). Taipei City, CN., pp: 16.
- Russell, E.W., 1973. Soil Condition and Plant Growth. 10<sup>th</sup> ed. Longman Ltd., London, U. K., pp: 849.
- Liu, Z., Q.Q. Zhu and L.H. Tang, 1983. Microelements in the main soils of China. Soil Sci., 135: 40-46.
- Elrashidi, M.A. and G.A. Oconnor, 1982. Boron sorption and desorption in soils. Soil Sci. Soc. Am. J., 46: 27-31.
- Goldbach, H.E., 1997. A critical review on current hypotheses concerning the role of boron in higher plants: suggestion for further research and methodological requirements. J. Trace Microprobe Tech., 15(1): 51-91.

26. Shorrocks, V.M., 1997. The occurrence and correction of boron deficiency. *Plant and Soil*, 193: 121-148.
27. Chang, S.S., 1993. Nutritional physiology of boron and the diagnosis and correction of boron deficiency and toxicity in crops. In: "Proceedings of the Symposium on Reclamation of the Problem Soils in the Eastern Taiwan", S.N. Hwang and G.C. Chiang, (Eds.). Chinese Soc. Soil Fert. Sci. and Hwalian District Agricultural Improvement Station, Taiwan, pp: 109-122.
28. Gupta, V.K. and B. Singh, 1985. Residual effect of zinc and magnesium on maize crop. *J. Indian Soc. Soil Sci.*, 33: 204-207.
29. Paull, J.G., B. Cartwright and A.J. Rathjen, 1988. Responses of wheat and barley genotypes to toxic concentrations of soil boron. *Euphytica*, 39: 137-144.
30. Riley, M.M., A.D. Robson, G.A. Dellar and G.W. Gartrell, 1994. Critical toxic concentrations of boron are variable in barley. *J. Plant Nutr.*, 17: 1701-1719.
31. Kelling, K.A., 2010. Soil and Applied Boron. From: <http://corn.agronomy.wisc.edu/Management/pdfs/a2522.pdf>.
32. Asad, L.M.B.O., D.C. Medeiros, I. Felzenswalb, A.C. Leitao and N.R. Asad, 2001. Effects of low iron conditions on repair of DNA lesions induced by Cumene peroxide in *Escherichia coli*. *Mutat. Res.*, 485: 339-344.
33. Rerkasem, B. and S. Lordkaew, 1992. Predicting grain set failure with tissue boron analysis. In *Boron Deficiency in Wheat*. Eds. C.E. Mann and B. Rerkasem, pp: 9-14. CIMMYT Wheat Special Report No. 11. CIMMYT, Mexico.
34. Rerkasem, B. and J.F. Loneragan, 1994. Boron deficiency in two wheat genotypes in a warm, subtropical region. *Agron. J.*, 86: 887-890.
35. Koehler, F.E., C.D. Moodie and B.L. McNeal, 1984. Laboratory Manual for soil fertility, Washington State Uni. Pullman Washington.
36. Thomas, G.W., 1982. Exchangeable cations. pp: 159-164. In A.L. Page, R.H. Miller and D.R. Keeney, eds., *Methods of Soil Analysis, Part 2*. 2<sup>nd</sup> Ed. American Society of Agronomy, Madison, WI.
37. Bingham, F.T., 1982. Boron. In: A.L. Page, (Ed.), *Methods of Soil Analysis. Part 2*, Am. Soc. Agron. Madison, WI., pp: 431-448.
38. Wolf, B.A., 1982. Comprehensive System of Leaf Analysis and its Use for Diagnosing Crop Nutrients Status. *Commun. Soil Sci. Plant Anal.*, 13: 1035-1059.
39. Hocking, P.J. and J.S. Pate, 1977. Mobilization of Minerals to Developing Seeds of Legumes. *Ann. Bot.*, 41: 1259-1278.
40. Wolf, B., 1974. Improvement in the Azomethine-H Method for Determination of Boron. *Commun. Soil Sci. Plant Anal.*, 5: 39-44.
41. SAS Institute, 2001. SAS user's guide of release version 8. 2. SAS Inst., Cary, NC.
42. Karimian, N. and A. Ghanbari, 1990. Evaluation of different extractants for prediction of plant response to applied P fertilizer in highly calcareous soils. 10<sup>th</sup> World Fertilizer Congress. Abstract, pp: 25.
43. Mortvedt, J.J., F.R. Cox, L.M. Shuman and R.M. Welch, 1991. *Micronutrients in Agriculture*, SSSA Books.
44. Soil and Plant Analysis Council, 1992. *Handbook of reference methods for soil analysis*, Athen, GA, USA.
45. Singh, B., S.K.A. Natesan, B.K. Singh and K. Usha, 2005. Improving zinc efficiency of cereals under zinc deficiency. *Current Sci.*, 88(1): 10.
46. Martins, D.C. and W.L. Lindsay, 1990. Testing soils for copper, iron, manganese and zinc. pp: 229-264. In: *Soil Testing and Plant Analysis*, 3<sup>rd</sup> ed., R.L. Westerman, ed. Soil Sci. Soc. Amer., Madison, WI.
47. Kao, M.M. and T.C. Juang, 1978. Comparison of Boron Test Methods for Sugarcane Soils in Taiwan. Rept. Taiwan Sugar Exp. Station, pp: 15-27.
48. Jonson, G.V. and P.E. Fixen, 1990. Testing soils for Sulfur, Boron, Molybdenum and Chlorine. In: R.L. Westermann, ed. *Soil testing and plant analysis*. 3<sup>rd</sup> Ed. Madison, WI (USA). Soil Science Society of America, pp: 265-273.
49. Soltanpour, P.N., 1985. Use of AB-DTPA soil test to evaluate elemental availability and toxicity. *Communication in Soil Science and Plant Analysis*, 16(3): 323-338.
50. Balali, M.R., M.J. Malakouti, H. Mashayekhi and Z. Khademi, 2000. Micronutrients Effects on Yield Increasing and their Critical Level Determination in Irrigated Wheat Soils. In: *Balance Nutrition of Wheat (A complication of Papers)*, M.J. Malakouti, (Ed.). Agricultural Education Publication, Washington, DC., pp: 121-134.
51. Feiziasl, V., G.H.R. Valizadeh, V. Toshieh, A.A. Taliee and V. Belson, 2003. Determination of micronutrients critical level in Northwestern of Iran for Dryland wheat. *Iran. J. Crop Sci.*, 5: 236-249.
52. Crowley, D.E. and W. Smith, 1996. Soil factors associated with zinc deficiency in Avacado. *Avocado Research Symposium*, pp: 17-21.



53. Erenoglu, B., M. Nikolic, V. Romhold and I. Cakmak, 2002. Uptake and transport of foliar applied zinc (65Zn) in bread and durum wheat cultivars differing in zinc efficiency. *Plant and Soil*, 241: 251-257.
54. Grzebisz, W., M. Wronska, J.B. Diatta and P. Dullin, 2008. Effect of zinc foliar application at early stages of maize growth on patterns of nutrients and dry matter accumulation by the canopy. Part I. Zinc uptake patterns and its redistribution among maize organs. *J. Elementol.*, 13: 17-28.
55. Wilhelm, N.S., R.D. Graham and A.D. Rovira, 1988. Application of different sources of manganese sulphate decreases take-all (*Gaeumannomyces graminis* var. *tritici*) of wheat grown in a manganese deficient soil. *Australian J. Agric. Res.*, 39: 1-10.
56. Savithri, P., R. Perumal and R. Nagarajan, 1999. Soil and crop management technologies for enhancing rice production under micronutrient constraints. *Nutrient Cycling in Agroecosystems*, 53: 83-92.
57. Potarzycki, J. and W. Grzebisz, 2009. Effect of zinc foliar application on grain yield of maize and its yielding components. *Plant Soil Environ.*, 12: 519-527.
58. Maralian, H., 2009. Effect of foliar application of Zn and Fe on wheat yield and quality. *African J. Biotechnol.*, 8(24): 6795-6798.
59. Barker, A.V. and D.J. Pilbeam, 2007. *Handbook of Plant Nutrition*. CRC Press, Taylor and Francis, USA.
60. Deleers, M., J.P. Servais and E. Wulfert, 1985. Micromolar concentrations of Al<sup>3+</sup> induce phase separation, aggregation and dye release in phosphatidylserine-containing lipid vesicles. *Biochim. Biophys. Acta.*, 813: 195-200.
61. Cakmak, I. and H.J. Braun, 1999. Zinc deficiency and genotypic variation in wheat. In: *Applying Physiology to Wheat Breeding*.
62. Vitti, G.C., 1992. Nutricao e crescimento de plantas citricas. In: L.C. Donaldiao, coord. *Seminario Internacional De Citros*, 2., Bebedouro, 1992. Campinas: Fundaçaõ Cargill, Resumos. Campinas, pp: 132-62.
63. Graham, R.D., R.M. Welch, D.L. Grunes, E.E. Cary and W.A. Norvell, 1987. Effect of zinc deficiency on the accumulation of boron and other mineral nutrients in barely. *Soil Sci. Soc. Am. J.*, 51: 652-657.
64. Singh, J.P., D.J. Dahiya and R.P. Norwal, 1990. Boron uptake and toxicity in wheat in relation to zinc supply. *Fert. Res.*, 24: 105-110.
65. Eraslan, F., N. Icek and E. Guneri, 2004. The effect of boron toxicity and gypsum application on growing ant nutrient element contents of pepper. *Turkey 3<sup>th</sup> National Fertilizer Congress, Agriculture- Industry- Environment*, 343- 350, 11- 13 October, Tokat, Turkey (in Turkish).
66. Rajaie, M., A.K. Ejraie, H.R. Owliaie and A.R. Tavakoli, 2009. Effect of zinc and boron interaction on growth and mineral composition of lemon seedlings in a calcareous soil. *International J. Plant Production*, 3(1): 39-49.
67. Swietlik, D. and J.V. Laduke, 1991. Productivity, growth and leaf mineral composition of orange and grapefruit trees foliar sprayed with zinc and manganese. *J. Plant Nutr.*, 14(2): 129-142.
68. Papadakis, I.E., K.N. Dimassi and I.N. Therios, 2003. Response of two citrus genotypes to six boron concentrations: concentration and distribution of nutrients, total absorption and nutrient use efficiency. *Aust. J. Agric. Res.*, 54: 571-580.
69. Shelp, B.J., 1988. Boron mobility and nutrition in broccoli (*Brassica oleracea* var. *italica*). *Ann. Bot.*, 61: 83-91.
70. Sotiropoulos, T.E., I.N. Therios and K.N. Dimassi, 1999. Calcium application as a means to improve tolerance of kiwi fruit (*Actindia deliciosa* L.) to boron toxicity. *Scientia Horticulturae*, 81: 443-449.