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Full Length Research Paper

Manganese, iron and copper contents in leaves of maize plants (*Zea mays* L.) grown with different boron and zinc micronutrients

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Micronutrients such as boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) play important physiological roles in humans and animals. Zn and B are the micronutrients most often deficient in maize, in Iran. A completely randomized factorial block design experiment was carried out at Fars province of Iran during the growing season in 2009 to evaluate the effects 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 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) fertilizers on Fe, Mn and Cu concentrations in the maize leaf. The results indicate that the use of B and Zn, by spraying, increased leaf Fe content. The presence of a high amount of B in the soil, and also Zn foliar spray, assisted the increase of Fe concentration in the leaf. In fact, there were synergisms between Zn and Fe as well as B and Fe. Reduction in the leaf concentration of Mn by B application may be due to the dilution effect or the antagonistic relationship between B and Mn. The presence of a high amount of B in the soil and the spraying of B prevented the increase of the leaf Mn content, by Zn application. An antagonism was seen between Zn and Mn, in that a high amount of Zn in the soil resulted in the decrease of the leaf Mn content.

Key words: Concentration, interaction, antagonism, synergism, deficiency.

INTRODUCTION

Maize (*Zea mays* L.) is the most important crop by volume among all cereal grain crops, such as wheat and rice, which are widely planted in subtropical and temperate agro-climatic regions throughout the world (Fageria et al., 1991). Maize has been previously considered to have lower B requirement than other cereals (Marten and Westermann, 1991). However, B deficiency has been reported in maize across five continents (Bell and Dell, 2008; Shorrocks, 1997) since it was first observed in the 1960s in the United States (Shorrocks and Blaza, 1973). The yield increases were observed for more than 10% in response to B application (Woodruff et al., 1987). B occurs in many rocks and soils at total concentrations of 5 to 50 mg kg^{-1}, and is normally present in plant leaf tissue at concentrations of 10 to 50 mg kg^{-1}. However, many species, including important cereals such as wheat, are quite sensitive to elevated B in their tissues, and they show severe toxicity symptoms of about 50 mg kg^{-1} at tissue level. Such level can be found in tissues when the available B exceeds 3 mg kg^{-1} in soil (Babaoglu et al., 2004). Studies on calcareous soil in the last decade show that Zn deficiency is the most detrimental to crop yield. In the case of calcareous soils, the conventional notion that micronutrients increase crop yield by 15 to 30% is an underestimated range (Malakouti, 2007).

Micronutrients are significantly affected by soil pH, decreasing with increasing soil pH. Solubility of Fe decreases a thousand fold for each unit increase in soil pH in the range of 4 to 9 (Lindsay, 1979), and consequently, most Fe deficiencies occur on calcareous soils. The activity (consequent bioavailability) of Mn, Cu and Zn decreases a 100-fold for each unit increase in soil pH. Amounts of exchangeable metals in soil are related to their concentrations in soil solutions, so soil pH affects exchangeable Fe, Mn, Cu and Zn similarly. Zinc is an essential element for normal crop growth and Zn deficiencies can severely impair crops and reduce yields

(Fageria et al., 2002; Gangloff et al., 2002). One of the widest ranges of biotic stresses in world agriculture arises from low Zn availability in calcareous soils, particularly in cereals. Zinc sulphate has traditionally been the “reliable” source of Zn fertilizer, but other sources of Zn are also available (Gangloff, 2006). Although the environment, soil type and past erosion each have an effect, the most important factors controlling plant available Zn are the soil pH, extractable soil P and extractable soil Zn. While the amount of Zn in the plant increases as the available soil Zn increases, increasing levels of soil P and pH are strongly associated with reduced levels of Zn in the plant (Murdock and Howe, 2001). The potential for Zn deficiencies is greatest in soils with low organic matter contents and pH levels above 7.0. In these situations, Zn deficiencies are easily corrected by applying fertilizers that contain high quantities of water-soluble Zn, such as organic Zn complexes or Zn chelates (Cakmak, 2008; Alloway, 2004). The original research that determined the adequate soil test levels of Zn for maize was also located on these soils and was based on a 0.1 N HCl extractant (Murdock and Howe, 2001).

Manganese is an essential plant nutrient acting as the key part of prosthetic groups in important processes. These processes include catalysis of the splitting of water in photosystem II (enzyme-S) and the scavenging of reactive oxygen species in the mitochondria by a Mn-containing superoxide dismutase (Mn-SOD) (Scandalios, 1993). Moreover, Mn is an activator in several important enzymes including phenylalanine ammonia- lyase (PAL), enzymes of the tricarboxylic acid cycle and the chloroplast RNA polymerase (Marschner, 1995). Manganese deficiency in plants is a significant global problem under a wide range of climatic conditions and soil properties (Reuter et al., 1988). It is usually a secondary nutritional disorder where ample soil resources of Mn are unavailable to plants due to oxidation, which is favoured by high pH and high oxygen concentration in the soil solution (Norvell, 1988). In Iran, Mn deficiency is currently recognized as the most important nutritional problem in the production of cereals. Manganese deficiency in Iran has traditionally been confined to sandy soils with neutral to slightly alkaline pH, soils developed on old marine sediments rich in carbonate and to soils rich in clay and organic matter. However, in recent years, Mn deficiency has also been observed on highly fertile clayish soils (Aref, 2010). Total soil Mn, however, only indicates the potential toxicity, whereas actual Mn toxicity is associated with forms that are either water soluble or easily reducible. Adams (1981) suggested a reducible (presumably NH_2OH-HCl extractable) Mn range of 50 to 100 mg kg^{-1} in soil, above which Mn toxicity would occur. The amount of available Mn^{2+} in soil mainly depends on the oxido-reduction processes, as well as on all the factors affecting these processes: soil pH, organic matter content, microbiological activity and moisture (Marschner, 1995).

In soils with a low pH value (below 6.0), Mn deficiency is likely to occur only if the total Mn content is very low (Marschner, 1995). If the pH value is below 5.5, high concentrations of readily mobile Mn^{2+} ions in the soil solution may lead to signs of toxicity. Raising soil pH generally decreases the organically bound Mn and increases amorphous and crystalline Mn forms (Zhang et al., 1997).

Iron is an essential trace element for all organisms. Although Fe is the fourth most abundant element in the earth’s crust, it is the third-most limiting nutrient for plant growth primarily due to the low solubility of the oxidized ferric form in aerobic environments (Yi et al., 1994). The oxidized Fe (III) has a very low solubility at basic pH, and high bicarbonate concentrations resulting in limited uptake by plant roots because it cannot be absorbed by root cells (Lucena et al., 2007). Iron deficiency is usually associated with high soil alkalinity, but it is also associated with excessive irrigation, prolonged wet soil conditions or poor drainage, and low soil temperature (Zekri and Obreza, 2009). Cool, wet weather enhances Fe deficiencies, especially on soils with marginal levels of available Fe. Poorly aerated or compacted soils also reduce Fe uptake by plants (Mortvedt, 2010). Iron deficiency is one of the most difficult deficiencies to correct, especially on calcareous soils. Plants and humans cannot easily acquire Fe from their nutrient sources although it is abundant in nature. Thus, Fe deficiency is one of the major limiting factors affecting crop yields, food quality and human nutrition. Therefore, approaches need to be developed to increase Fe uptake by roots, transfer to edible plant portions and absorption by humans from plant food sources (Yuanmei and Zhang, 2010). Over one third of the world’s soils are considered Fe-deficient (Yi et al., 1994). Iron deficiencies are widespread in Iran because of the generally low micronutrient availability in soils and because of increasing nutrient demands from increasingly intensive cropping practices.

Copper is an essential plant micronutrient, required for the protein components of several enzymes (Marschner, 1995). As such, when present in excess quantities, Cu is also highly toxic to plant growth potentially, causing damage resulting in complete inhibition of growth (Kopittke and Menzies, 2005). However, in Iran where soil pH is typically alkaline in nature (pH ≥ 8.0), toxic levels should not occur. In soil, Cu is relatively immobile, since it binds strongly with organic matter and it seldom leaches, and its availability to plants strongly depends on the soil type, namely: organic matter content and pH (Kopsell and Kopsell, 2007; Burkhead et al., 2009). Uptake of Cu by plants is affected by many factors including the soil pH, the prevailing chemical species, and the concentration of Cu present in the soil. Once it is inside the plant, Cu is sparingly immobile. Accumulation and expression of toxic symptoms are often observed with root tissues (Barker and Pilbeam, 2007). The rate of

Cu uptake in plants is among the lowest of all the essential elements (Kabata-Pendias and Pendias, 1992). Uptake of Cu by plant roots is an active process, affected mainly by the Cu species. Plants differ in their susceptibility to Cu deficiency with wheat (*Triticum aestivum* L.); oats, sudangrass (*Sorghum sudanense* Stapf.) and alfalfa being highly sensitive; and barley, maize and sugar beet being moderately sensitive (Barker and Pilbeam, 2007). Thus, iron deficiency in nutrient solution culture increased Cu and N leaf contents uniformly along maize leaf blades (Mozafar, 1997).

Plant analysis is a chemical evaluation of nutritional status. Concentrations of essential elements found in the indicator tissue reflect the nutritional status of plants. Proper interpretation of plant analysis results is critical to the effective use of this management tool. Guidelines for interpretation of analytical results have been developed over the years based on research, surveys and experience (Campbell, 2009). Just like in soil sampling, it is important to collect a representative plant tissue sample. This involves taking samples from many plants (25 to 50, depending on the size of the plant part) throughout the entire area of interest. The best sampling time and plant part of maize is initial silk and ear leaf, respectively (Reuter and Robinson, 1997). The interpretation of plant analysis results is based on the scientific principle that healthy plants contain predictable concentrations of essential elements. A number of researchers have offered schematics showing the relationship between maximum yield and concentrations of essential elements (Dow and Roberts, 1982). Therefore, this study was inevitable in determining the relationship between Zn and B with Cu, Mn and Fe and tissue Fe, Cu and Mn concentration in maize leaves.

MATERIALS AND METHODS

The experiment was undertaken in a calcareous soil in the farm of Aref in Abadeh Tashk, Fars province of Iran, on maize (*Zea mays* L.), cultivar "Single Cross 401", during the 2009 growing season. A site that had a potential for soil Zn and B deficiency was chosen. The site is situated at 29° 43' 44'' N, 53° 52' 07'' E and 1580 m above mean sea level, and it has cold winter and warm summer (Semiarid) (Aref, 2011). A completely randomized factorial block designed experiment with three replications was carried out during the 2009 season to evaluate the effects of Zn and B fertilizers on Fe, Mn and Cu concentrations in the leaves of maize. Factor one included 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), while factor two consisted of 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).

Composite surface soil samples, collected from the surface horizon (0 to 30 cm) of the soil before the experiment were initially air-dried, were crushed to pass through a 2 mm mesh sieve and analyzed for the properties shown in Table 1. The soil analyses were carried out by the methods of Pansu and Gautheyrou (2006). The soil was deep, well-drained, loam, high calcareous and Aridisiol. Soil texture was determined by hydrometer method, while soil pH and EC$_e$ were measured at a 1:2.5 soil/water ratio and saturated extract, respectively. However, the organic matter (OM) content was determined by Walkley and Black procedure. Soil available K was determined by 1 M NH$_4$OAc extraction and K assessment in the extract by flame photometer, while soil available P was measured by Olsen method. Available Fe, Zn, Mn and Cu in the soil were first extracted by diethylenetriaminepentaacetic (DTPA) and were then read by atomic absorption. Nonetheless, soil available B was extracted by hot water and measured by azomethine-H colorimetric method (Pansu and Gautheyrou, 2006).

Nitrogen, P and K used at 180, 70 and 75 kg ha^{-1} according to the recommendation from sources of urea (with 46% N), triple super phosphate (with 46% P$_2$O$_5$) and potassium sulfate (with 50% K$_2$O), were respectively added to all treatments (plots). Half of the urea was used when planting and the remainder was used two times: (1) at the vegetative growth and (2) when the maize ears were formed. Potassium and P were used before planting, while Zn and B, were from zinc sulfate and boric acid sources, respectively, were used by two methods: adding of urea to the soil and spraying. Addition of urea 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 twice (the first one was at the vegetative growth stage and the other one was after maize ears formation). The seeds of the maize were sown in May 2009 at a recommended spacing of 70 by 20 cm in plots; however, each experimental plot was 8 m in length and 3 m in width.

In order to determine Fe, Cu and Mn concentrations, leaf samples were taken from the second and third leaves, from the top of plant at silking stage. The samples 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. Fe, Mn and Cu concentrations were determined by atomic absorption spectrophotometer (Benton, 2001). All micronutrient concentrations were expressed in mg kg^{-1} DW.

Standard analysis of variance techniques were used to assess the significance of treatment means by ANOVA using the Statistical Analysis System (SAS), while treatment (fraction) means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Physical and chemical properties of the experimental soil

Selected physical and chemical properties of this site are presented in Table 1. A soil test can be an important management tool in developing an efficient soil fertility program, as well as monitoring a field for potential soil and water management problems. A soil test provides basic information on the nutrient supplying capacity of the soil.

According to the other researches, available K was high, and available P was low. The critical level of available P (Bray II) for maize is 10 to 15 mg kg^{-1} (Howeler, 1990). Olsen and Sommers (1982) reported that available P concentration was 10 mg P kg^{-1} with Olsen method for upland crops. The critical level of available P in Iran soils for majority of crops is 20 mg kg^{-1} soil. This implied that it was necessary to apply phosphate fertilizer for any crop planted at this site.

Phosphorus is unique among the anions in that it has low mobility and availability in soils. It is difficult to manage because it reacts so strongly with both solution and solid phases of the soil (Hodges, 2010). In acid soils,

Table 1. Initial soil test information collected in the spring prior to experiment establishment.

Soil test parameter	Test level	Test rating
Soil pH (1:2.5)	8.2	Alkaline
Soil texture	Loam	-
Electrical conductivity (EC, ds m^{-1})	2.41	Low
Organic matter (%)	0.59	Low
Nutrients (mg kg^{-1})		
P	12.1	Low
K	229	High
Fe	1.65	Low
Mn	8.14	High
Zn	0.32	Low
Cu	0.62	Low
B	0.78	Low

Al and Fe dominate P fixation, while Ca compounds fix P in alkaline soils. Due to the fact that most Fars soils are alkaline, P fixation is dominated by Ca compounds. This soil was calcareous; therefore, available P was low and P availability was greatest at soil pH between 6 and 7. Phosphorus can be rapidly fixed (also referred to as sorption) in forms that are unavailable to plants, depending on the soil pH and type (Al, Fe and Ca content). Cereal soils in Iran are primarily calcareous, with a pH of above 7.8, an OM of less than 1.0%, and a total neutralising value (TNV) exceeding 10.0%. The overuse of P-fertilizers with P accumulation in soils reduces the availability of the other nutrients below the critical demands in cereals (Malakouti, 2007).

Olfati et al. (1999), on the basis of their studies in Iran, reported that K critical level in different locations for wheat varied from 140 (mg kg^{-1}) in Boushehr and Iranshahar to 350 (mg kg^{-1}) in Lorestan, and was 241 (mg kg^{-1}) throughout the country. The availability of soil K depends primarily on the types and amounts of soil minerals present; as such, soil solution and exchangeable K are considered as readily available for plant uptake (Hodges, 2010). Soils testing less than 100 mg kg^{-1} may respond to K fertilization; besides, application of K fertilizer at soil test levels greater than 100 mg kg^{-1} was not justified for most field crops based on the current information (James and Topper, 1993).

The available Fe, Zn, Cu and B in the soil were lower than the critical level, but the available Mn was above the critical level. The soil of this research was calcareous with alkaline pH; therefore, available Zn and B were low. The critical levels of Fe, Zn and B have been determined by many scholars. Rezaei and Malakouti (2001) reported that critical levels of Fe, Zn and B in soils of Iran were 4.8, 1.1 and 1.0 mg kg^{-1} soil, respectively. Johnson and Fixen (1990) stated 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 were 5.0, 1.5, 0.5, 1.0 and 1.0, respectively. The actual total Fe content of a soil may exceed 50,000 mg kg^{-1}; however, the portion available to plants may be less than 5 mg kg^{-1} (Hodges, 2010). Page et al. (1982) classified Fe and Zn as: 0-5 mg kg^{-1} (very low), 6 to 10 mg kg^{-1} (low) and 11 to 16 mg kg^{-1} (medium) for Fe, and 0.0 to 0.5 mg kg^{-1} (very low), 0.6 to 1.0 mg kg^{-1} (low), 1.1 to 3.0 mg kg^{-1} (medium) and >3.0 mg kg^{-1} (high) for Zn. Critical range of B extractable with hot water related to soil texture, pH and plant species were 0.0 to 0.4 mg kg^{-1} (very low), 0.5 to 0.8 mg kg^{-1} (low) 0.9 to 1.2 mg kg^{-1} (medium) and 1.3 to 2.0 mg kg^{-1} (high). Chang et al. (1983) studied the soil B status of Taiwan and found that the level of HWS-B was affected by the type of parent material, the organic matter content, the duration of leaching and the irrigation water.

Zn is one of the essential elements for plants and humans, but it is deficient (less than 1.00 mg kg^{-1} DTPA-extractable Zn) in most calcareous soils and, consequently, in plant and human diets. The critical level for DTPA-extractable Zn is 0.8 mg kg^{-1} soil (James and Topper, 1993). Sturgul (2010) reported that the optimum Zn soil test ranges are 3.1 to 20 mg kg^{-1} for all soil textures. The need for supplemental Zn applications should be confirmed with plant analysis, in that scalped or severely eroded soils are more apt to be Zn deficient. Also, sands, sandy loams and organic soils are more likely to be Zn deficient than other soil types. Severe soil compaction can also reduce Zn availability. In addition, cool weather during the growing season may also induce Zn deficiency in high demand crops.

Leaf Fe concentration

Application of different levels of Zn showed no significant effect on the leaf Fe content relative to the zero Zn level ($p<0.05$), but Zn spraying increased the leaf Fe content relative to the application of Zn to the soil. The soil was calcareous with high soil pH; therefore, Zn was soon rendered as insoluble. Zinc availability is dependent on soil pH, in that as soil pH increases, Zn availability decreases. 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 Zn and B are soon rendered insoluble. Plant nutrient availability is strongly tied to the activity of H^+ in the soil solution. Decreasing soil pH directly increases the solubility of Mn, Zn, Cu and Fe (Hodges, 2010). Zinc is complexed with $CaCO_3$ in alkaline (pH 8.2) soils of Iran. After applying Zn fertilizers, the activity and extractability of Zn added to soils in water soluble forms continually and slowly decreases, and Zn changes to more stable forms through slow reactions with soil constituents (Shuman, 1991). Foliar spraying under these conditions could be much more efficient than any other application of Zn to the soil. Therefore, there was a synergism between Zn and Fe in this research. Zinc foliar spray aided Zn in affecting the increase of Fe concentration in the leaf. The results are in contradiction with those of Erdal et al. (2003) who showed that the Fe, Cu, Mn, N, P, K, Ca and Mg concentrations of maize plants decreased with Zn fertilization from soil. Rajaie et al. (2009) showed that Zn application decreased the concentration of Fe, Mn and Cu in lemon 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 the dilution effect or antagonistic relationship between Zn and other micronutrients.

The lowest and highest mean Fe concentration in the leaf (108.8 and 122.8 mg kg^{-1}) was seen at 16 kg ha^{-1} Zn and Zn foliar spray levels; but it had no significant difference from the zero Zn level. The leaf Fe concentration was sufficient according to the results of researchers. In most plant species, deficiencies occur when the Fe content of leaves was below 10- 80 mg kg^{-1} (Hodges, 2010). Vitosh et al. (1994) reported that sufficiency ranges of Fe, Cu and Mn at ear leaf of initial silk were 21 to 250, 6 to 20 and 20 to 150 mg kg^{-1}. Studies focusing on plant tissue analysis as a diagnostic tool have emphasized economy and ease of plant sampling. In maize for example, the youngest fully developed leaf is taken before tasseling, but after tasseling the flag leaf (leaf opposite and below the ear node) is collected (James and Topper, 1993). The data reported in the literature on nutrient absorption and removal by maize and other crops vary greatly, depending on the fertility of the soil, the yields obtained, and the plant parts removed in the harvest.

Application of different levels of B on the leaf Fe concentration was significant ($p<0.05$). The use of 6 kg ha^{-1} B increased leaf Fe content from 106.4 to 119.5 mg kg^{-1}, showing a 12.3% increase relative to the zero B level. The use of 3 kg ha^{-1} B and B spraying had no significant effect on the leaf Fe content relative to the zero and 3 kg ha^{-1} B levels. Therefore, the presence of a high amount of B in the soil assisted the increase of Fe concentration in the leaf. In fact, there was a synergism between Fe and B. Similar results were reported by other researchers. Patel and Golakiya (1986) showed that B application, up to 2 mg kg^{-1} soil, increased the uptake of N, P, K, Fe, Cu and B. Rajaie et al. (2009) reported that there was a significant increase in shoot concentration of Fe, Mn and Cu with increasing B levels; although the effect of B on concentration of Fe, Mn and Cu was an accumulation effect rather than an increased uptake which resulted from B. 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, leaf P, K, Ca, Mg, Fe, Mn and Cu concentrations fell quite rapidly at first and then decreased gradually or leveled off.

The lowest and highest mean leaf Fe content (106.4 and 119.5 mg kg^{-1}) was not seen at Zn and 6 kg ha^{-1} B levels, respectively. The effect of Zn-B interaction on the leaf Fe concentration showed B application in any Zn levels which had no significant effect on the Fe concentration in the leaf. The use of 24 kg ha^{-1} Zn at B spraying level decreased leaf Fe content from 124.3 to 90 mg kg^{-1}; but at other B levels, it had no significant effect on the leaf Fe content (Table 2).

All treatments, except the treatment of Zn and B spraying, showed significant difference on the Fe concentration in the leaf as compared to the control. The highest leaf Fe content (132.3 mg kg^{-1}) was obtained by joint use of Zn and B spraying, in which 24% increase was shown as compared to 106.7 mg kg^{-1} of the control.

Leaf Mn concentration

The main effect of Zn on the leaf Mn concentration was insignificant ($p<0.05$), but the effect of B on the leaf Mn concentration was significant ($p<0.01$) (Table 3). The highest mean Mn concentration in the leaf (153.1 mg kg^{-1}) was not seen at any B level. The use of B on the soil and spraying it decreased leaf Mn content relative to the no B level. Therefore, reduction in the leaf concentration of Mn may be due to the dilution effect or the antagonistic relationship between B and Mn. The results are in contradiction with those of Rajaie et al. (2009) who stated that the use of B significantly increased Mn concentration in the corn shoot. Also, they found that the effect of B on concentration of Fe, Mn and Cu has an accumulation effect rather than an increased uptake which resulted from B. 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 2. The effect of Zn and B on the leaf Fe concentration (mg kg^{-1})*.

B (kg ha^{-1})	Zn (kg ha^{-1})					
	0	8	16	24	Zn foliar spray	Mean of B levels
0	106.7^{bcd}	100.7^{cd}	106.0^{bcd}	106.0^{bcd}	112.7^{abcd}	106.4^{b}
3	115.7^{abc}	105.0^{bcd}	112.3^{abcd}	114.0^{abc}	128.3^{ab}	115.1^{ab}
6	118.3^{abc}	123.0^{abc}	111.3^{abcd}	128.7^{ab}	118.0^{abc}	119.5^{a}
B foliar spray	124.3^{abc}	107.7^{bcd}	106.3^{bcd}	90.0^{d}	132.3^{a}	112.1^{ab}
Mean of Zn levels	116.3^{ab}	109.1^{b}	108.8^{b}	109.4^{b}	122.8^{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 the leaf Mn concentration (mg kg^{-1})*.

B (kg ha^{-1})	Zn (kg ha^{-1})					
	0	8	16	24	Zn foliar spray	Mean of B levels
0	133.0^{bcd}	153.3^{abc}	182.0^{a}	154.3^{ab}	143.0^{abc}	153.1^{a}
3	139.3^{abc}	158.0^{ab}	127.3^{bcd}	138.7^{abc}	92.7^{d}	131.2^{b}
6	123.3^{bcd}	112.7^{bcd}	112.7^{bcd}	144.3^{abc}	127.0^{bcd}	124.0^{b}
B foliar spray	113.7^{bcd}	149.7^{abc}	121.3^{bcd}	108.3^{cd}	133.7^{bcd}	125.3^{b}
Mean of Zn levels	127.3^{a}	143.4^{a}	135.8^{a}	136.4^{a}	124.1^{a}	

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

The lowest mean Mn concentration in the leaf (124 mg kg^{-1}) was seen at high B level (6 kg ha^{-1} B). According to the other researches, leaf Mn content was sufficient. Due to the fact that the soil available Mn in the soil was higher than the critical levels, the Mn uptake by corn increased and was sufficient. Manganese levels in plants vary between species and from site-to-site much more than those of other macro and micronutrients owing to the link between soil pH and Mn availability, as well as to interspecies variation in the redox potential of the roots and the nature of their acid exudates (Marschner, 1995; Coga et al., 2009). Differing soil pH can result in changing levels of Mg and Mn in plant tissue (Sturgul, 2010). Wide ranges of optimal Mn contents in plants are reported in literature. Plants take up only Mn^{2+} which is, besides weak complexes, dominating in tissue, but its concentrations are variable, depending on soil conditions and plant species. The concentration range in plants is observed frequently between 30 and > 1000 mg kg^{-1} in dry matter (Marschner, 1995). Bergman (1992) reports values between 30 and 100 mg kg^{-1} dry matter (DM), while Fregoni (1998) reports a range of 50 to 500 mg kg^{-1} DM. For maize, a plant tissue analysis showing a value of 16 mg kg^{-1} for Mn would indicate that the nutrient status category is the critical range. Nutrients may need to be added to bring plant tissue levels into the sufficient range and improve crop yield. If nutrient applications to the current crop are not feasible, they can be made before planting future crops. Reuter and Robinson (1997) classified Mn concentration in ear leaf of maize at initial silk as: <15 mg kg^{-1} (deficient), 16 to 19 mg kg^{-1} (critical value or marginal), 20 to 150 mg kg^{-1} (sufficient or adequate/normal/ optimal), 151 to 200 mg kg^{-1} (high) and >200 mg kg^{-1} (toxic or excessive).

The effect of Zn-B interaction on leaf Mn concentration was significant ($p<0.05$). The use of B at low levels of Zn (0 and 8 kg ha^{-1} Zn) showed no significant effect on the leaf Mn content, but at high Zn levels (16 and 24 kg ha^{-1} Zn) and Zn spraying, it decreased leaf Mn content. Therefore, the presence of a high amount of Zn in the soil assisted the reduction of the leaf Mn content, that is, an antagonism was seen between Zn and Mn. Similar results were reported by Rajaie et al. (2009) who concluded that the use of Zn reduced the concentration of Fe, Mn and Cu in plant shoot. 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.

The use of 16 kg ha^{-1} Zn at zero B level increased the leaf Mn content from 133 to 182 mg kg^{-1}, showing a 37% increase relative to the no B level; but at other B levels, applying Zn to the soil had no significant effect on the leaf Mn content. The presence of a high amount of B in the soil and B spraying prevented the increase of the leaf Mn content, by Zn application.

All treatments, except the treatment with the highest leaf Mn content (application of 16 kg ha^{-1} Zn) had significant effect on the leaf Mn content relative to the control. Application of 16 kg ha^{-1} Zn, with a leaf Mn content of 182 mg kg^{-1}, showed 37% increase relative to the control, with a leaf Mn content of 133 mg kg^{-1}.

Table 4. The effect of Zn and B on the leaf Cu concentration (mg kg^{-1})*.

B (kg ha^{-1})	Zn (kg ha^{-1})					
	0	**8**	**16**	**24**	**Zn foliar spray**	**Mean of B levels**
0	7.1^{ab}	7.5^{a}	6.5^{abc}	6.4^{abc}	7.1^{ab}	7.0^{a}
3	6.4^{abc}	7.2^{ab}	6.1^{abc}	6.7^{abc}	5.8^{abc}	6.4^{a}
6	5.2^{bc}	7.1^{ab}	4.9^{c}	7.1^{ab}	6.8^{abc}	6.2^{a}
B foliar spray	6.1^{abc}	6.8^{abc}	5.5^{abc}	5.7^{abc}	6.7^{abc}	6.2^{a}
Mean of Zn levels	6.2^{ab}	7.1^{a}	5.8^{b}	6.5^{ab}	6.6^{b}	

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

Leaf Cu concentration

The effect of different levels of Zn on the leaf Cu concentration was insignificant ($p<0.05$), but application of 16 kg ha^{-1} Zn reduced leaf Mn content relative to the 8 kg ha^{-1} Zn level (Table 4). The highest mean Mn concentration in the leaf (7.1 mg kg^{-1}) was seen at 8 kg ha^{-1} Zn level, but it showed no significant difference in the no B level. As such, the effect of different Zn levels and Zn-B interaction on the leaf Mn content was insignificant ($p<0.05$). Copper concentration in the leaf was sufficient according to the other researches. So, Cu tissue levels below 2 mg kg^{-1} are generally inadequate for plants (Kabata-Pendias and Pendias, 1992). A critical Cu concentration of Canadian prairie soils for cereal crops production was reported as 0.4 mg kg^{-1} (Karamanos et al., 1986).

Although plant analysis was first used in production agriculture to diagnose potential deficiencies, it has now been developed into an important management tool in monitoring the nutritional status of healthy crops (Campbell, 2009). The effects of time of sampling, variety or hybrid and environmental factors, such as soil moisture, temperature, light quality and intensity, may significantly affect the relationship between nutrient concentration and response. Thus, it is essential that the time of sampling, stage of growth and character of growth prior to sampling be known and considered when interpreting a plant analysis result (Hodges, 2010).

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