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BORON TOXICITY IN IRRIGATED COTTON (*GOSSYPIUM HIRSUTUM* L.)

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Abstract

Boron toxicity is a serious concern in irrigated agriculture throughout the world for sustainable crop production. A greenhouse experiment was conducted to determine the critical level of boron (B) toxicity for cotton in soil and plant tissues in an arid climate. B levels of 0, 0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 15.0, 20.0 and 25.0 mg kg^{-1} were applied in solution form to soil before the sowing of the crop. Toxicity symptoms (yellowing and necrosis in patches between veins and tips and margins of leaves) first appeared on older leaves at 5 mg B kg^{-1} soil level and increased in severity with increasing levels. Dry matter yield declined significantly ($p \le 0.05$) with increasing levels of B. The maximum dry matter yield (29.10g/pot) was recorded at 2 mg B kg^{-1} soil level. The 90% of the maximum dry matter yield was obtained at B level of 5 mg kg^{-1} soil. At 5 mg B kg^{-1} soil, leaf tissues maintained contents at 198 mg B kg^{-1} dry weight. The concentration of B in various plant parts was found in the order of leaves>shoot>root. The addition of higher levels of B caused significant ($p \le 0.05$) reduction in N, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+} and Fe^{2+} contents whereas P, K^{+} and Cu^{2+} contents increased significantly ($p \le 0.05$) in leaf tissues.

Introduction

Maintaining adequate level of nutrients including B in soil is essential for the normal growth of plants. However, B usually causes toxicity when it is applied in level more than needed for normal growth (Marschner, 1995). Toxicity of B is most commonly found in arid and semi -arid regions (Nable *et al.,* 1997). Boron toxicity in the crop plants occurs because of higher B concentration in irrigation water or higher applications of B fertilizer to correct deficiency or use of compost material containing high B content. It may occur also due to fly ash or soils developed from marine sediments (Nable *et al.,* 1997). Boron is transpired in the plants mainly through xylem and accumulates more at the leaf tips and margins of older leaves than in the younger parts (Bennet, 1993; Bergmann, 1992). Initial symptoms may include the yellowing of the tips and margins of the older leaves. As severity of the disorder increases, the chlorotic areas later on become necrotic. The necrosis progresses from the leaf tips and margins towards the midrib and base of the leaf. This gives the leaf a scorched appearance and eventually the entire leaf dies and falls from the plant (Kabata-Pendias & Pendias, 1992).

Cassman (1993) reported that in cotton plant toxicity developed as yellowing of leaf margins and in patches between leaf veins that became necrotic later on. The necrotic areas contained 2700-6400 mg B kg^{-1} leaf dry weight. Silva *et al*., (1979) observed that cotton plants showing symptoms of B toxicity contained > 590 mg B kg^{-1} dry matter under greenhouse conditions. Aitkin & McCallum (1988) reported that boron concentration of 1.9 mg L^{-1} in soil solution caused yield reduction in sunflower. Bergmann (1992) pointed out that B content ranging from 100 to 1000 mg kg^{-1} of dry weight could induce boron toxicity. Reisenauer *et al*., (1973) reported that most of the crop plants showed B toxicity symptoms in soils having more than hot water soluble 5 mg B kg^{-1}. Lovatt & Bates (1984) reported that as B accumulated in the leaves of the plants supplied with excess B in summer squash, leaf conductance of water vapour, CO_2 fixation and chlorophyll content of the older leaves were reduced in relevance to that in plants supplied with sufficient B. Similarly, Loomis & Durst (1992) found that leaf cupping resulted due to inhibition of cell wall expansion at higher levels of B in the rooting medium. Boron toxicity has been reported in dicotyledonous species grown in the Southern Australian cereal belt (Paull *et al*., 1992). Many reports indicate reduction in growth, grain and straw yield of barley and wheat due to B toxicity (Gupta, 1983; Cartwright *et al*., 1984; Yau & Saxena, 1997).

The use of high analysis fertilizer is one of major causes of boron deficiency (Fageria *et al*., 2002) and the addition of boron fertilizers without chemical soil analysis has caused toxicity to certain crop plants (Nabel *et al.,* 1997). The reason being that range between boron deficiency and toxicity is very narrow. In soils, deficiency may take place at levels less than 0.5mg kg^{-1} soil hot water soluble B, while soil containing more than 5 mg B kg^{-1} soil could cause toxicity (Rashid & Ryan, 2004). Boron toxicity is generally confused with fungal diseases. However, recent research has demonstrated this nutritional disorder in barley, wheat, lentil, medicas and rice in Mediteranean type soil climate (Rashid & Ryan, 2004). The literature regarding boron toxicity in cotton is scanty and inconclusive under varying eco-edaphic conditions. Therefore, this study was inevitable to determine the relationship between plant growth and tissue boron concentration and also to determine critical boron concentration in the soil.

Materials and Methods

The experiment was undertaken in a naturally lit greenhouse at University College of Agriculture, Bhauddin Zakariya University, Multan, Pakistan. The site is situated at: longitude: 71°, 30.79′ E; latitude: 31 °, 16.4′ and altitude: 128 m. The sunshine hours ranged from 6.75–11.75 with day and night temperatures being 38.8°C and 28.7°C, respectively. The relative humidity ranged from 62.2 to 47.2% during the span of experiment.

A bulk of silt loam was collected from Ap horizon. The soil was air- dried, crushed and sieved through 2 mm sieve. The soil analyses were carried out by the methods of Ryan *et al.,* (2001). The soil was deep, well-drained, coarse silty, moderately calcareous, hyperthermic, Typic Haplocambids (Anon., 1998). The chemical analyses revealed that soil had: EC_e 2.1 dS m^{-1}; pH 8.1; organic matter content: 0.76%; $CaCO_3$; 7.4%; NH_4OAc-extractable-K, 155 mg kg^{-1}; $NaHCO_3$ – DTPA available-P, 9 mg kg^{-1}; and HCl–extractable B, 0.47 mg kg^{-1}. Before sowing of the crop different boron levels from sufficiency to toxicity (0.0, 0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 15.0, 20.0 25.0 mg kg^{-1}) were developed in soil by using boric acid solution (H_3BO_3, 17.5% B). The boron solution was sprayed on air dried soil and incorporated manually. The pots (33 cm diameter and 36 cm height) lined with polyethylene sheet were filled with 20 kg soil and packed to a bulk density ≈ 1.04 mg m^{-3} (Paull *et al*., 1988). The water holding capacity of the soil was 33% and moisture of the soil was maintained up to 70% of the field moisture capacity with de-ionized water. The soil in the pots was equilibrated for 7 days (Aitken & McCallum, 1988). A basal dose of N (urea), P_2O_5 (triple super phosphate) and K_2O (potassium sulphate) @ 200, 100, and 70 kg ha^{-1}, respectively were mixed thoroughly into the soil. The experiment was arranged according to completely randomized design with four replications. Cotton seed (cv. CIM-473) was delinted with sulphuric acid and dried. A total of 10 seeds were dibbled in each pot and plants were thinned to two at 15 days after sowing (DAS). The moisture content was monitored and maintained at soil moisture up to 70% by weighing the pots regularly.

The plants were uprooted carefully at 60 DAS and data on plant structure were recorded. The plants were divided into roots, shoots and leaves .The plant material was washed with de-ionized water and blotted. The material was dried in a thermo-ventilated oven at 65 ± 5°C upto constant weight. The dry matter yield (DMY) was recorded. The dried material was ground in a John Wiley mill and passed through a 40 mesh screen. The ground material was dry-ashed at 550°C for 6 hours in a muffle furnace. Then the ash was taken in 0.36N H_2SO_4 (Gains & Mitchell, 1979) and the B concentration was determined by spectrophotometer at 420 nm wavelength using azomethine-H method (Bingham, 1982). Boron concentration in leaf blades (excluding petioles) were determined by dry ashing (Gaines & Mitchell, 1979) and colorimetry using azomethine-H (Bingham, 1982).The other nutrients in leaf were determined, i.e. Zn, Mn, Cu, Fe, Ca, Mg, P, K following the method of Ryan *et al*., (2001).

Statistical analysis: The experimental data were analyzed statistically by employing "MSTAT C" on a personal computer (Anon., 1989) and employing least significant differences to segregate the treatment impact.

Results and Discussion

Symptoms of B toxicity appeared as yellowing on leaf margins and in patches between leaf veins. The symptoms started in 15 days older leaves in soil treated with 5 mg B kg^{-1}. The symptoms were severe at higher B levels ranging from 5.0 to 25.0 mg kg^{-1}. The chlorotic areas became faded brown which eventually turned into necrotic areas. The necrosis progressed from leaf tips and margins towards the midrib and at the base of leaf. The leaves showed scorched appearance and at last entire leaf died prematurely and fell down (Fig. 1). The similar symptoms were reported by Bennet (1993) and Bergmann (1992).

Data on main stem height, number of nodes on main stem and internodal length differed significantly ($p\leq0.05$) with B fertilization (Table 1). The main stem height increased from 38.7 to 46.10 cm as B level increased from 0 to 2.0 mg B kg^{-1} soil, whereas the addition of 5 mg B kg^{-1} soil or higher dose caused significant reduction in main stem height ($p\leq0.05$). The other parameters of plant structure, i.e. number of nodes per plant and inter nodal length followed the similar pattern as that of main stem height. The addition of B up to 3.0 mg kg^{-1} soil caused increase in internodal length. However, the internodal length decreased due to addition of B @ 5 mg kg^{-1} soil. Similarly, the addition of higher quantity of B also caused reduction in the number of nodes on main stem. Main stem height was reduced by 14.1% at 5 mg B kg^{-1} as compared to 3.0 mg B kg^{-1} soil. The results are in conformity to the findings of Gupta (1983) and Sotiropoulos *et al.*, (2002) who reported growth reduction in several crop plants due to higher levels of B. Significant ($p\leq0.05\%$) reduction in plant height, node number and dry matter yield of cotton in response to increasing B is due to the adverse effects of B on plant metabolic activities. Under conditions of excess B supply, its concentration in the cytosol may rise and cause metabolic disturbances by formation of complexes with NAD^+ of the ribonucleotide units that form a key part of the RNA structure (Loomis & Durst, 1992).

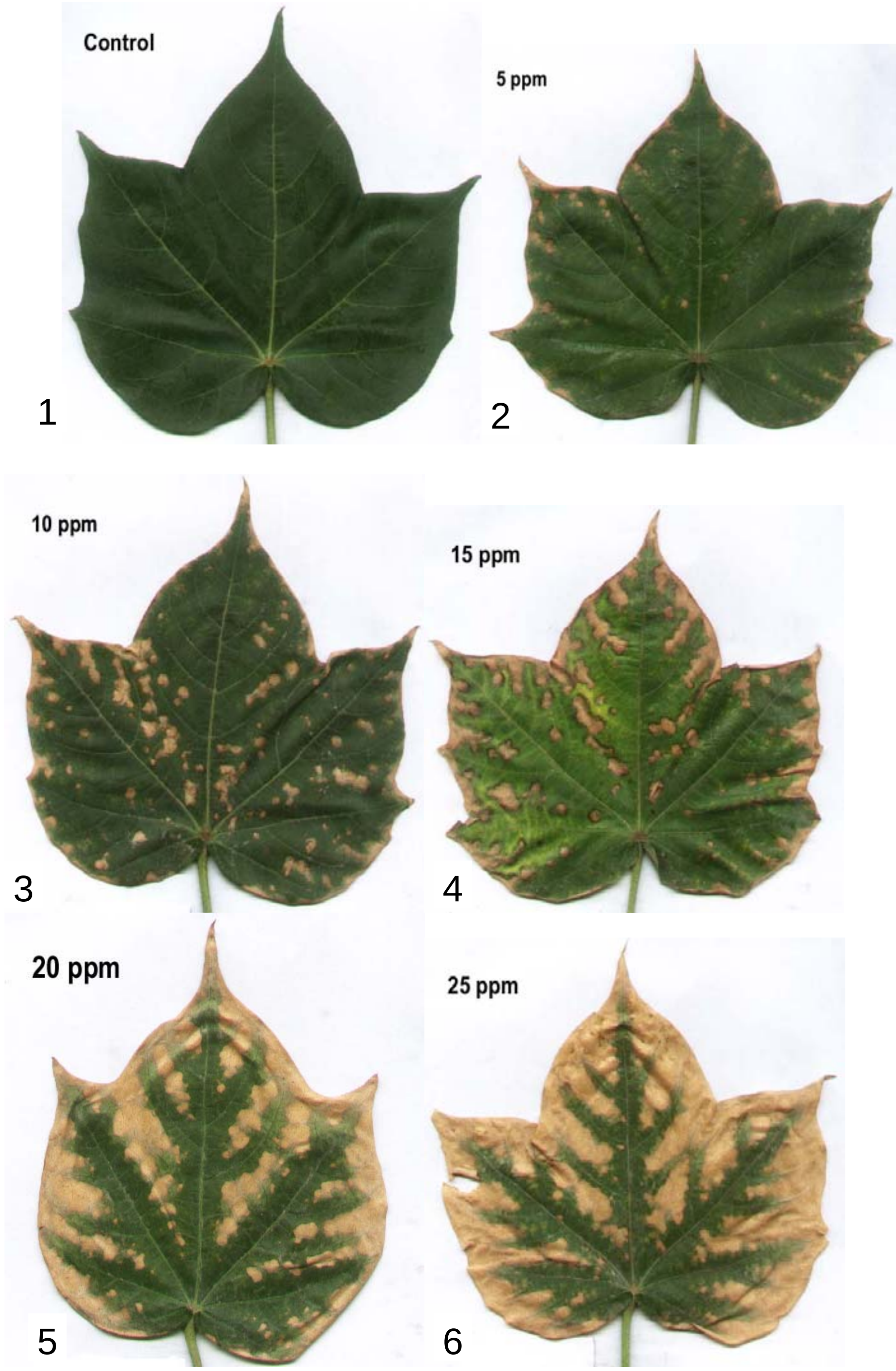

Fig. 1. Toxicity symptoms of different B levels in cotton leaves; Plates 1-6.

Table 1. Effect of different levels of boron on plant structure.

B levels (mg kg^{-1})	Main stem height (cm)	No. of nodes plant^{-1}	Internodal length (cm)
0.0	36.0	15.3	2.41
0.5	38.7	16.0	2.43
1.0	43.3	17.0	2.56
2.0	46.1	17.0	2.71
3.0	43.1	17.0	2.54
5.0	37.0	16.0	2.40
10.0	31.4	15.0	2.10
15.0	28.6	15.0	1.90
20.0	23.3	14.0	1.67
25.0	16.3	11.0	1.49
LSD ($p \leq 0.05$)	**1.289**	**1.187**	**0.137**

Table 2. **Effect of different levels of soil applied B on dry matter production and relative dry matter yield.**

B levels (mg kg^{-1})	Shoot dry wt. (g pot^{-1})	Root dry wt. (g pot^{-1})	Total dry matter (g pot^{-1})	Relative dry matter yield (%)
0.0	20.60	4.10	24.70	84.70
0.5	21.50	4.30	25.80	88.49
1.0	22.30	4.30	26.60	91.20
2.0	24.40	4.70	29.10	100.0
3.0	23.00	4.60	27.60	94.60
5.0	21.90	4.40	26.30	90.20
10.0	19.50	3.90	23.40	80.26
15.0	16.10	3.40	19.50	66.88
20.0	12.30	2.60	14.90	51.0
25.0	11.60	2.10	13.70	47.0
LSD ($p \leq 0.05$)	**2.214**	**0.340**	**1.797**	

The adverse effects of B on plant metabolic activities are more probably related to chlorosis and necrosis, loss of photosynthetic capacity, and eventually reduction in plant productivity. Lovatt & Bates (1984) reported that boron accumulated in the roots of summer squash when excess B was applied. The accumulation of B subsequently inhibited the elongation and lateral root development of squash plants.

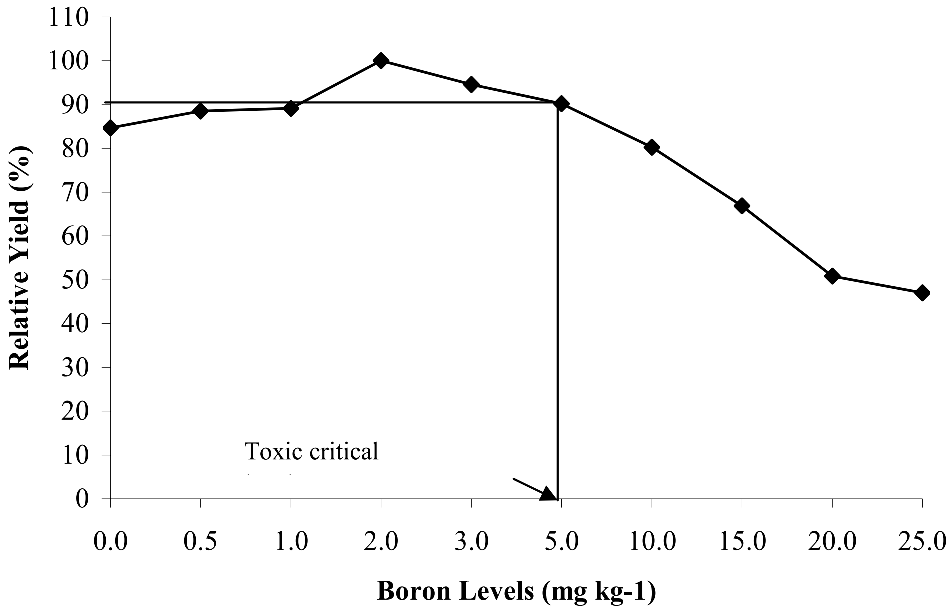

Fig. 2. Effect of B levels on relative dry matter yield.

Boron fertilization upto 2 mg kg^{-1} soil increased the root and shoot dry weights significantly ($p \leq 0.05$) and further increase in B level caused gradual decrease (Table 2). About 90% of the maximum dry matter yield was obtained at 5 mg B kg^{-1} soil (Fig. 2). Ulrich & Hills (1990) reported that the critical level of a nutrient was the concentration occurring in a specific plant part at 90% of the maximum yield. This study has revealed that 5 mg B kg^{-1} soil is the toxic critical level of B for cotton plant. The further increment in B beyond this reduced the yield markedly. In the present study, necrotic areas developed on leaves between veins and loss of leaves due to toxicity inhibited photosynthetic process and exerted a negative impact on cotton growth. Reduction in dry matter yield has been reported earlier by Cartwright *et al.,* (1984), Alpaslan & Gunes (2001) and Sotiroponlos *et al*., (2002). The root dry matter yield also showed the same trend in response to B application. Root dry weight was significantly reduced ($p \leq 0.05$) by 49% at higher B. This might be due to the accumulation of boron in roots that resulted in reduced elongation and lateral root development (Lovatt & Bates, 1984). The analysis of root portion revealed high B concentration of 65 mg kg^{-1} root dry matter (Table 3). The negative impact of high B on root growth has been demonstrated earlier (Riley *et al*., 1994; Reid *et al*., 2004).

The partitioning of B in various plant tissues showed significant variations with increasing B level (Table 3). The B was assimilated in the order of leaf > shoot> root. On an average root, shoot and leaf absorbed 36, 57 and 247 mg B kg^{-1} dry matter, respectively. The leaf with toxicity symptoms at 5 mg B kg^{-1} soil maintained B concentration at 198 mg kg^{-1}leaf dry weight. This critical level is also associated with 90% of the maximum relative dry matter yield. This value lies in the intermediate range for B toxicity in cotton as described by Bradford (1966). Our results differed with those mentioned by Sakal & Singh (1995) who reported more than 522 mg B kg^{-1} dry matter concentration for toxicity in cotton plant. The lower concentration of B in root as compared to leaves is also reported by Oertli & Roth, (1969) and Nable (1988). The movement of B within the root and shoot is passive and absorbed B in root moves with transpiration stream into the shoot (Kochian, 1991). The root portion serves as a source of nutrients from where the nutrients are translocated to different growing parts of the plants. As a result comparatively higher B concentration was found in aerial parts of the plant (Table 3). The results reported by Nable (1988) and Oertli & Khol (1961) also support the current research findings.

A variable response of N, P, K, Ca, Mg, Zn, Cu, Mn and Fe contents with increasing B levels was observed (Table 4 and 5). Nitrogen concentration showed 5.2% increase up to 2 mg B kg^{-1} soil and beyond this level it started decreasing. The same trend was observed in corn by Mozaffar (1989). Phosphorus and K responded positively to different B levels. Our results demonstrate that the P concentration (Table 4) in the leaves increased significantly ($p \leq 0.05$). Previous reports relating to the B-P relationship refer to B deficiency environment, which decrease P uptake (Robertston *et al.,* 1974; Pollard *et al.,* 1997). The lowest concentration (0.21%) was registered in control and the maximum was noted at 25 mg B kg^{-1} soil. Similarly, the K concentration increased significantly ($p \leq 0.05$) in leaves with the increasing B levels. The K increase of 26% was recorded in leaf at highest level. In our study B fertilization at different levels enhanced translocation of P and K to the aerial part of the plant. The increase in P and K concentration due to B application was reported by Lopez-Lefebre *et al.,* (2002) in tobacco. The results also indicate that B toxicity exerts a strong influence on Ca and Mg absorption and/or transport. The highest concentration of Mg was determined in control (0.61%) and the lowest concentration (0.26%) was registered at 25 mg B kg^{-1} soil showing an overall decrease of 57% at the highest level. Bowen (1981) observed antagonistic relationship between B and Mg. Similarly Ca concentration in leaves decreased significantly ($p \leq 0.05$) with increasing levels of B. The decline was 31% at the highest level (25 mg B kg^{-1} soil) as compared to control. The significant decrease in Mg and Ca and increase in K might be due to the competition between these elements at uptake sites (Cramer *et al.,* 1991).

The concentration of Zn, Mn and Fe first gradually increased upto level of 3 mg B kg^{-1} soil and beyond this level it started decreasing with further increase in B levels, while the Cu concentration in leaves increased with B supply. Decreases in Mn and Ca concentrations in kiwi fruit leaves with increasing B levels were reported by Sotiropoulos *et al*., (2002), Ca in corn (Mozafar, 1989), Ca in *Vicia faba* (Muhling *et al*., 1998) and Mg in tobacco (Lopez-Lefebre *et al*., 2002). The decrease in Zn, Fe and Mn and increase in Cu concentration were also reported in groundnut and cotton (Gopal, 1975; El-Gharably & Bussler, 1985). In these studies, B interferes in translocation of Zn, Fe and Mn. Similar results regarding Cu, Fe, Zn and Mn were also observed in sunflower (*Helianthus annus* L.) with boron supplies by Gomez Rodriguez *et al*., (1981).

Conclusion

The results of this study show that the toxicity symptom of B appeared at the level of 5 mg kg^{-1} soil. The B content associated with B toxicity symptoms was 198 mg kg^{-1}. The growth parameters increased from 0-5 mg kg^{-1} B levels but thereafter reduced sharply.

Table 3. Effect of different levels of soil applied B on B accumulations ($mg\ Kg^{-1}$) in different plant organs.

B levels ($mg\ kg^{-1}$)	Plant organs		
	Leaf	Shoot	Root
0.0	39.0	21.0	12.0
0.5	51.0	29.0	16.0
1.0	65.0	33.0	21.0
2.0	93.0	39.0	26.0
3.0	108.0	47.0	30.0
5.0	198.0	60.0	37.0
10.0	269.0	69.0	43.3
15.0	374.0	78.7	48.0
20.0	547.0	88.7	57.0
25.0	721.0	101.0	65.0
LSD ($p \leq 0.05$)	**2.843**	**2.867**	**3.095**

Table 4. Effect of different levels of soil applied B on macro nutrients concentration in leaf tissues (%).

B levels ($mg\ kg^{-1}$)	N	K	P	Ca	Mg
0.0	4.12	2.26	0.21	2.63	0.61
0.5	4.15	2.31	0.27	2.51	0.61
1.0	4.26	2.39	0.31	2.41	0.57
2.0	4.35	2.43	0.37	2.33	0.51
3.0	4.34	2.48	0.40	2.19	0.45
5.0	4.30	2.53	0.42	2.12	0.41
10.0	4.10	2.56	0.47	2.06	0.41
15.0	3.91	2.71	0.48	2.00	0.34
20.0	3.83	2.74	0.51	1.85	0.28
25.0	3.69	2.85	0.55	1.81	0.26
LSD ($p \leq 0.05$)	**0.0794**	**0.0458**	**0.0458**	**0.102**	**0.0648**

Table 5. Effect of different levels of soil applied B on micro nutrients concentrations in leaf tissue.

B levels ($mg\ kg^{-1}$)	Nutrient concentration ($mg\ kg^{-1}$)			
	Zn	Fe	Mn	Cu
0.0	40.0	229.5	23.3	6.5
0.5	46.0	231.4	26.9	6.8
1.0	51.0	240.0	29.3	7.1
2.0	54.0	245.0	32.0	7.4
3.0	54.3	242.0	33.0	7.4
5.0	50.0	330.0	30.0	7.6
10.0	41.0	218.0	29.4	7.8
15.0	43.0	200.0	25.3	8.3
20.0	38.0	193.0	22.4	8.9
25.0	32.0	182.0	19.7	10.1
LSD ($p \leq 0.05$)	**4.822**	**4.743**	**2.067**	**0.1124**

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