Egypt. J. Agron. **Vol. 28**, No. 1, pp. 1-13 (2006)

Boron-Zinc Relationship in Cotton Plants Grown under Low or High Lime Levels in the Soil

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COTTON plants (*Gossypium barbadense* L. var. Giza 85) were grown in pots on two soils differing in calcium carbonate content (2.0 % and 10.0 %) to study boron-zinc relationship as well as status of certain nutrients as affected by foliar application of boron, zinc and their combinations. Growth of cotton shoots was significantly affected by foliar application of boron, zinc and their combined treatments. The highest biomass accumulation in shoots of the plants grown on low lime soil was obtained by (25 ppm B) or (25 ppm B + 50 ppm Zn) , but the combined treatment (25 ppm B+ 50 ppm Zn) was superior in the plants grown on high lime soil content. The same treatments also increased other nutrient concentrations and uptake by cotton shoots. There was a certain relationship between boron and zinc from one side and boron and other nutrients from the other side, including uptake, concentrations and balance, which suggests that boron may play a key role among other nutrients. Thus, boron/element ratios in the shoot tissues, which led to the highest biomass accumulation, can be used as an indicator for growth in soils which contain such calcium carbonate levels.

Keywords: Boron, Zinc, Foliar fertilization, Lime stress, Cotton .

Boron is an essential micronutrient for higher plants (Loomis and Durst, 1992). Its deficiency causes decrease in plant growth and yield. Some of major B-deficiency disorders are the inhibition of NADH-reductases (Barr *et al.*, 1993) and ferricyanide-inducible reductase activity in the roots (Goldbach *et al.*, 1990 and Findeklee *et al.*, 1997). Unlike other nutrients, boron transport in plants differs among species. In some species, boron is phloem immobile (Brown and Shelp, 1997). Most of annual crops, which do not contain polyols are phloem immobile-B and described as sensitive plants to B-deficiency.

To correct boron deficiency, it can be added as soil applications (Blamey *et al.*, 1997 and Eguchi & Yamanda, 1997b,) or as foliar feeding. However, unfavorable soil conditions such as high pH values and/or high $CaCO_3$ levels were found to generally restrict the uptake and translocation of micronutrients (El-Fouly, 1983 and Marschner, 1995). On the other hand, in sandy or sandy loam soils, about 10% of the soil applied B was found to be absorbed by the plant roots, 30-40 % left in the soil and 40-60 % leached from the top soil (Eguchi and Yamanda, 1997a). In such a case, boron-fertilization through soil seems to be less effective. Boron foliar fertilization was found to increase ion uptake (Pollard *et al.*, 1977) and interfere with K, Ca and Mn, which finally positively influenced the yield (Goldbach *et al.*, 2000; Reinbott *et al.*, 1997 and Schon & Blevins, 1990).

Since, the relationship between boron and zinc is still unclear. The present work aimed to study B-Zn relationship and its interaction with other nutrients through foliar application of boron, zinc and their combinations on the shoots of cotton plants (boron sensitive). The study included plants grown on soil contains a relatively high level of $CaCO_3$ and others grown on soil contains low $CaCO_3$ level.

Material and Methods

Sowing and agricultural practices

Cotton seeds (*Gossypium barbadense* L. var. Giza 85) were sown in black-plastic pots filled with 5.0 kg soil (2% $CaCO_3$) in the greenhouse of the Plant Nutrition Institute, University of Bonn, Germany. Before sowing, half of the pots received pure $CaCO_3$ to increase their content to reach 10.0 %. Then, the pots received 0.4 g ammonium sulfate (20.6 % N), 0.2 g potassium sulfate (50 % K_2O) and 0.1 g super mono-phosphate (15.5 % P_2O_5) per kg soil. Irrigation took place daily to keep soil moisture at 60 % capacity. Two weeks after sowing, the plants were thinned to leave 3 plants/pot.

Treatments

Treatments were carried out as foliar spray at 21 days after sowing in 3 replicates as follows:

1) Control
2) +B (25 ppm boron)
3) ++B (50 ppm boron)
4) +Zn (50 ppm zinc)
5) +B +Zn (25 ppm boron + 50 ppm zinc)
6) ++B +Zn (50 ppm boron + 50 ppm zinc)
7) ++ Zn (100 ppm zinc)
8) +B ++Zn (25 ppm boron + 100 ppm zinc)

Boron treatments were prepared from pure boric acid (H_3BO_3), and Zn-EDTA was used for zinc treatment preparations.

Analysis

Plant material

The plants were harvested 10 days after spraying. Shoots were weighed for fresh weight and then, washed with tap water and boron-free distilled water, oven dried at 70° C in plastic-free boron containers for 24 hr and ground. One-gram sample was dry-ashed in a muffle furnace at 550°C for 6 hr using 3.0 N HNO_3. The residue was, then, suspended in 0.3 N HCl.

Soil samples

Representative soil samples were taken after soil preparation and before fertilization. The samples were air-dried and passed through a 2.0 mm sieve pores. Some soil physical and chemical characteristics are shown in Table 1. Soil mechanical analysis was carried out using the hydrometer method (Bouyoucos, 1954); pH and E.C (electric conductivity): soil/water suspension (1:2.5); calcium carbonate: Calcimeter method (Alison and Moodle, 1965); organic matter: potassium dichromate method (Walkely and Black, 1934).

Soil phosphorus was extracted using sodium bicarbonate (Olsen *et al.*, 1954). Potassium and magnesium were extracted using ammonium acetate (Chapman and Pratt, 1978), while Fe, Mn, Zn and Cu were extracted using DTPA (Lindsay and Norvell, 1978). Boron was extracted and measured according to Wolf (1971).

TABLE 1. Physical and chemical characteristics of the used soil.

Physical characteristics		Nutrient content	
pH	7.0 M	***Exchangeable macronutrients*** (mg/100g soil)	
E.C. (dS/m)	0.4 M		
$CaCO_3$ (%)	2.0 L	P	2.3 M
O.M.(%)	0.3 vL	K	12.0 L
		Mg	17.4 L
Sand (%)	69.2	Ca	142 vL
Silt (%)	14.8	***Available micronutrients*** (mg/kg soil)	
Clay (%)	16.0	Fe	24.6 H
Texture	Sandy loam	Mn	20.7 H
		Zn	5.2 H
		Cu	2.6 vH
		B	1.02 M

L = Low, vL = very low, M = Moderate, H =High, vH = very high
(Nutrient status evaluated according to Ankerman and Large, 1974)

Measurements

Phosphorus was determined photometrically using the Molybdate-Vanadate method and measured using the UV-VIS spectrophotometer (Perkin-Elmer Lambda 2). Potassium and calcium were measured in the extract using flamephotometer (Jenway PFP7). Magnesium, iron, manganese, zinc and copper were measured using the atomic absorption spectrophotometer (Zeiss PMQ3). Boron was extracted according to Wimmer and Goldbach (1999) and measured using UV-VIS-spectrophotometer (Perkin-Elmer Lambda 2).

Data analysis

Data were statistically analyzed using Costate Statistical Package (Anonymous, 1989).

Results and Discussion

Growth

Growth of cotton shoots grown on soil which contains low or high $CaCO_3$ level was significantly affected by foliar treatments of B, Zn and their combinations (Fig. 1). Highly significant biomass accumulations in the shoots were obtained using the treatments (+B+Zn) and (+B) for plants grown on low lime content soil. In the shoots grown on soil which contains high lime, significantly positive biomass accumulation was obtained by applying the treatment (+B+Zn). This can be attributed to the integration and balance of nutrients caused by applying proper boron doses.

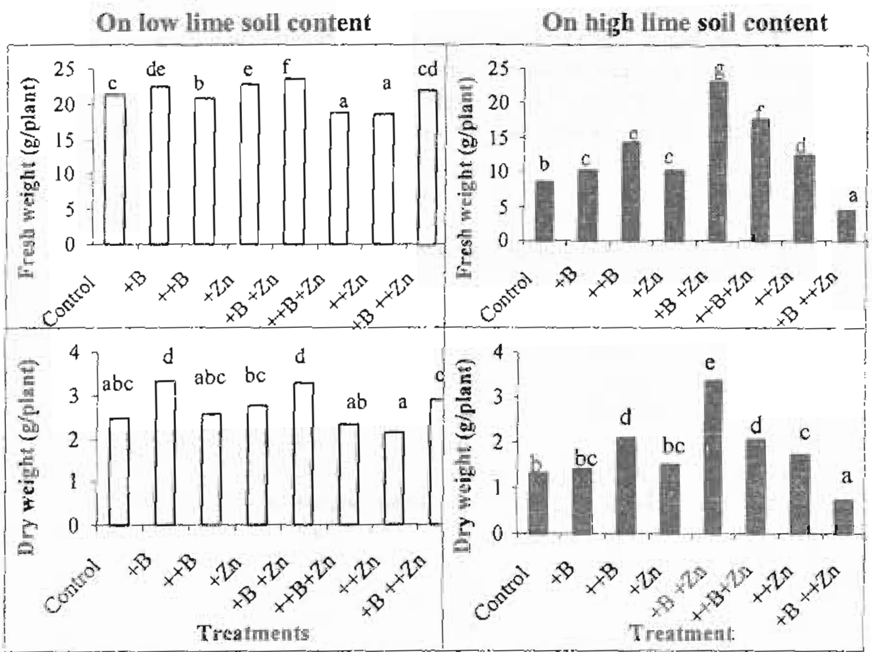

Fig. 1. Fresh and dry weights of cotton shoots as affected by foliar fertilization of B, Zn and their combinations (bars with same letters are not significantly different, P= 0.05) **.**

Nutrient concentrations

Foliar treatments of boron, zinc and their combinations increased both B and Zn-concentrations in the shoot tissues of the plants grown on both low and high $CaCO_3$ levels in the soil. Besides, they also led to Fe, Mn and Cu concentration increases (Fig. 2).

Zinc concentrations were proportional to boron concentrations with all treatments, even in case of Zn addition without boron or boron only without Zn. This relationship is more clear in the plants grown on high $CaCO_3$ level where, zinc availability is severely restricted. This means that Zn absorption and translocation is strictly controlled by boron and most of zinc deficiency cases in the crops sensitive to boron deficiency like cotton are related to boron deficiency. Iron, Mn and Cu show similar trend, except Fe with the treatment (+B++Zn) in the plants grown on low $CaCO_3$ level and Cu in the plants grown on both $CaCO_3$ levels. This may related to the antagonistic effect of high zinc concentrations with both Fe and Cu (Adriano *et al.*, 1971). Potassium and calcium concentrations were also increased and nearly showed the same trend of the above mentioned nutrients (Fig. 3). It was also found that concentration of both elements respond negatively to the high Zn-concentrations in the tissues caused by the treatment (+B++Zn). This may support the suggestion of Goldbach *et al.* (2000) that due to the less negative charges on the cell wall caused by boron deficiency, a reduction in the portion of Ca bound to the cell wall occurred. Potassium concentration increase was also found by Cakmak *et al.* (1995) in the leaves of sunflower as a response of B re-supplementation which, was attributed to the reduction of K-leakage occurred in the absence of boron.

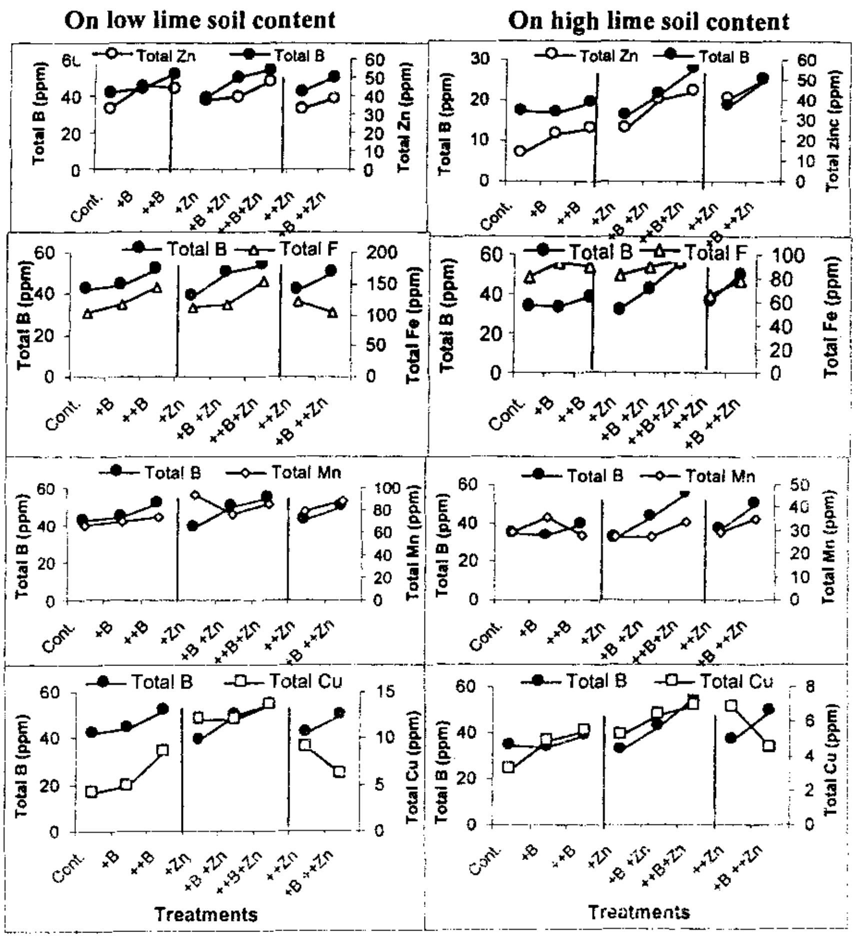

Fig. 2. Boron, zinc, iron, manganese and copper concentrations in cotton leaves as affected by foliar fertilization of boron, zinc and their combinations

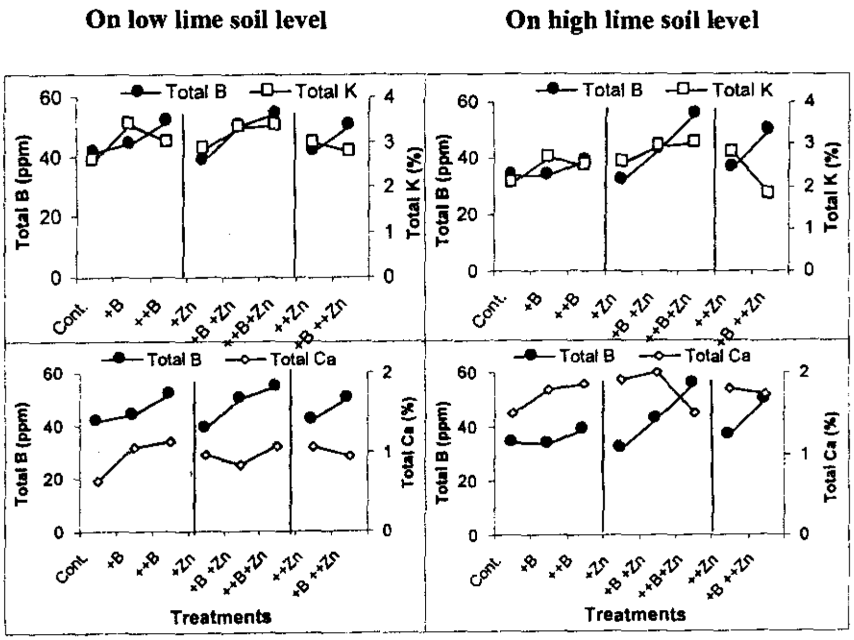

Fig. 3. Potassium and calcium concentrations in cotton leaves as affected by foliar fertilization of boron, zinc and their combinations.

Nutrient uptake

Uptake of nutrients by cotton shoots differed significantly according to the applied treatments (Fig. 4 & 5). The highest uptake of B, Zn, Cu, K and Ca by the shoots grown on low lime soil level was detected as response to the treatments (+B) or (+B+Zn). Meanwhile, the treatment (+B+Zn) was superior to increase nutrient uptake by the shoots grown on high lime soil level.

Acting on the membrane level, boron deficiency was reported to inhibit membrane uptake of number of nutrients (Blaster-Grill *et al.*, 1989 and Cakmak *et al.*, 1995) and affects nutrient transport capacity due to lowering ATPase (Lawrence *et al.*, 1995), NAD(P)H (Barr *et al.*, 1993) and ferricyanide-inducible reductase activity in the roots (Goldbach *et al.*, 1990 and Findeklee *et al.*, 1997). Thus, proper B- doses could improve their availability, translocation and physiological functions within the plant tissues to meet the proper requirements of the plant.

Nutrient balance

Boron could also control the assimilation of nutrients in the plant tissues. Boron/nutrient ratios as affected by boron, zinc and their combined treatments are shown in Table 2.

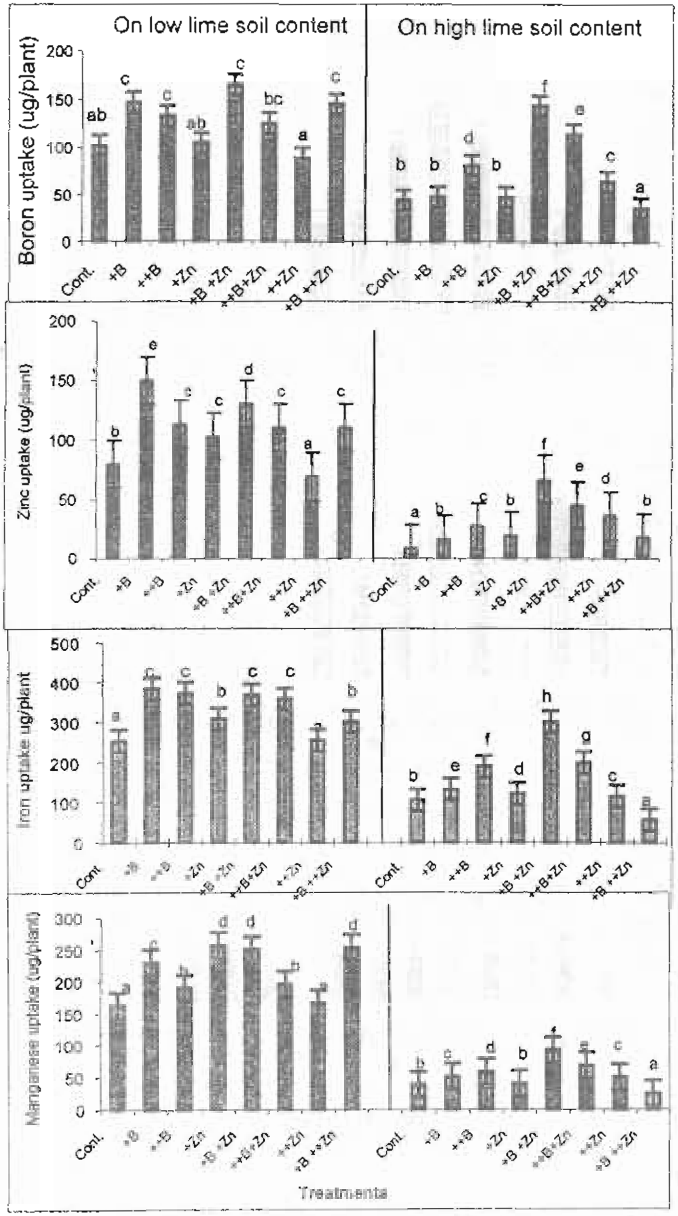

Fig. 4. Boron, Zn, Fe and Mn-uptake by shoots of cotton plants grown on low and high lime soil levels as affected by foliar application of boron, zinc and their combinations .

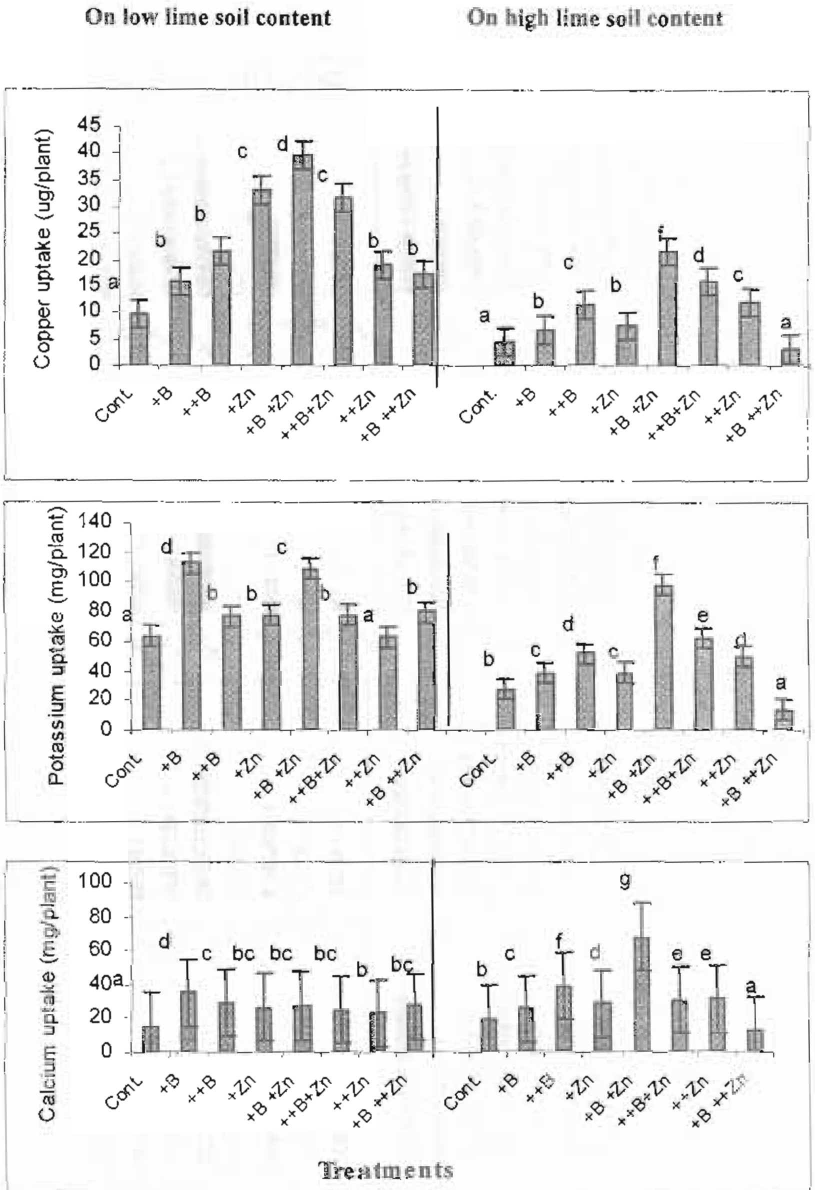

Fig . 5. Copper, K and Ca-uptake by shoots of cotton plants grown on low and high lime soil content as affected by foliar application of boron, zinc and their combinations (bars with same letters are not significantly different, P = 0.05) .

TABLE 2. Boron/nutrient ratios in cotton shoot tissues (31 days age) grown on low or high lime soil level as affected by foliar applications of boron, zinc and their combinations.

Treatments	Nutrient ratios in shoots of cotton plants grown on low lime soil level					
	B/Zn	B/Mn	B/Fe	B/Cu	K/B	Ca/B
Control	0.8	0.36	0.40	10.4	323	152
+B	1.0	0.64	0.38	9.2	766	228
++B	0.9	0.70	0.36	6.1	577	219
+ Zn	1.0	0.41	0.34	3.2	735	250
+B +Zn	0.8	0.66	0.43	4.2	660	166
++B+Zn	0.9	0.64	0.35	4.0	620	199
++Zn	0.8	0.53	0.34	4.7	707	256
+B++Zn	0.8	0.57	0.48	8.3	552	188
Treatments	Nutrient ratios in shoots of cotton plants grown on high lime soil level					
	B/Zn	B/Mn	B/Fe	B/Cu	K/B	Ca/B
Control	4.9	1.15	0.42	10.6	608	441
+B	2.9	0.93	0.36	7.0	791	531
++B	3.00	1.38	0.43	7.2	638	480
+ Zn	2.5	1.18	0.39	6.2	797	595
+B +Zn	2.1	1.55	0.48	6.7	685	496
++B+Zn	2.5	1.65	0.58	8.0	539	267
++Zn	1.8	1.27	0.56	5.4	760	489
+B++Zn	2.0	1.42	0.64	11.1	360	346

Boron/element concentration ratios that realized the highest biomass accumulation in a certain period may be considered as a base of good nutrient assimilation. Nutrient ratios which gave the highest dry weight of cotton shoots (31 days age) grown on low $CaCO_3$ level in the soil were obtained by the treatments (+B) and (+B+Zn). They were in the order of B/Zn = 0.8-1.0, B/Mn = 0.64-0.66, B/Fe = 0.38-0.43, B/Cu = 4.2-9.2, K/B = 660-766 and Ca/B = 166-228. These ratios are supposed to stimulate functions of cell wall synthesis, lignification, carbohydrate metabolism, RNA metabolism, IAA synthesis, phenol metabolism and membrane function (Loomis and Durst, 1991), which in turn, led to a good growth. Other treatments are supposed to disturb one or more of the above-mentioned processes that cause a growth limitation.

Nutrient ratios in the plant shoots grown on high lime soil content were higher than those calculated for the plants grown on low lime soil level. They were in the order of B/Zn = 2.1, B/Mn = 1.55, B/Fe = 0.48, B/Cu = 6.7, K/B = 685 and Ca/B = 496. Compared to the ratios calculated for those grown under low lime conditions in the soil, this indicates that under lime stress conditions, more boron percentage is required to maintain optimal cell wall and membrane physical properties. However, to senergize boron, zinc is essential and both elements are required in low doses (25 ppm B +50 ppm Zn in the spray solution) to achieve good growth.

An overview on the nutrient concentrations, uptake and balance in relation to boron, it is obvious that boron is directly or indirectly controlled their absorption and/or translocation which, suggests that boron plays a key role in controlling these processes for other nutrients.

Conclusions

From the present work, it can be concluded that:

-There is a synergistic relationship between boron and zinc absorption, translocation and physiological behavior in cotton tissues. More studies should be done to confirm this relationship in other plant species and discover its quality in different plants.

-Soil B-concentrations was estimated as moderate levels are not adequate for good growth of cotton plants.

-Foliar B-treatments could increase B, Zn and other nutrient uptake and concentrations in cotton shoots grown on soil which contains low or high lime level which, suggests that boron may plays a key role in controlling such processes in plant tissues.

-Nutrient ratios, which gave the highest biomass accumulation, can be considered as a base to achieve good growth.

-In low lime soil level, an additional foliar B-treatment in a dose as low as 25 ppm in the spray solution can improve plant nutrient status resulting in good growth. However, plants grown on high lime soil level, must be treated with a moderate doses of B and Zn to obtain best growth.

Acknowledgments: The authors wish to thank Prof. Dr. Heiner Goldbach, Head of Plant Nutrition Institute, Bonn University, Germany and his staff for sincere help during the course of the study. The first author is very grateful to the German Academic Exchange Service (DAAD) for financing his stay in Germany.

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(*Received* 30/11/2003;
accepted 26/10/2004)

العلاقة بين عنصرى البورون والزنك فى نباتات القطن النامية على مستوى منخفض أو مستوى مرتفع من كربونات الكالسيوم فى التربة

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استنبتت بذور نبات القطن فى أصص بلاستيكية على تربة تحتوى على تركيزين من كربونات الكالسيوم (٢ % ، ١٠ %) لدراسة العلاقة بين عنصرى البورون والزنك، وكذلك تأثير التغذية الورقية بالبورون أو الزنك أو مخلوط منهما على محتوى النباتات النامية من العناصر الأخرى. وجد أن نمو نباتات القطن قد تأثر معنويا بالتغذية الورقية بكل من العنصرين أو مخلوط منهما، وأن أقصى وزن جاف للنباتات النامية على تربة منخفضة المحتوى من كربونات الكالسيوم قد تحقق عندما استخدمت معاملة (٢٥ جزء فى المليون بورون فقط) أو (٢٥ جزء فى المليون بورن + ٥٠ جزء فى المليون زنك) ، بينما كانت المعاملة (٢٥ جزء فى المليون بورن + ٥٠ جزء فى المليون زنك) هى الأفضل بين كل المعاملات من حيث تراكم المادة الجافة فى النباتات النامية على التربة العالية المحتوى من كربونات الكالسيوم. ولقد أدت نفس المعاملة إلى زيادة تركيز ومعدل امتصاص العناصر المغذية الأخرى بالإضافة إلى البورون والزنك.

ولقد تبين من الدراسة أن هناك علاقة خاصة بين البورون والزنك من جهة وكذلك بين البورون والعناصر المغذية الأخرى من جهة ثانية تشمل التركيز والامتصاص وتوازن العناصر داخل أنسجة النبات، مما يحدو إلى اقتراح أن عنصر البورون قد يلعب دورا رئيسيا تجاه العناصر الأخرى بالنبات، وبناء على ذلك فأن نسب البورون إلى العناصر المغذية الأخرى والتى تؤدى إلى أفضل نمو وتراكم للمادة الجافة قد تصلح لأن تكون أساسا لتعديل نسب العناصر للحصول على نمو جيد للنباتات التى تنمو على مثل هذه التركيزات من كربونات الكالسيوم فى التربة.