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THE EFFECT OF APPLICATION OF PHYTOTOXIC LEVELS OF BORON ALONG WITH SULPHUR ON NUTRIENT CONTENT IN COTTON (G*ossypium hirsutum* L.*)*

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Abstract

This study was carried out determining the effect of phytotoxic boron along with sulphur fertilizers on nutrient content in cotton in the research field of the department of field crops at Dicle University between 2013 and 2014. In the trial designed in randomized complete split block, the effect of two sulphur doses (0-100 kg da^{-1}) and four boron doses (0, 2.5, 5 and 15 mg kg^{-1}) on nutrient concentrations of cotton leaves were examined. According to the results, 5 and 15 mg kg^{-1} of B treatments with and without sulphur did not cause any visible toxic symptoms in cotton. Moreover, the treatments resulted in uptake of N, P, K, Fe, Zn and Mn nutrients in sufficiency ranges whereas Ca, S, Mg, Cu and Mo concentrations were lower than required concentration ranges of these nutrients. B content of leaves was found in sufficient range in plots where phytotoxic level of B was used. The effect of S application over N, K, Ca, B, Mn and Mo content of leaves showed decrease of these nutrients' concentrations but an increase the concentrations of S, Mg, Fe, Zn and Cu. In addition to this, the content of Mo was the lowest among nutrients. In terms of B and S interaction, P concentrations showed inconsistent results during the study.

Key words: c*otton, sulphur, boron toxicity, nutrient content.*

INTRODUCTION

Sulphur (S) has an important role in synthesis of essential amino acids and chlorophyll and nitrogen fixation mechanism in leguminous plants (Kopáček et al., 2014). Also, S is either a component of molecules such as RNA, DNA, amino acids, proteins, sulfolipids, flavonoids, lipids, glucosinates, polysaccharides, and nucleotide or a factor used in sustaining hormonal activities.
Boron (B) is one of the most essential nutrients for plant growth and should be applied to plants sufficiently (Tanaka and Fujiware, 2008) and be available in soil throughout vegetation period of cotton (Bogiani et al., 2014).
B toxicity symptoms appear in crops in many arid regions where irrigation water or soil has excessive B content (Karabal et al., 2003). Besides, B toxicity may emerge through either fertilization or mining (Nable et al., 1997).
Although the tolerance of plants to boron toxicity is different, this mechanism is not fully understood yet. However, it was thought that boron tolerance of plants is related to limiting factors making B available for plant roots (Cord et al., 2010). Clay content, soil moisture, soil temperature, pH (Goldberg et al., 1997) and carbonates (Keren et al., 1985) are factors affecting B availability in soils. High soil pH along with clay minerals play important role in B adsorption from soil solution. When pH is below 7, boron is available in soil as $B(OH)_3$ (boric acid), which is not favored by clay minerals. As pH increases the boric acid concentration decreases and $B(OH)_4$ (borate anion) concentration increases resulting in reduction of B absorption by plants (Hu and Brown, 1997). Consequently, in order for B be available in soil and be taken up by plants pH should be slight acid or neutral (Herrera-Rodriguez et al., 2010)

Other than these factors, it is reported that adding calcium to soil reduces B toxicity (Tariq and Mott, 2007) and several authors reported that calcium fertilization reduced effects of boron toxicity in tomato (Singaram and Prabha, 1997) and in wheat (Turan et al., 2009).

Similar to these results a strong negative relationship also was reported between boron content of cotton and calcareous soils with high pH by Ahmed et al., 2013.

Few studies about B and S fertilization are present in cotton.

However, it is not well kown how phytotoxic levels of B affect cotton which is grown in clay soils with high calcium content. Therefore, in this study the effect of sulphur on sufficient and toxic levels of B in cotton (*Gossypium hirsutum* L.) was investigated.

In this respect, the effect of the boron and sulphur treatments and their interactions on macro and micronutrients of cotton leaves were examined.

MATERIALS AND METHODS

Soil sampling and analysis

The study was conducted in cotton (*Gossypium hirsutum* L.*)* during 2013 and 2014 at the experimental field of the Faculty of Agriculture, Dicle University, Diyarbakir, Turkey.

Composite soil samples (0-30 cm) were taken from the field at different locations in October, 2013. The samples were analyzed as described by Ryan et. al. (2001).

The SO_4-S analysis was performed by Fox et. al. (1964) while boron content of the soil was determined by hot water method of Dible et al. (1954), which is a modified method of Berger and Troug (1944).

The soil was clay, moderately alkaline (pH 7.8), non-saline (EC, 0.66 m S cm^{-1}), moderately calcareous ($CaCO_3$, 11.70 %) and low in organic matter (0.97 %).

The nutrient compositon of the soil was determined as follows: Olsen P (5.18 mg kg^{-1}), NH_4OAc-extractable K (723.67 mg kg^{1}), NH_4OAc-extractable Ca (18517.67 mg kg^{-1}), NH_4OAc-extractable Mg (1173.67 mg kg^{-1}), NH_4OAc-extractable Na (423 mg kg-1), DTPA-extractable (1.21 mg kg^{-1}), Fe DTPA-extractable Mn (2.74 mg kg^{-1}), DTPA-extractable Zn (0.78 mg kg^{-1}), DTPA-extractable Cu (1.30 mg kg^{-1}), SO_4-S (4.89 mg kg^{-1}) and hot water extractable-B (0.58 mg kg^{-1}). In order to observe pH changes, the soil pH was measured three times during the cultivation: at thinning, at first squaring and at first flowering.

Experimental design and growth conditions

Sulphur and boron doses were applied as treatments arranged in randomized complete split block design with four replications.

Main plots were treated with two doses of elemental S (0-100 kg S da^{-1}) whereas subplots were applied with four boron levels (0, 2.5, 5 and 15 B mg kg^{-1}) as disodium octaborate tetrahydrate ($Na_2B_8O_{13}\cdot4H_2O$, 20.9 % B).

All treatments were broadcast and incorporated prior to planting with basal fertilizer as 20-20-0 compound fertilizer (35 kg da^{-1}).

The incomplete nitrogen was completed using ammonium nitrate (33 %) as 30 kg da^{-1} before the first irrigation.

Furrow irrigation was adopted as irrigation method and the irrigation was applied when necessary. Plots were formed 10 m long with 5.6 m wide consisting of 8 rows with 70 cm spacing.

Overseeded plots were thinned to one plant with 20 cm spacing. Stonevielle 468 (ST 468) was used as the variety of seed cotton. The standard plant protection management practices together with agronomic practices were employed for the crop cultivation.

Determination of Macro and Micro Nutrients of the Leave Samples

Total nitrogen (N) levels of leave samples were analysed by Dumas method using elemental combustion system (Coshtech ecs 4010). Phosphorous (P), potassium (K), calcium (Ca), sulphur (S), magnesium (Mg), molybdenum (Mo), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and boron (B) concentrations of leave tissues were analyzed, as described by Halvin ve Soltanpour (1980), using ICP-OES (Varian 4.1.0).

Table 1. pH values by years

Uygulama	2013			2014		
	pH1	pH2	pH3	pH1	pH2	pH3
S	7.4	7.55	7.73	7.71	7.57	7.75
S	7.53	7.53	7.73	7.51	7.48	7.65
S	7.77	7.57	7.77	7.61	7.52	7.87
S	7.74	7.52	7.63	7.63	7.42	7.75
S+2.5 ppm B	7.43	7.59	7.75	7.64	7.44	7.65
S+2.5 ppm B	7.59	7.53	7.81	7.75	7.52	7.82
S+2.5 ppm B	7.74	7.51	7.82	7.68	7.52	7.84
S+2.5 ppm B	7.69	7.53	7.86	7.71	7.56	7.75
S+5 ppm B	7.37	7.61	7.65	7.77	7.52	7.68
S+5 ppm B	7.57	7.54	7.78	7.69	7.55	7.83
S+5 ppm B	7.68	7.53	8.11	7.64	7.54	7.94
S+5 ppm B	7.68	7.52	7.8	7.65	7.68	7.87
S+ 15 ppm B	7.6	7.51	7.73	7.62	7.53	7.86
S+ 15 ppm B	7.6	7.63	7.76	7.68	7.42	7.79
S+ 15 ppm B	7.73	7.57	7.87	7.64	7.45	7.87
S+ 15 ppm B	7.73	7.58	7.69	7.61	7.42	7.89
Control	7.58	7.65	7.85	7.6	7.8	8
Control	7.7	7.8	7.93	7.74	7.94	7.98
Control	7.75	7.72	8.1	7.66	7.86	7.94
Control	7.65	7.69	7.93	7.68	7.68	7.87
2.5 ppm B	7.62	7.75	8	7.79	7.86	8.03
2.5 ppm B	7.71	7.7	7.8	7.77	7.81	8.1
2.5 ppm B	7.63	7.74	8.09	7.65	7.74	8
2.5 ppm B	7.73	7.79	8.05	7.56	7.71	7.92
5 ppm B	7.63	7.75	7.99	7.67	7.85	7.99
5 ppm B	7.62	7.74	8.05	7.65	7.84	8.1
5 ppm B	7.83	7.78	8.06	7.69	7.75	7.86
5 ppm B	7.64	7.62	7.97	7.63	7.52	7.77
15 ppm B	7.6	7.7	8.07	7.61	7.67	8.05
15 ppm B	7.75	7.79	8.04	7.65	7.84	8
15 ppm B	7.77	7.73	8.03	7.64	7.75	8.03
15 ppm B	7.62	7.65	7.91	7.62	7.7	8.04

RESULTS AND DISCUSSIONS

pH values

The pH values of the plots with S are not much different from the plots without S at the first measurement.

It has been determined that the values obtained at the beginning of squaring in the S applied plots showed a noticable decrease compared to the plots without S.

This may be explained slow decompositon process of the elemental S into the soil.

On the other hand, the pH of the plots without S increases gradually (Table 1).

Nutrient Contents

First of all, no visible toxicity symptoms originating from any of B levels were observed. The effect of B and S applications on nutrient contents of cotton leaves were shown in Table 2 and Table 3. N, P, K, Ca and Cu concentrations of leaves showed inconsistencies depending on years and B application levels.

The concentrations of N, P, K, Ca and Cu were found significant only in 2013 at B4, B3, B2, B3 and B3 levels, respectively. As for S, Mg and B contents, they showed significant difference regarding B levels during both years. The highest nutrient concentrations of S, Mg and B were obtained at B4, B3 and B4 levels, respectively. Similarly, Fe, Zn, Mo and Mn concentrations significantly differed in 2013 and 2014.

Nonetheless the highest values of Fe and Mn were uneven in terms of B levels.

Conversely, Zn and Mo contents showed smooth results at B3 and at B3, respectively, by years.

The effect of S application on N, P and K content of leaves was found insignificant during 2013 and 2014. By contrast Ca, B, Mo,and Mn concentrations significantly differed and lower concentrations of these elements were determined depending on S application during both years.

Nevertheless, S, Mg and Fe contents of leaves significantly increased after S application in the years of the study.

As for Zn and Cu concentrations of cotton leaves, they were found significant only in 2013 showing inconsistency between years (Table 2 and Table 3).

Ahmed et al. (2008) and Turan et al., (2009) stated that B toxicity symptoms became apparent when 5 mg B kg^{-1} applied to soil in cotton and in wheat respectively.

Our results contradicted these results.

The lack of B toxicity symptoms in both with and without S plots may be explained with physical and chemical soil properties of experimental area.

Soils with high content of organic matter, oxide minerals, clay minerals, and carbonates play an important role in reduction of the phytotoxic effects of excessive B since these organic or mineral surfaces serve as sinks for B with their adsorption sites.

Once B is adsorbed by any mineral or organic surfaces, it is neither available nor toxic to plants. Therefore, soils with high clay content have enormous potential in attenuation of boron toxicity (Goldberg et al., 2005).

pH is another factor for B to be adsorbed onto organic and mineral surfaces (Goldberg and Forster, 1991).

The adsorption of B onto Al and Fe oxides and clay minerals such as kaolinite, montmorillonite and illite intensifies at low pH levels.

Nonetheless, while the sorption of B peaks in pH range 7-8 for Al and Fe oxides, the adsorption peak shifts to pH 8-10 for clays (Goldberg and Forster, 1991).

At these pH ranges, B forms primarily as borate anion and cannot be available to plants (Herrera-Rodriguez *et al*., 2010; Römheld and Marshner, 1991).

The pH level of the experimental field was moderately alkaline and in spite of S application the lowest pH was recorded as 7.37 in 2013 (Table 1).

However due to the buffering capacity of the soil, pH values returned to their initial state in the following weeks in which the adsorption of B may be the highest in this pH range.

Table 2. Nutrient content of cotton leaves by years and applications

	N (%)		P (mg kg^{-1})		K (mg kg^{-1})		Ca (mg kg^{-1})		S (mg kg^{-1})		Mg (mg kg^{-1})	
Applications/Years	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
B1 (0 mg kg^{-1})	3.85b	3.90	2744.50c	2703.75b	19158.13b	19396.25b	11324.63c	11425.88	2139.38c	2136.25c	2353.25a	2345.50a
B2 (2.5 mg kg^{-1})	3.80b	3.90	2883.75b	2963.75a	20993.88a	20351.63a	11906.00bc	12161.38	2311.75b	2277.88bc	2366.25a	2389.38a
B3 (5 mg kg^{-1})	3.78b	3.92	3006.63a	2948a	19539.13b	19726.75ab	12639a	12659.13	2297.75b	2390.13ab	2315.13a	2337.38a
B4 (15 mg kg^{-1})	4.13a	4.11	2710.00c	2741.63b	19139.00b	19168.38b	12148.88ab	12549.50	2517.88a	2520.38a	2248.13b	2187.75b
LSD	0.21*	-	99.03**	115.75	829.31**	715.69*	615.36**	1046.11	118.11**	157.29**	52.19**	92.64**
S1 (100 kg S da^{-1})	3.82	3.93	2837.44	2830.69	19539.56	19485.25	11516.44b	11803.75b	2516.06a	2537.94a	2378.44a	2349a
S2 (0 kg S da^{-1})	3.96	3.99	2835	2847.88	19875.50	19836.25	12492.81a	12594.19a	2117.31b	2124.38b	2262.94b	2281b
LSD	-	-	-	-	-	-	435.12**	739.71*	83.52**	111.22**	36.90**	65.51*
General Mean	3.89	3.96	2838.22	2839.28	196707.53	19660.75	12004.63	12198.97	2316.69	2331.16	2320.69	2315

Mean values with different letters in each column represent significant difference at the ** $P < 0.01$ and *$P < 0.05$ level based on LSD test.

Table 3. Nutrient content of cotton leaves by years and applications

	B (mg kg^{-1})		Fe (mg kg^{-1})		Zn (mg kg^{-1})		Mo (mg kg^{-1})		Mn (mg kg^{-1})		Cu (mg kg^{-1})	
Applications/Years	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
B1 (0 mg kg^{-1})	13.04c	13.40d	140.25c	139.75c	28.74b	29.04b	1.93b	2.09b	34.25b	33.80b	16.84b	15.85
B2 (2.5 mg kg^{-1})	15.87b	15.98c	156.00a	152.63ab	28.84b	28.72b	2.18a	2.26a	35.00b	37.06ab	18.23a	16.85
B3 (5 mg kg^{-1})	20.13a	18.47b	141.63c	141.75bc	31.73a	30.70a	2.12a	2.17ab	35.75b	37.45a	19.04a	17.62
B4 (15 mg kg^{-1})	20.51a	20.31a	149.63b	154.63a	28.28b	28.83b	2.23a	2.28a	38.25a	39.79a	16.13b	16.55
LSD	1.32**	0.83**	4.40**	8.39*	0.61**	1.02*	0.12**	0.09*	1.30**	2.53*	0.66**	-
S1 (100 kg S da^{-1})	13.33b	13.48b	158.56a	156.19a	30.11a	29.69	1.90b	1.95b	33.56b	34.23b	17.95a	17.08
S2 (0 kg S da^{-1})	21.45a	20.60a	135.19b	138.19b	28.69b	28.96	2.33a	2.45a	38.06a	39.82a	17.18b	16.35
LSD	1.87**	1.17**	6.23**	11.86**	0.86**	-	0.17**	0.12**	1.84**	3.58**	0.93*	-
General Mean	17.39	17.04	146.88	147.19	29.40	29.32	2.12	2.20	35.81	37.02	17.56	16.72

Mean values with different letters in each column represent significant difference at the ** $P < 0.01$ and *$P < 0.05$ level based on LSD test.

Calcareous soils are also important with regard to adsorption of B. The high ionic strength of soil solution, particularly with divalent ions, increases boron adsorption (Majidi et al., 2010). Clays saturated with calcium (Ca) are more prone to adsorb B compared to sodium (Na) saturated clays (Harter, 1991). Moreover, B retention by calcite in calcareous soils increases in low pH and adsorption is reversed after reaching peak level at 9.5. Since calcite removal decreases B retention in calcareous soils, calcite is conceded as a sink for B. Therefore, toxic effect of excess of B in calcareous soils may be abated by the presence of carbonates (Goldberg and Forster, 1991). The high calcium content along with clay soil of the experimental field may cause more adsorption of excessive B and prevent observation of B toxicity in the study.

Depending on clay mineralogy of soil, increasing soil temperature is another factor affecting adsorption and desorption of B. B adsorption increases between pH 3-7 range and peaks at 7.5-10 in all different clay types (Goldberg et al., 1993). The air temperature in the trial field increases from April to August and reaches its highest value within this month. Due to the increased air temperature, the soil temperature increases. The pH values of the plot without sulfur application showed a uniform increase in pH values for two study years. Therefore, this may have caused the high doses of applied B to be adsorbed to the soil.

Soil temperature along with soil moisture also affect diffusion of ions to plant roots causing different crop responses from one growing season to another (Schaff and Skogley, 1982). In this vein, in our study, pH decrease took place in soil samples taken after planting in sulphur treated plots but after a short period pH increased reaching 7.7-7.8 peak range. Nevertheless, in plots without sulfur pH constantly increased becoming stable at 7.8-8 range. This pH ranges along with increasing soil temperatures during cultivation period of cotton may bring about retention of B on oxide minerals and clays decreasing phytotoxic effect of high B treatments.

In terms of macro and micro nutrient contents of leaves, the evaluation was made with respect to sufficiency ranges reported by Mithchel and Baker (2000). According to this, the uptake of N, P, K, Fe, Zn and Mn were found within sufficiency ranges as response to B and S treatments. Ca, S, Mg and Cu content of leaves were sufficient depending on treatments. As for B concentrations, they were found sufficient in higher doses of B treatments. Mo content of leaves established very low in B and S treated plots. Furthermore, sulphur treatment decreased the concentrations of N, K, Ca, B, Mn and Mo. The uptake of S, Mg Fe, Zn, and Cu increased. As for P concentration, it did not show consistency as response to sulphur fertilization. In this study Mo concentration particularly was found very low, ranging from 1 to 2.45 mg kg^{-1}. This range was extremely low compared with that in study in which zero Mo treated sand resulted in obtaining 3 mg kg^{-1} Mo in cotton (Joham, 1953). As for Mn concentration values they were between 25-350 mg kg^{-1} which is accepted in sufficiency range (Dordas, 2009). Mn concentration levels observed in this study were within sufficiency range. Besides, Anderson and Boswell (1968) and Joham et al. (1967) reported that Mn content of cotton leaf tissues grown in sand was between 14-2000 mg kg^{-1} and above or below this range of Mg concentrations should be interpreted as deficiency or toxicity levels. Ahmed et al. (2008) found that different B rates decreased Ca, Mg, Mn, Zn and Fe contents of leaf tissues of cotton while they increased B, P, K and Cu concentrations significantly. Ahmed et al. (2011), reported that N, P, K, Cu, Fe Zn, and B constitution of cotton increased depending on different levels of B. However, Ca, Mg, and Mn concentrations of different parts of the cotton decreased. Turan et al. (2010) observed that application at 0, 1, 3 and 9 kg B ha^{-1} increased N, P, K, Mg, Fe and Mn concentrations of lucerne while it decreased Ca, Zn, and Cu concentrations. Razmjoo and Henderlong (1997) observed no important P, Ca, Mg content increase after S, K, B and Mo fertilization in alfalfa (*Medicago sativa* L.). Moreover, S application increased K content of alfalfa while B treatment did not change K levels significantly. Aires et al. (2007) suggested that S fertilization along with N significantly increased the uptake of N, S, K, Ca, Mg, Na, Cl and Si in broccoli sprouts

(*Brassica oleracea* var. *italica*). Salvagiotti et al. (2009) reported that the effect of S treatment on N uptake in wheat depended on the highest rate of N fertilizer. This can be explained by the interaction between S and N fertilizers Jamal et al. (2010) suggested that inadequate S fertilizer affects N metabolism negatively and vice versa. Rahman et al. (2011) observed that the application S at 5 t ha^{-1} together with N at 0.34 t ha^{-1} in maize (*Zea mays*) increased uptake of Fe, Mn and Zn in calcareous soil. Mamatha (2007) reported that application of 25 kg ha^{-1} Zn, S and Zn fertilizer in cotton resulted in highest uptake of N, P, K, S, Fe and Zn nutrients in clay soil.

CONCLUSIONS

Overall, 5 and 15 mg kg^{-1} of B treatments with and without sulphur did not cause any visible toxic symptoms in cotton. Therefore, toxic levels of B for clay soils with high Ca content should be determined. Also, a special caution should be taken for cotton cultivation in soils having insufficient Ca, S, Mg and Mo as regards B and S fertilizers.

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