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Interactive Effects of Boron and Humic Acid on the Growth and Nutrient Status of Maize Plant (*Zea mays* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Development of the methods decreasing boron (B) toxicity to agricultural crops is a high priority. The use of alternative organic material sources such as leonardite based humic substances (H.A.) could be used to control B balance in soils. For this aim, a pot experiment, based on a completely randomized design with three replications, was conducted using the soil of calcareous usthochrepts. In the research, maize variety of ADA-9510 (*Zea mays* L.), which was obtained from

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Central Anatolia Region, was used. In the experiment, leonardite based humic substance (12% humic acid) at the levels of 0, 60, 120 mg kg^{-1} were used as humic material source, which was developed by Turkish Coal Corporations Foundation. Boron fertilizer at the levels of 0, 10, 20 and 30 mg B kg^{-1} were used in the form of H_3BO_3. The plants were harvested after 56 days, and dry weights in top of maize plants were recorded. Macro and micro nutrient concentrations of the plants were also determined. Dry matter yields of maize plants were significanly affected by the applications of H.A. and B fertilizer, whereas dry matter yield was decreased by the application of higher B at the rate of 30 mg kg^{-1} without H.A application. Thus, results of this study clearly showed that there was a close sinergism between the H.A. and B applications with regard to B toxicity tolerance of maize plants. The maximum dry matter yields of 50.71 and 51.09 g pot^{-1} were obtained by the applications of 20 mg B kg^{-1} together with H.A. applications at the rates of 60 and 120 mg kg^{-1}, respectively. Depending on H.A. applications, B contents of maize plants varied between 32.18 and 35.02 mg kg^{-1}.

Keywords: Maize plant; humic acid; leonardite; boron fertilizer; boron toxicity.

1. INTRODUCTION

Humic substances, which are considered as essential parts of soil organic matter, are living materials arising from rotting of plant and animal products. It is estimated that the global extent of carbon, in soil organic matter is 3.0-3.5x10 tons. Seventy to eighty percent (70-80%) of carbon in soil is comprised of humic substances [1,2]. Starting materials of humic substances are decay products of lignin and vanillin [3]. Their fundamental structures are accepted as aliphatic compound complex macromolecules which form bonds with amino-acid, amino sugar, peptide and aromatic groups [4]. Humic substances arouse attention of health, food and agricultural sector due to their adsorption, absorption properties, their ion exchange capacity, redox property, diffraction or emulgator property, their nutritional value and other similar qualities [5,6,7,8,9]. Positive relations between, humic substances obtained from the sources of natural organic matter (Leonardite, turf etc.) and plant development are presented by various scientific researches [10,11,12].

Direct effects of humic compounds to plant development arise from their impacts on root development and nutrient element absorption metabolisms of plants and enriching of nutrient element availability [13,14,15]. Petit [1] has revealed that micro molecular structured fulvic and humic acids, which exist in the structure of humic substance, are absorbed easier by roots of plants and they increase nutrient element availability. Nardi et al. [10] have stated that humic compounds affects the development of plant positively with a few mechanisms, as well as they have positive effects on root and sucker development, they increase the availability of nutrient elements and they provide resistance towards different stress conditions. On the contrary, researches about the interactive relations between humic substance application in the cultivated areas of Turkey, and plant nutrient elements, are still insufficient and current scientific data, about plant development and fruit quality factors on the basis of different soil and plant types [16,17].

Recently considerable relation has been given to solve the boron (B) toxicity problems, especially in the dry regions of the World [18]. B deficiency or toxicity is a particularly widespread micronutrient problems in maize, leading to severe depressions in maize production and nutritional quality of the crop. Boron toxicity occurs mainly in dry areas, especially in alkaline soils. It has been demonstrated that B toxicity tolerance as well as drought tolerance are needed in dry areas having high levels of subsoil B [19]. When B is released from soil minerals, or is mineralized from organic matter, or is added to soils by means of irrigation or fertilization, part of it remains in the soil solution while part of it is adsorbed by the particles of soil [20]. The solubility of B in the soil solution is controlled mainly by B adsorption reactions. However, crop sensitivity to B deficiency or toxicity vary widely depending on some agro-physiological mechanisms together with soil and other environmental interactions affecting B availability and optimal plant growth. Thus there are a number of soil and environmental factors that affect B uptake by plants [21,22,23,24].

Hence, development of new methods to decrease B toxicity is a high priority on agricultural fields, where the soils have high levels of B. The strongest evidence that organic matter affects the availability of soil B is derived from studies that show a positive correlation between levels of soil organic matter and the amount of hot water soluble B [25]. The use of alternative organic material sources such as leonardite based humic substances could be used to control B toxicity and balance on these soils. The objective of this study was to evaluate the tolerance capacity to B toxicity of maize leaves (*Zea mays* L.) as affected by increasing boric acid treatments under the humic acid applications obtained from low calorie leonardite sources.

2. MATERIALS AND METHODS

In this study, a pot experiment, based on a completely randomized design with three replications, was conducted using the soil of calcareous usthochrepts. The air dried soils for pot experiment were secreened to pass through a 2 mm mesh. In the research, maize variety of ADA-9510 (*Zea mays* L.), which was obtained from Central Anatolia Region, were used. In the experiment, leonardite based humic substance (12% humic acid) developed by Turkish Coal Corporations Foundation was used at the levels of 0, 60, 120 mg kg^{-1} as humic material source. Boron fertilizer at the levels of 0, 10, 20 and 30 mg B kg^{-1} were used in the form of H_3BO_3. Phosphorous fertilizer was used at the level of 100 mg P kg^{-1} and applied in the H_3PO_4 form. Additionally, for normal plant growing, 100 mg N kg^{-1} ammonium nitrate were applied. According to need the other plant nutrient elements were also applied in equal amounts for each plant as fertilizer, irrigation and other controls were made routinely. The plants were harvested after 56 days, and dry weights in top of wheats were recorded. Plants were then washed thoroughly in distilled water and dried in the oven set at 68℃., and dry matter yields were recorded. Boron concentrations in the tops of maize plants were determined by the method of Azometin-H [26]. Total macro (N, P, K, Ca, Mg) and other micro nutrient (Fe, Cu, Zn, Mn) concentrations in the tops of maize plants were also determined by routine methods.

The composite soil samples used for the experiment were air-dried and ground to pass through a 2 mm sieve for further analysis. The extractable soil B contents were determined by ICP [27]. In the experimental soil, available P analysis was made by the method of Olsen et al. [28]. Determinations were also made for saturation percent [29]. $CaCO_3$ [30], pH [31], electrical conductivity (EC) [29] and organic matter contents [32] for both topsoil and subsoil samples. Some physical and chemical analysis results of the soil used in the study were presented. Silt, sand and clay contents were 26.24, 18.06 and 55.70, respectively. Average value of $CaCO_3$ was 87.9 g kg^{-1}, pH was 8.12, organic matter content was 1.69% and EC was 300 µmhos cm^{-1}. Available P_2O_5 and K_2O contents were 0.39 and 80 kg da^{-1}, respectively. Available B, Fe, Cu, Zn, Mn contents were 9.23, 16.03, 2.18, 0.21 and 5.93 mg kg^{-1}. The collected data were analyzed by using MSTAT program.

3. RESULTS AND DISCUSSION

3.1 Dry Matter Yield of Maize Plants

Humic acid applications together with boron fertilizer positively affected dry matter yield of maize plants (Table 1). It has been found that the effect of H.A. application to dry matter yield of maize was statistically significant (**$P < 0.01$). Effect of B fertilizer on the dry matter yield of maize plant was also statistically significant (**$P < 0.01$) (Table 1). By increasing H.A. dose up to 120 mg kg^{-1}, dry weights were significantly increased within each B treatment. The highest average dry matter yields of 47.03 gr pot^{-1} and 47.56 gr pot^{-1} were obtained at 60 mg kg^{-1} and 120 mg kg^{-1} H.A. applications, whereas the lowest yield of 20.11 gr pot^{-1} was obtained at control (without H.A. application). Positive effects of H.A. applications to plant development and dry matter amount have also been presented in some other researches [16,33,34].

By increasing of B fertilizer dose up to 20 mg kg^{-1}, dry weights were significantly increased within each H.A. treatment. However, after higher B dose of 30 mg kg^{-1}, dry matter yield of maize plant decreased. Our results are consistent with the earlier observations under different soil conditions. In that studies, dry matter yield of maize was increased by increasing B levels, but higher levels of B decreased dry matter yiled of maize [35]. Boron toxicity has also been report to affect various developmental processes in plants [23,36,37,38]. Thus, considering the narrow range between optimum and toxic B concentrations, it is necessary to be careful when applying B fertilizers to the soils [39,40].

Table 1. Effect of humic acid and boron applications on the dry matter yield of maize plant, gr pot^{-1}

B	H.A., mg kg^{-1}			Mean
mg kg^{-1}	0	60	120	
0	17.19 e	44.52 cd	43.45 cd	35.05 b
10	23.71 d	46.52 bc	48.52 abc	39.58 a
20	22.60 de	50.71 a	51.09 a	41.46 a
30	16.92 e	46.38 bc	47.19 abc	36.83 b
Mean	20.11 b	47.03 a	47.56 a	

*F test: Humic Acid (HA): 0.671**, Boron level: 0.228**, HA x Boron: 1.418**
**, ** Significant at the P < 0.05 and P < 0.01 level, respectively.*
Figures with identical letters within columns are not significantly different
Means with identical letters are not significantly different

On the other hand, effect of the interaction of H.A. and B on the dry matter yield of maize was found statistically significant (**P < 0.01). Dry matter yields of maize plants were increased by the applications of H.A. and B fertilizer, however, they were decreased by the application of higher B at the rate of 30 mg kg^{-1} without H.A application. Thus, the results of this study clearly showed that there was a close sinergism between the H.A. and B applications with regard to B toxicity tolerance of maize plants. The maximum dry matter yields of 50.71 and 51.09 g pot^{-1} were obtained by the applications of 20 mg B kg^{-1} together with H.A. applications at the rates of 60 and 120 mg kg^{-1}, respectively. The results showed that the H.A. treatments increased the available B contents particularly in the upper part of the soil profile, thus, H.A. enhanced the retention and availability of B in the soil and hence increased bioavailability of B for maize plants. The findings are consistent with Kaptan et al. [41]. H.A. positively affect the water-holding capacity, cation exchange capacity, fertilizer retention and microbial activity of the soil [42]. Moreover, H.A. increase root vitality and nutrient uptake and contribute to improvement of yields. Humic acid creates soluble or insoluble organic complexes with organic compounds, metals and minerals. Angin et al. [43] reported that H.A. additions can be an effective way to remediation of B, but their optimum performance depends on degree of soil B contents.

3.2 Macro Nutrient Contents of Maize Plants

Effect of H.A. applications on the N and P contents of maize plant were found statistically significant (**P < 0.01), whereas non-significant effect was found for K, Ca, Mg contents. Depending on H.A. applications, average N contents of maize plants significantly increased from 2.79% (at 0 mg H.A. kg^{-1}) to 3.67% (at 120 mg H.A. kg^{-1}). Humic based fertilizers and mineral contents are the excellent combination which provides the ideal environmental condition for plant growth and nutrient uptake capacity of plants. The use of H.A. is a promising natural resource to be utilized as an alternative for increasing crop production. Thus, in a similar study, statistical analysis of the data showed that H.A. application significantly affected the total N level in wheat stem and increased from 1.3% to 3.3% [44].

Phosphorus contents of maize plants also showed a significant increase depending on the H.A. applications (P < 0.01) (Table 2). Phosphorus contents of maize plants varied between 0.45 and 0.63% depending on H.A. applications from 0 to 120 mg kg^{-1}. The highest P content of 0.63% was obtained from H.A. treatment of 120 mg kg^{-1}. Many studies have revealed that the availability of P in fertilizer added to alkaline soil could be increased by the application of H.A. Humic acid decrease P fixation and provide more water soluble P for the plant, thus P uptake and content of plants increased by the presence of humic acids [45,46].

The K, Ca and Mg contents of maize plants varied, 2.41-2.50%, 0.78-0.87% and 0.48-0.54%, respectively depending on H.A. applications from 0 to 120 mg kg^{-1}. On the other hand, the effect of B application on the N, P, K, Ca and Mg contents of maize plant were found statistically non-significant. Nitrogen contents of maize plant varied from 3.19 to 3.38% depending on B treatments from 0 mg kg^{-1} to 30 mg kg^{-1}. Phosphorus contents of maize plant varied from 0.48-0.60% depending on B treatments from 0 mg kg^{-1} to 30 mg kg^{-1}. Potassium contents of maize plant varied from 2.37 to 2.54% depending on B treatments from 0 mg kg^{-1} to 30 mg kg^{-1}. Calcium contents of maize plant varied from 0.77 to 0.87% depending on B treatments from 0 mg kg^{-1} to 30 mg kg^{-1}. Magnesium contents of maize plant varied from 0.48 to 0.57% depending on B treatments.

3.3 Micro Nutrient Contents of Maize Plants

Effect of H.A. applications on the B and Zn contents of maize plant were found statistically significant (**$P < 0.01$), whereas non-significant effect was found for Fe, Cu, Mn contents (Tables 3-4). Depending on H.A. applications, B contents of maize plants significantly increased (Table 3). Depending on H.A. applications, B contents of maize plants varied between 35.03 mg kg^{-1} and 40.09 mg kg^{-1}, and by the applications of B they ranged between 33.61 mg kg^{-1} and 40.92 mg kg^{-1}. Humic substances enhanced solubilisation and availability of nutrients in the soils positively. Therefore, they increased B availability in the soils account for its a chelation effects [43,47]. Thus, high level B treatments showed a boron accumulation in the upper part of maize plants.

Table 2. Effect of humic acid and boron applications on macro nutrient status of maize plant

B	**H.A., mg kg^{-1}**			**Mean**
mg kg^{-1}	**0**	**60**	**120**	
		N, %		
0	2.72	3.24	3.62	3.19
10	2.98	3.56	3.55	3.36
20	2.66	3.69	3.78	3.38
30	2.81	3.21	3.71	3.24
Mean	2.79 b	3.43 ab	3.67 a	
		P, %		
0	0.47	0.58	0.62	0.56
10	0.31	0.59	0.55	0.48
20	0.49	0.62	0.69	0.60
30	0.52	0.60	0.64	0.59
Mean	0.45 b	0.60 a	0.63 a	
		K, %		
0	2.39	2.45	2.27	2.37
10	2.42	2.29	2.56	2.42
20	2.64	2.35	2.63	2.54
30	2.53	2.53	2.44	2.50
Mean	2.50	2.41	2.48	
		Ca, mg kg^{-1}		
0	0.97	0.69	0.95	0.87
10	0.64	0.88	0.80	0.77
20	0.73	1.02	0.67	0.81
30	0.76	0.90	0.78	0.81
Mean	0.78	0.87	0.80	
		Mg, mg kg^{-1}		
0	0.49	0.39	0.58	0.49
10	0.57	0.62	0.51	0.57
20	0.63	0.48	0.45	0.52
30	0.42	0.41	0.62	0.48
Mean	0.53	0.48	0.54	

Means with identical letters are not significantly different

Table 3. Effect of humic acid and boron applications on micro nutrient status of maize plant

B mg kg^{-1}	HA, mg kg^{-1} 0	60	120	Mean
		B, mg kg^{-1}		
0	32.18 c	33.62 c	35.02 bc	33.61 c
10	35.29 bc	40.05 b	36.29 bc	37.21 b
20	34.73 c	40.22 b	47.82 a	40.92 a
30	37.92 bc	39.72 b	41.26 ab	39.63 ab
Mean	35.03 b	38.40 ab	40.09 a	
		Fe, mg kg^{-1}		
0	79.28	96.28	99.96	91.84
10	63.50	84.43	87.42	78.45
20	96.73	103.00	100.85	100.19
30	84.83	85.32	78.13	82.76
Mean	81.09	92.26	91.59	
		Cu, mg kg^{-1}		
0	23.91	29.03	19.79	24.24
10	14.27	17.25	21.34	17.62
20	28.72	18.42	20.62	22.59
30	18.25	32.70	16.31	22.42
Mean	21.29	24.35	19.52	
		Zn, mg kg^{-1}		
0	56.17	61.52	74.28	63.99
10	49.63	85.85	65.59	67.02
20	60.19	68.11	67.72	65.34
30	57.63	56.72	63.20	59.18
Mean	55.91 b	68.05 a	67.69 a	
		Mn, mg kg^{-1}		
0	76.21	92.29	75.28	81.26
10	110.85	79.83	93.62	94.77
20	88.52	111.46	89.27	96.42
30	92.03	77.92	80.53	83.49
Mean	91.90	90.38	84.68	

Figures with identical letters within columns are not significantly different
Means with identical letters are not significantly different

Table 4. Combined variance analysis of the effect of humic acid and B applications on the macro and micro nutrient status of maize plant

	N	P	K	Ca	Mg	B	Fe	Cu	Zn	Mn
HA	**	**	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
B	n.s	n.s.	n.s	n.s	n.s	**	n.s	n.s	n.s.	n.s
HA x B	n.s	n.s.	n.s	n.s	n.s	*	n.s	n.s	n.s.	n.s

**, ** Significant at the $P < 0.05$ and $P < 0.01$ level, respectively. n.s.: non significant*

Humic acid application had also significant effect on the average Zn contents of maize plants. While Zn content was 55.91 mg kg^{-1} in control, it reached the levels of 68.05 mg kg^{-1} and 67.69 mg kg^{-1} depending on the increasing H.A. levels of 60 mg kg^{-1} and 120 mg kg^{-1}, respectively. The Fe, Cu and Mn contents of maize plants varied between 81.09-91.59 mg kg^{-1}, 19.52-24.35 mg kg^{-1} and 84.68-91.90 mg kg^{-1}, respectively depending on H.A. applications. A greenhouse study was conducted to determine the effects of low-rate commercial H.A. on zinc (Zn) availability of spring wheat yields, in both a calcareous soil and a non-calcareous soil. The results of that study also indicated that H.A. applications increased Zn content of plants due to increasing Zn availability in the soil depending on H.A. applications [46,47].

On the other hand, the effect of B applications on the B content of maize plants were found statistically significant (**$P < 0.01$), whereas non-significant effect was found for Fe, Cu, Zn, Mn contents (Tables 3-4). By increasing B doses, B contents of maize plants significantly increased (Table 3). Depending on B doses, B contents of maize plants increased from 33.61 mg kg^{-1} at control to 40.92 mg kg^{-1} and 39.63 mg kg^{-1} at the B levels of 20 and 30 mg kg^{-1}, respectively. The Fe, Cu and Mn contents of maize plants varied between 78.45-100.19 mg kg^{-1}, 17.62-24.24 mg kg^{-1} and 81.26-96.42 mg kg^{-1}, respectively depending on H.A. applications. In a similar study, increasing rates of B applied to maize plant increased B concentration of the plant in a calcareous soil under greenhouse conditions [48,49,50].

4. CONCLUSION

While B is an essential plant nutrient for the growth and development of plants, relatively small amounts of B are required to support the process of plant growth. The understanding of the effects of boron (B) toxicity and deficiency on the growth and nutrients accumulation of crops is required to substantiate the need of adequate dosage for their survival and optimal production. On the other hand, the use of alternative organic material sources such as leonardite based humic substances could be used to control the B toxicity and balance on these soils. Thus, crop sensitivity to B deficiency or toxicity will also vary widely depending on humic substance contents of soils together with many other environmental interactions affecting B availability and optimal plant growth. As a conclusion remark, results of this study clearly showed that there was a close sinergism between the H.A. and B applications with regard to B toxicity tolerance of maize plants. Dry matter yields of maize plants were significanly affected by the applications of H.A. and B fertilizer, whereas dry matter yield was decreased by the applications of higher B rates without H.A applications. The maximum dry matter yields of 50.71 and 51.09 g pot^{-1} were obtained by the applications of 20 mg B kg^{-1} together with H.A. applications at the rates of 60 and 120 mg kg^{-1}, respectively. Boron application together with H.A. application had also significant effect on the average B contents of maize plants The study have revealed that H.A. application to soils will lead to higher tolerance of maize plants to B toxicity. The results also showed that higher tolerance of maize plants to B toxicity under the application of H.A. could also be used in breeding programs in order to decrease detrimental effects of B toxicity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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