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Original Article

Determination of the Effect of Humic Acid on Growth and Development Parameters of Parsley (*Petroselinum sativum* Hoffm.) Grown in Boron Soil

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Abstract

Boron is an important micronutrient, required for all plant growth, and critical for high yield and quality of crops. The aim of the present research was to determine the effects of boron on pot-grown parsley (*Petroselinum sativum* Hoffm.). The experimental design consisted of four treatments using Hoagland-Arnon (1950) nutrient solutions with two different boron concentrations (B1 - 15 ppm and B2 - 150 ppm), each with and without 10 ml humic acid addition (HB1 and HB2), and controls with full strength Hoagland-Arnon solutions. Growth analyses of the parsley revealed that 15 ppm boron application caused an increase in root length leaf fresh and dry weight root fresh and dry weight and leaf area compared to control values. 150 ppm B (B2) concentration decreased all growth parameters compared to controls. The two humic acid treatments (HB1 and HB2) did not increase any of those growth parameters either in controls (C) or in the two boron (B1 and B2) concentrations. Analysis by (ICP-MS) revealed that B content in the leaves increased gradually in B1 and B2, as well as in both humic treatments where in HB2 it increased to 99.38% compared to B1. In the leaves, Mn, Zn and Fe contents behaved the same as B, increasing in all treatments, with the amounts in HB2 being significantly greater than in C, B1 and B2 leaves.

Keywords: boron; growth; humic acid; parsley; *Petroselinum sativum*; nutrient uptake

Introduction

Boron (B) is a non-metal element with uneven distribution in the earth's crust but widely found in both the lithosphere and hydrosphere, ranging from 5-10 mg kg^{-1} in rocks (Shorrocks, 1997), and 3-30 μg kg^{-1} in rivers (Power and Woods, 1997). It increases rock acidity and is associated with organic matter, showing elevated concentrations in some coals (Kabata-Pendias and Pendias, 1999).

In the soil, B combines chemically with oxygen to form several minerals, such as the tourmaline group containing hydroxides and silicates. B is readily soluble after weathering of rocks, producing common anions such as BO_{2-}, $B(OH)_4^-$, $B_4O_7^{2-}$ H_2BO^3, of which the last two occur in soil solutions at a pH above 7. B is likely retained by clay in soil solutions and its concentration is relatively high, between 67 and 3000 μg L^{-1} (Kabata-Pendias and Pendias, 2001).

Boron is an essential element for plants, where it is required as a micro-level nutrient. Its mobility is limited in phloem in some species whereas it can move freely in certain plants. For instance, B is immobile in clementine hybrid of mandarin (Papadakis *et al.*, 2004), however in fruits such as *Pyrus*, *Malus* and *Prunus* genera, which have high sorbitol content, B is mobile in phloem as a B-sorbitol complex molecule (Brown and Hu, 1996). Boron concentration in most soil types is higher under low precipitation or reduced irrigation, due to lack of leaching to deeper layers. At increasing concentrations in the soil, beyond that needed for normal growth, B becomes toxic to the plant (Marschner, 1995; Reid, 2010).

Boron can be absorbed by plants as boric acid (H_3BO_3) or borate anion ($B(OH)_4^-$) forms (Carter and Gregorich, 2008). The availability of B to plants is affected by the pH, structure, temperature and organic content (i.e. humus) of soil. pH has a key role in B absorption by plants, since B is present in the form of boric acid while soil is neutral or slightly acidic (Goldberg, 1997; Herrera-Rodriguez *et al.*, 2010).

Generally the mobility of boron is limited among plant organs. Boron has crucial roles such as sugar transport, inducing changes in structural and functional features of

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membranes, affecting metabolic pathways of carbohydrates, RNA and indoleacetic acid (IAA), for respiration and phenol metabolism. It is also involved in the formation and stabilization, as well as lignification, of cell walls (Loomis and Durst, 1991). Directly or indirectly, cell division, elongation and photosynthesis in leaves is affected by B concentration (Parvaiz and Prasad, 2012). Boron is associated with nodule formation in the root of fabaceae members, so affects nitrogen metabolism (Kacar *et al*., 2006). In plants, B concentration in tissues, and essential mineral nutrients, were affected by transpiration when reduced water transport occurred, resulting in toxic levels of B being reduced but, also resulting in nutrient deficiencies (Hu and Brown, 1997; Brown and Shelp, 1997).

The understanding of B toxicity is rather limited and fragmented although it is of considerable agronomic importance. The presence of excessive amounts of B is most common in arid and semiarid regions, which are more commonly affected by salt and alkaline soils compared to lands with B deficiency (Gupta *et al*., 1985; Gupta, 1993, 2006). It is rather difficult to improve land with excessive B concentration. One of the methods of improving soil highly contaminated with B is to grow plants resistant to B toxicity, and the other is to wash out the soil with water which has a very low level of B (Nable *et al*., 1997). The visible common symptoms of B toxicity are the appearance of chlorotic and/or necrotic spots on the tip or edges of older plants leaves (Wilcox, 1960; Nable *et al*., 1997; Lacey and Davies, 2009).

Humic substances are deposited in soil and sediments, being naturally formed by the decay of organisms such as plants, animals and microorganisms over millions of years. Leonardite is the most important organic source of humic acid. It formed in proximity to lignite beds but differs in that humic substances contain high oxygen, macro-and micro-nutrients (Tipping, 2002).

Humic substances are key components in making soil structure friable. In the soil, bacteria synthesize organic matters (eg. carbohydrates) and humic substances, leading to formation of a soil aggregate together with clay and silt. These formations help create an ideal crumb structure in the top layer of soil, making it friable which improves tilth and gives more porous openings (Hoffman *et al*., 1993).

Humic acids (HA) increase cation exchange capacity (CEC) and readily form salts with more than 60 different inorganic minerals available to plants (Pettit, 2004; Stevenson, 1994). The inorganics in the soil are bound to naturally occurring humic acids, which are important for ion exchange and making metal complexes (chelates) which are taken up by roots and through the cell membrane (Tipping, 2002; Kulikova *et al*., 2005; Akinci, 2011).

The available studies reveal that humic acids promote both plant growth and root development. For instance, in relation to root stimulation, length and dry weight of root increase in maize (Sharif *et al*., 2002; Eyheraguibel *et al*., 2008), in *Helianthus annuus* L. (Kolsarici *et al*., 2005), root dry weight increase in tomato and cucumber (Atiyeh *et al*., 2002); root development in ryegrass (Bidegain *et al*., 2000), root fresh and dry weight increase in tomato and eggplant (Dursun *et al*., 1999), and positive effect on tomato root fresh and dry weights (Adani *et al*., 1998).

Humic application positively affects mineral nutrient absorption by roots in some plants, such as enhanced uptake of nitrogen, phosphorus, K^+, Ca^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} and Fe^{3+} in maize (Eyheraguibel *et al*., 2008); similarly in maize Zn^{2+}, Fe^{3+}, Mn^{2+} and Cu^{2+} (Sharif *et al*., 2002); in ryegrass increased nitrogen, potassium, copper and manganese content (Bidegain *et al*., 2000). For tomatoes grown in hydroponic cultures prepared with peat- or leonardite-derived humic acids, at concentrations of 20 mg L^{-1} and 50 mg L^{-1}, the iron content in the roots increased by 113% and 123% respectively for peat-sourced humic acid, and by 135% and 161% for leonardite-derived humic treatment (Adani *et al*., 1998).

HA effects on plant growth have been reported by various researchers. Studies indicate that HA causes increased weights of above-ground parts of plants such as common wheat (*Triticum aestivum* L.) (Malik and Azam, 1985); sunflower (*Helianthus annuus* L.) (Kolsarici *et al*., 2005); strawberry (*Fragaria ananassa* v. Tribute); tomato (*Lycopersicon esculentum* v. Rutgers), marigold (*Tagetes patula* v. Antigua Gold F1), pepper (*Capsicum annuum grossum* v. King Arthur) (Arancon *et al*., 2003); corn (*Zea mays* L.) (Eyheraguibel *et al*., 2008; Tan and Nopamornbodi, 1979).

Humic acid caused increases in dry weights of leaf and stem of maize of 53% and 100% repectively (Eyheraguibel *et al*., 2008). The same study found that adding HA to maize seedlings increased stem length by 72.5%. In tomato plants, when HA and N, P, K fertilizers were applied together the number, fresh and dry weights of leaves as well as quantity and quality of fruits increased (Abdel-Mawgout *et al*., 2007). According to Ferrara *et al*. (2007) application of HA to grape enhanced stem length and enlarged fruit size and weight compared to controls. In sunflower (*Helianthus annuus* L.), HA treatment increased stem length, and fresh and dry weights of seedlings (Kolsarici *et al*., 2005). Sharif *et al*., (2002) stated that 50 and 100 mg/kg HA derived from lignites and applied with N, P, K caused 20% and 23% increases in maize stem weight grown in pots.

The effects of HA on mineral constituents of various plants has been reported by many scientists. For instance, Mackowiak *et al*. (2001) revealed that HA enhanced the uptake of Fe, Cu and Zn in the leaves of wheat (*Triticum aestivum* L.) grown in a hydroponic medium. In maize, HA and N, P, K application increased N content in the plant and soil, and the uptake of micronutrients such as Zn, Fe, Mn and Cu in seedlings grown in a pot experiment (Sharif *et al*., 2002). Bidegain *et al*. (2000) reported that using poplar sawdust as an HA source for ryegrass increased P content in the leaves, with an accompanying increase in leaf dry weight and enhanced uptake of N, K, Cu and Mn. Different concentrations of HA application to tomato and egg plant caused increased N, P, K, Na, Mg, Ca, Mn, Fe, Zn and Cu content in the leaves compared to control plants (Dursun *et al*., 1999). In tomato (*Lycopersicom esculentum* L.) grown in hydroponic medium and treated with HA prepared from peat or leonardite, Adani *et al*. (1998) found that the former sourced HA caused increased N, P, Fe and Cu and the latter increased N, P and Fe uptake. The most appreciable nutrient was Fe, in which concentrations of 20 mg L^{-1} and 50 mg L^{-1} of the hydroponics peat humic increased Fe by 41% and 33% and leonardite caused increases of 31% and 46% compared to controls respectively. Another study with the tomato revealed that 1280 mg/l of HA enhanced the contents of P, K, Ca, Mn, Fe, Mg and Z in the stem of tomato seedlings significantly (David *et al*., 1994). Fagbenro and Agboola (1993) stated that using three different HA concentrations on teak (*Tectona grandis* L.) increased uptake of the nutrients P, K, Mg, Ca, Zn, Fe and Cu, but decreased Mn in the seedlings grown in growth room conditions.

Parsley (*Petroselinum sativum* Hoffm.) is a very common and popular aromatic culinary herb and is cultivated in many parts of the world. It is a biennial plant belonging to the Apiaceae family. In Turkey, approximately 40% of commercially produced parsley, or 58.190 tonnes, was grown in Hatay province during the 2016 season (BUGEM, 2017).

The objectives of the present study were to assess the effect of HA on parsley under two different boron concentrations and to determine the uptake of essential nutrients (Na^{+}, K^{+} Fe^{3+}, Zn^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+} and B^{3+}) in the leaves of plants grown in pot experiments during 2013-2014.

Materials and Methods

In the experiment, seeds of parsley (*Petroselinum sativum* Hoffm.) 'Giant of Italy' cultivar, which has strongly aromatic leaves and prefers to grow in high moisture soil and a warm climate, were obtained from Vilmorin trade mark by Anadolu seed production and marketing company. The liquid Humic (BIOTOTAL is a trade product of Genta LTD, Turkey) which was used in the present study, contains organic, humic, fulvic and water soluble K_2O ratios as given in Table 1.

Growing conditions

The seeds of parsley were planted in plastic pots containing a 3:1 mixture of torf (GARDOL) and perlite (Taşper Perlit San.Tic. Ltd. Şti. - www.tasper.com.tr). After a month, the germinated plantlets were transferred into plastic pots of 10 cm diameter and 8 cm height, each containing 130 g of the same mixture of growing media. The experimental sets were prepared in five blocks each of seven replicates with four individual plants. These were arranged in a completely randomized block design with seven replicates for each of five treatments as follows: controls (C) containing only Hoagland-Arnon (1950); two boron concentrations (15 and 150 ppm, denoted B1 and B2 respectively); and two treatments with the same two boron concentrations plus 10 mL humic acid (HB1 and HB2) (Table 2). Following transfer to pots, the plants were watered with Hoagland alone for two weeks, after which the experimental solutions were applied for a three-week experimental period (Fig. 1).

Table 1. Humic acid components used

Components	Volume (%w/w)
Total organic substances	15
Total HA+fulvic acid	15
Water-soluble K_2O	3
pH	8-10

The pots with a seedling in each were set up as blocks using a completely randomised method (Mead and Curnow, 1983) at 23±2 °C. The moisture level of the growth medium was maintained at 55% ± 5 and sets were exposed to 4000-4200 lux plant fluorescence intensity for 14 h/10 h day and night periods respectively (Akinci *et al*., 2009). Growth parameters at the harvesting point, 55 days after transplanting, were determined by measuring plant height (PH), root length (RL), leaf area (LA), average leaf area (ALA); fresh weight of leaves (LFW), stalk (SFW), and roots (RFW), with dry weights of leaves (LDW), stalk (SDW) and roots (RDW) evaluated after 24 hours oven drying at 80 °C to fully remove tissue water.

Nutrient analysis

The oven-dried leaves of parsley were weighed and then crushed using a mortar and pestle to powderise them according to the method of Kacar (1972). The powder was transferred into an Erlenmeyer flask and 6 ml nitric acid + perchloric acid was added to it. The acid mixture was put in a water bath at 40 °C for 30 minutes to digest. A 1 ml extract was obtained after the supernatant was removed by heating at 150-180 °C on a hot plate.

Fig. 1. Parsley treatments in plastic pots at harvesting time. K: Control, B1: 15 ppm boron, B2: 150 ppm boron, HB1: 15 ppm B+ humic acid, HB2: 150 ppm B+ humic acid

Table 2. Boron and humic acid used

Experimental groups	Contents and preparations
Control (C)	Full strength Hoagland solution (Hoagland-Arnon, 1950)
15 ppm B (B1)	2ml B is dissolved in Hoagland solution and volume made up 1 L
150 ppm B (B2)	20 ml B is dissolved in Hoagland solution and volume made up 1 L
15 ppm B+ Humic acid (HB1)	2 ml B and 10 ml HA are dissolved in Hoagland and volume made up 1 L
150 ppm B +Humic acid (HB2)	20 ml B and 10 ml HA are dissolved in Hoagland and volume made up 1 L

The residue was made up to 100 ml with distilled water and kept in coloured bottles. Na and K were determined by flame photometer (Jenway PEP7) and Fe, Zn, Ca, Mn, Mg, and B by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (7500A Agilent Technologies) at the Department of Environmental Engineering, Faculty of Engineering, in Marmara University (Fig. 2).

Statistical analysis

The data from growth parameters and nutrient analyses were subjected to NCSS (Statistical software program, V9 for Windows) for paired-sample T-test. Terms were considered to be significant at the level of $P < 0.05$ (Duncan's Multiple-Comparison Test to determine significance of differences between means). Means are indicated with standard error (± s.e.) in tables and graphs as error bars.

Results and Discussion

Effect of Boron and HA on growth of parsley seedlings

The growth parameter results from the present study, for two levels of B, controls (Hoagland), and humic acid treatments, are presented in Table 3.

B application on parsley caused gradual reduction in plant heights with increasing concentrations (15 and 150 ppm B), by 2.66% and 15.34% in B1 and B2 respectively. Both decreases were significant compared to controls at 95% confidence level. HA addition to boron treatment caused a further reduction plant height, of 19.92% in HB1 and 18.86% in HB2 compared with controls. The decreases in both HB1 and HB2 were significantly greater than those in B1 and B2 at 17.73% and 4.15% respectively (α=0.05). The application of 15 ppm B seemed not to have a harmful effect on parsley, however the ten-fold increase in B concentration in the B2 treatment caused a significant inhibition of growth. The result from B2 agrees with other reports indicating reduction in growth of grain and straw yield in barley and wheat caused by toxic levels of B (Gupta, 1983; Cartwright *et al*., 1983; Yau and Saxena, 1997). Nable *et al*. (1990) also stated that plants exposed to high B levels typically show reduced growth of shoots and roots.

There were no positive affect of HA application mitigating B toxicity either in B1 or in B2 plant heights. There are no reports of HA effects on B toxicity to parsley, however it is known that soil containing humus can increase B uptake by plant roots (Goldberg, 1997).

Table 3. The effect of different treatments on growth parameters in Parsley

Parameters	Treatments				
	C	**B1**	**B2**	**HB1**	**HB2**
Plant height PH					
(cm) s.e	15.025 ± 0.467	14.625 ± 0.446	12.719 ± 0.428*/**	12.031 ± 0.355*/**	12.194 ± 0.548*/**
Root length (RL)					
(cm) s.e	13.294 ± 0.616	15.013 ± 0.404*	12.713 ± 0.598**	12.244 ± 0.499**	10.906 ± 0.703*/**
Leaves fresh weight (LFW)					
(g) s.e	1.293 ± 0.068	1.508 ± 0.067*	1.003 ± 0.048*/**	0.880 ± 0.032*/**	0.705 ± 0.089*/**/***
Leaves dry weight (LDW)					
(g) s.e	0.184 ± 0.008	0.218 ± 0.014*	0.156 ± 0.00**	0.126 ± 0.010*/**	0.104 ± 0.012*/**/***
Stalk fresh weight (SFW)					
(g) s.e	1.687 ± 0.185	1.590 ± 0.124	1.233 ± 0.075*/**	1.000 ± 0.058*/**	0.883 ± 0.099*/**
Stalk dry weight (SDW)					
(g) s.e	1.180 ± 0.019	0.180 ± 0.013	0.138 ± 0.005**	0.101 ± 0.005*/**/***	0.098 ± 0.014
Root fresh weight (RFW)					
(g) s.e	0.753 ± 0.139	0.893 ± 0.150	0.725 ± 0.047	0.505 ± 0.040**	0.325 ± 0.063*/**/***
Root dry weight (RDW)					
(g) s.e	0.212 ± 0.036	0.234 ± 0.049	0.146 ± 0.007**	0.102 ± 0.013**	0.057 ± 0.013*/**
Leaf area (LA)					
(mm^2) s.e	24611 ± 1715	28770 ± 1230	18348 ± 1666*/**	17632 ± 793*/**	14875 ± 1913*/**
Average leaf area (ALA)					
(mm^2) s.e	1253.5 ± 6.330	1285.0 ± 76.314	1001.0 ± 41.795**	912.0 ± 35.649*/**	801.5 ± 28.324*/**

C: Control, B1:15 ppm boron, B2:150 ppm boron, HB1: 15 ppm boron+ humic acid, HB2: 150 ppm boron+humic acid. *: Significantly different from C; **: Significantly different from B1;***: Significantly different from B2

The mean of root length (RL) increased (12.94%) significantly in the B1 treatment compared to controls at the 95% confidence level. Contrary to this, in B2 and the two humic acid treated samples, RL significantly decreased. The decrease in both HA (HB1 and HB2) was 18.45% and 14.16% respectively compared to B1 and B2, significant at the level of α= 0.05. Like PH, RL was affected under high concentration of B, and HA did not reduce the harmful effect of this toxic level of B. 15 ppm B application increased RL significantly, (12.94%) indicating this concentration may stimulate plant root development. Although known that HA promotes plant root growth, HA was not effective in the presence of B.

The present experiment produced LFW of 1.508 g in B1 and 1.003 g in B2. The increase (16.62%) in B1 was significant to compared to controls, but in contrast LFW decreased significantly in B2 (22.42%) compared to C and B1. The effects on dry weights of leaves were similar to those for fresh weights, with an increase in B1 (18.47%) but decrease in B2 (15.21%) with both changes significant compared to C values. HA treatment did not moderate the B-induced reduction in leaf fresh and dry weights of parsley. The data obtained from B1 (15ppm) showed that the concentration of B absorbed by roots was less than that causing any toxic effect on leaf growth and development. The toxic level was reached by 150 ppm B treatment, in which the LFW and LDW decreased significantly compared to C and B1. The toxic level effect in leaves did not decline after HA treatments in HB1 and HB2 in which both fresh and dry weight of leaves continued to be lower.

Leaf stalk fresh weight (SFW) slightly decreased (5.4%) in B1 although there was no change in dry weight (SDW) in this treatment. In the B2 treatment, significant reductions were found for SFW and SDW (26.97% and 23.33% respectively), the former significantly less than C and B1 but the latter different only from B1. The reductions were maintained in both HA applications; the decrease in HB1 was 37.10% in SFW and 43.88% in SDW, both significant compared to B1 treatments. Fresh and dry weights in HB2 application were 28.32% and 28.98% less than B2 respectively, but neither of these was not significant.

Although the toxic concentration of B varies across plant species, the B concentration causing growth inhibition in B2 treatments of parsley seedlings agreed with studies of chickpea (*Cicer arietinum* L.) (Bayrak *et al*., 2005), common bean (*Phaseolus vulgaris* L.) (Hamurcu and Gezgin, 2007); grain and straw yield of barley and wheat (Gupta, 1983; Cartwright *et al*., 1983; Yau and Saxena, 1997), and cotton (*Gossypium hirsutum* L.) (Ahmed *et al*., 2008).

The root fresh and dry weights behaved like the other parameters, increasing in B1 then decreasing in the other treatments. The root fresh and dry weight increases were 18.59% and 10.37% respectively in B1 compared to controls. In B2, both fresh and dry weights reduced by 3.71% and 31.13% with the latter (RDW) decrease being significant compared to B1. This finding agrees with the report by Nable *et al*. (1990) that a reduction in root growth of plants exposed to high B levels is typical.

Humic acid treatments did not induce any recovery after loss of root weight from boron treatments. For HB1 and HB2 fresh and dry weights significantly decreased compared to B1 roots. The decrease of fresh weights in HB1 was 43.46%, and in HB2 55.17%, significant reductions compared to B1 and B2 respectively. Greater reductions were found for dry weights, at 56.14% and 60.95% respectively for HB1 and HB2, with the former significant compared to B1.

The mean leaf area increased (16.89%) in the B1 treatment compared to controls but the increase was not significant at the 95% confidence level. Contrary to this, the LA in B2 decreased by 25.44% and was significantly different from C and B1. On the other hand, like for the other growth parameters, HA did not affect leaf area enlargement, and both HB1 and HB2 decreased by 38.71% and 18.92% respectively. The reductions in those were significant compared to controls and B1 treatments.

Average leaf area (ALA) was determined by the average of replicates in pots divided by the number of leaves of those of plants. The ALA figures were similar to LA where B1 had a slight increase (2.51%). The decrease of ALA in B2 was 20.14% compared to C, however the reduction was only significantly different from B1 at the 95% level of confidence. The decrease in HB1 (29.02%) rather than in B1, and in HB2 (19.93%) differed from B2, with the changes in both HB1 and HB2 significant compared to both C and B1 treatment values. Regarding boron effect on plants, some reports state that reduced growth of various parts of plants, such as roots and shoots, is a typical signal of plants exposed to high B levels (Nable *et al*., 1990). Reid *et al*. (2004) stated that the cause of inhibition of growth is mostly related to toxicity of boron accumulating in mature tissues, causing retardation of many cellular processes which is enhance in light by photo-oxidative stress.

There is no existing data on the effect of HA on boron toxicity, although there are some published studies reporting effects of B alone on plant growth.

The overall finding on growth parameters in B1 and B2 indicating that concentrations of B over 15 ppm are harmful to parsley plantlets agrees with the findings by Eaton (1944) who stated that more than 15 ppm B content had a toxic effect on parsley.

Effect of B and HA on nutrient content of parsley

The concentration of selected macro and micro nutrients, namely boron, magnesium, calcium, manganese, iron, zinc, sodium and potassium, were determined in parsley plants grown in C, B1 and B2 solutions with either 15 ppm or 150 ppm boron, with and without added HA. The results from the present study of the nutrient concentrations among the treatments determined at the harvesting stage are presented in Fig. 1.

Boron

In the present investigation, B concentration decreased in B1 and B2 applications by 50.59% and 67.56% respectively compared to controls, but B2 had a higher B content (33.54%) than in B1 (Fig. 2.a). This result does not agree with the findings of other studies that B content mostly increases in plants in correlation with increased B concentration in soil (Eaton, 1944; Jame *et al*., 1982). Boron availability to plants can occur in two ways: passive and active absorption. According to Brown *et al*. (2002) and Tanaka and Fujiwara (2007), in the passive process boric acid absorption by roots takes place where B is available in adequate or excessive quantity and B passively diffuses across the lipid bilayer. In contrast, active boron uptake by roots occurs under low B conditions (Dannel *et al.*, 2000 and 2002; Stangoulis *et al.*, 2001). It is difficult to evaluate from the present study whether boron was absorbed actively or passively. Although B content was slightly higher in B2 than B1 this was probably a result of the passive absorption mechanism (Brown *et al.*, 2002); Tanaka and Fujiwara (2007)) since B concentration in B1 seemed to be adequate and the B2 concentration was excessive for parsley growth.

The solutions with humic acid, which were prepared by adding 15 ppm and 150 ppm B in Hoagland, caused increases in the B concentrations in HB1 (81.86%) and HB2 (100.87%) compared to B1 and B2 respectively (Fig. 2.a). The increase in HB2 was significant at the level of 95% confidence. This result from the present study of a positive effect of HA in parsley leaves supports the report indicating that soil humus level may be linked with higher B absorption by roots (Goldberg, 1997).

Sodium-Potassium

Sodium content changes in parsley leaves in the treatments were similar to the behaviour of K, although the percentage of Na was lower than K by around 50% (Fig. 2.c). The content of Na varied in all treatments but was highest in B1. Na increased by 7.35% in B1 and decreased by 17.63% in B2 compared to controls, of which the latter was significant. Na accumulation decreased in both HA treatments. In HB1, Na reduced by 14.53% without significance, but the reduction in HB2 (1.63%) was significantly different from B1. It is known that a high amount of K uptake may cause increased B-deficiency symptoms (Fageria *et al.*, 2011).

The proportionally higher amount of K compared to Na content was greatest in B1, at 19.54%, with values for the other treatments, B2, HB1 and HB2, at 3.67%, 21.74% and 4.62% respectively (Fig. 2.b). Increase of K accumulation in B1 treatment may be a result of B-K synergistic relationship (Gezgin and Hamurcu, 2006), where B accumulation shows a reduction while K absorption reaches a maximum level (Cikili *et al.*, 2013).

Application of HA caused K in HB1 to differ significantly from B1 at the level of 95% confidence level. At the same significant level, the K content decreased by 4.62% in HB2 compared to B1. Previous studies revealed that HA application caused accumulation of K in some plants (Karaman *et al.*, 2012; Mohamed, 2012) but sometimes it may reduce K uptake (Cimrin *et al.*, 2001). The results obtained from the present study agreed with research stating that HA increased K content, particularly the significant difference between HB1 and B1.

Calcium

Ca content in the leaves of parsley was found to be between 828 mg/g and 1296 mg/g dry weight (Fig. 2.d). Ca accumulation slightly reduced in B1 but it increased in other treatments in the order B2, HB1, HB2. The increased Ca concentration in B2 was only by 3.37% compared with controls and the difference was not significant. Ca accumulation in 150 ppm boron treated seedlings (B2) increased by 4.07% without any significance. The HA

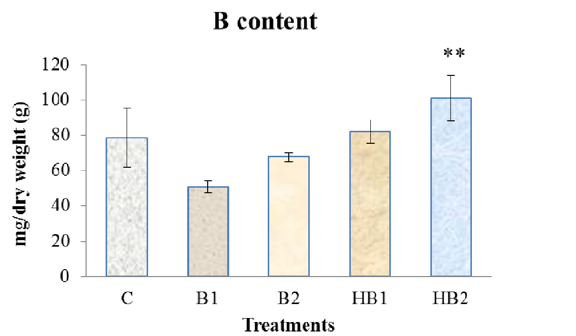

Fig. 2a. Nutrient contents in parsley (*Petroselinum sativum* Hoffm) leaves under treatments of 15ppm boron (B1), 150 ppm boron (B2), 15 ppm boron +humic acid (HB1), 150 ppm + humic acid (HB2) and Hoagland (C). *: Significantly different from C; **: Significantly different from B1; ***: Significantly different from B2

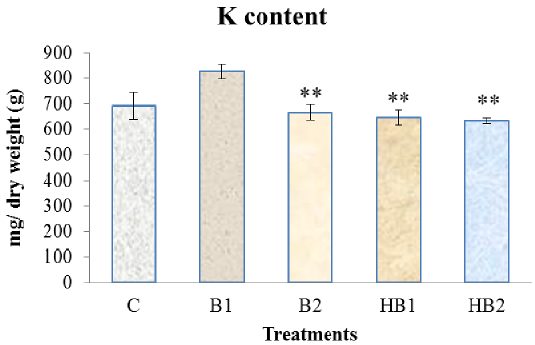

Fig. 2b. Nutrient contents in parsley (*Petroselinum sativum* Hoffm) leaves under treatments of 15ppm boron (B1), 150 ppm boron (B2), 15 ppm boron +humic acid (HB1), 150 ppm + humic acid (HB2) and Hoagland (C). *: Significantly different from C; **: Significantly different from B1; ***: Significantly different from B2

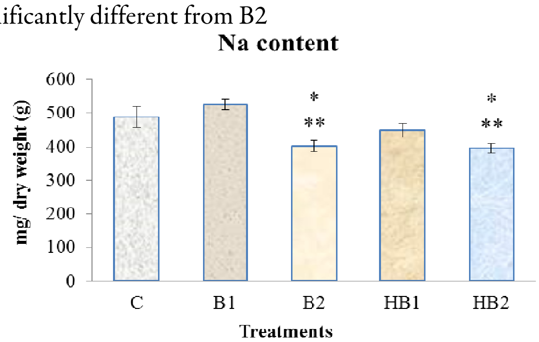

Fig. 2c. Nutrient contents in parsley (*Petroselinum sativum* Hoffm) leaves under treatments of 15ppm boron (B1), 150 ppm boron (B2), 15 ppm boron +humic acid (HB1), 150 ppm + humic acid (HB2) and Hoagland (C). *: Significantly different from C; **: Significantly different from B1; ***: Significantly different from B2

treatments caused increases of 17.00% in HB1 and 45.12% in HB2, however neither was significant at the 95% confidence level. The accumulation of Ca has an antagonistic relationship with B (Gezgin and Hamurcu, 2006), and it is known that high levels of calcium increase B deficiency symptoms. A previous report indicated that B uptake had a negative effect on Ca absorption (Kord *et al.*, 2010), nevertheless the data obtained from the present study agreed with the study finding that HA increased Ca content (Savasturk, 2008). On the other hand, another study found that HA caused a negative effect on Ca uptake on maize seedlings (Cimrin *et al*., 2001).

Magnesium

Magnesium content accumulation was small, and similar to Mn, among the nutrients analysed by the present study. This ratio may be related to high levels of Potassium which reduce Mg uptake (Fageria *et al*., 2011).

The concentration of Mg increased in B1 treatment which has risen up to 111.11% where Mg content increased by only 23.33% compared to controls but neither change was significant at the 95 confidence level (Fig. 2.e). In terms of HA application, Mg content decreased from 114.0 mg/g in B1 to 76.7 mg/g in HB1, a reduction of 32.71% which was not significant. In HB2, the amount of Mg increased 31.98% compared to B2 treatments but this change was also not significant. Since there is an antagonistic relationship between B and Mg (Gezgin and Hamurcu, 2006), the increased B concentration (150 ppm) caused a reduction in uptake of Mg. With HA addition to B and Hoagland solutions, the positive changes in Mg in both HB1 and HB2 compared to controls supports previous reports by Savasturk, (2008); Karaman *et al*., 2012).

Manganese

Mn concentration changes appeared the same as for iron in all treatments of parsley leaves. Mn content decreased by 18.85% in B1 while it increased in B2 by 11.42% compared to controls but the differences were not significant (Fig. 2.f). HA addition affected Mn absorption significantly (α= 0.005) in HB1 (71.43%) and 55.02% in HB2 compared to B1 and B2 respectively. Although there was no clear relationship between available Mn-B uptake from the soil by plants through synergistic or antagonistic ways (Gezgin and Hamurcu, 2006), the results from the present study agreed with reports by Karaman *et al*., (2012) which underlined that HA causes increased Mn uptake.

Iron

Fe content in parsley leaves was one of highest along with Ca, K, Na. Iron accumulated in parsley leaves in B2 and both humic treatments (i.e. HB1 and HB2) compared to controls (Fig. 2.g). Fe concentration showed a slight decrease in B1 (11.83%) though this was not statistically significant, but increased by 24.79% in B2 compared to leaves in controls. HA increased Fe concentration in both HB1 and HB2 treatments, which differed from B1 and B2 by 77.48% and 54.95% respectively. Both HA changes were significant at the 95% confidence level (Fig. 2.g). Although some reports state that Fe content varies in different species, and generally legumes have more Fe than grasses, parsley is one example where the Fe accumulation that occurred in the present study increased after HA application.

Zinc

Zn changes in parsley leaves were similar to B, Fe and Mn, with a slight decrease in B1, and gradually increasing across B2, HB1 and HB2 treatments (Fig. 2.h). The content of Zn ranged between 63 and 121 mg/g dry leaf weight. In the present investigations, Zn concentration decreased by 18.85% in B1 compared to C but the reduction was not significant The Zn content was decreased in B1 (18.00%) while it increased by 4.11% in B2 compared to controls. After humic addition, Zn concentration increased 50.17% and 50.12% in HB1 and HB2 treatments respectively, of which the latter was significant from the Zn contents in C, B1 and B2 at the confidence level of 95% (Fig. 2. h). It is known that there is a synergistic relationship between B and Zn uptake from the soil (Gezgin and Hamurcu, 2006). However, in *Citrus aurantifolia* L., B content decreased on application of Zn (Rajaie *et al*., 2009). Therefore, Zn application is recommended to reduce B toxicity in boron-rich soil but, conversely, decreased Zn concentration may not result from B application (Adiloglu and Adiloglu, 2006; Rajaie *et al*., 2009). According to Hosseini *et al*. (2007) and Aref (2011), in plant tissue, in terms of concentration, boron and zinc are antagonistic. However, Sinha *et al*. (2000) reported that Zn and B had a positive interaction in mustard (*Brassica nigra* (L.) Koch) and in tomato (*Lycopersicon esculentum* Mill., cv. 'Lale') Zn and B treatments increased Zn concentration (Gunes *et al*., 2000).

The reports from Karaman *et al*. (2012), Savasturk (2008), and Mohamed (2012) stated that HA causes increase in Zn absorption, in agreement with the results from the present study which revealed that HA increased Zn content up to 50% in both HA applications.

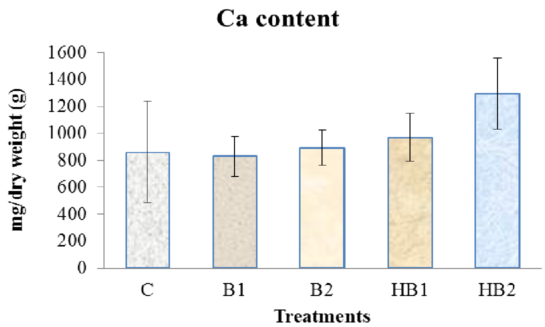

Fig. 2d. Nutrient contents in parsley (*Petroselinum sativum* Hoffm) leaves under treatments of 15ppm boron (B1), 150 ppm boron (B2), 15 ppm boron +humic acid (HB1), 150 ppm + humic acid (HB2) and Hoagland (C). *: Significantly different from C; **: Significantly different from B1; ***: Significantly different from B2

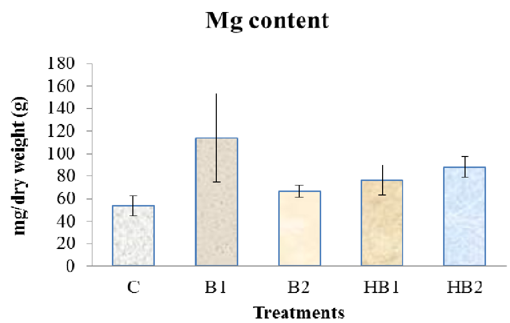

Fig. 2e. Nutrient contents in parsley (*Petroselinum sativum* Hoffm) leaves under treatments of 15ppm boron (B1), 150 ppm boron (B2), 15 ppm boron +humic acid (HB1), 150 ppm + humic acid (HB2) and Hoagland (C). *: Significantly different from C; **: Significantly different from B1; ***: Significantly different from B2

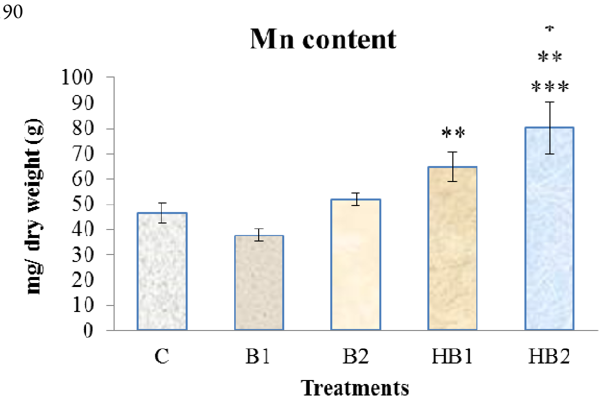

Fig. 2f. Nutrient contents in parsley (*Petroselinum sativum* Hoffm) leaves under treatments of 15ppm boron (B1), 150 ppm boron (B2), 15 ppm boron +humic acid (HB1), 150 ppm + humic acid (HB2) and Hoagland (C). *: Significantly different from C; **: Significantly different from B1; ***: Significantly different from B2

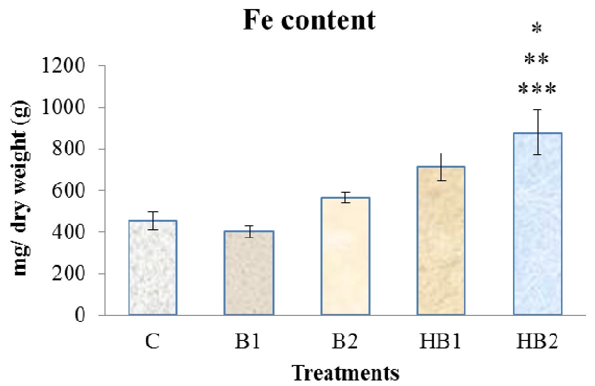

Fig. 2g. Nutrient contents in parsley (*Petroselinum sativum* Hoffm) leaves under treatments of 15ppm boron (B1), 150 ppm boron (B2), 15 ppm boron +humic acid (HB1), 150 ppm + humic acid (HB2) and Hoagland (C). *: Significantly different from C; **: Significantly different from B1; ***: Significantly different from B2

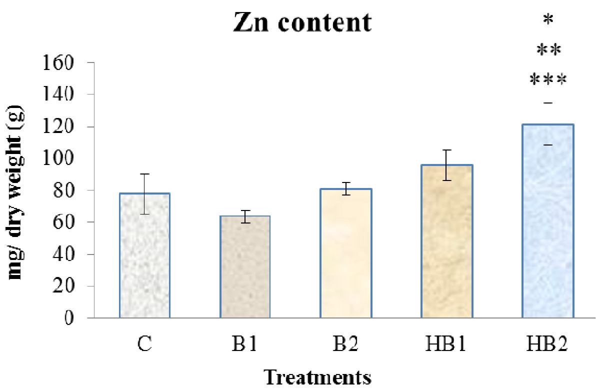

Fig. 2g. Nutrient contents in parsley (*Petroselinum sativum* Hoffm) leaves under treatments of 15ppm boron (B1), 150 ppm boron (B2), 15 ppm boron +humic acid (HB1), 150 ppm + humic acid (HB2) and Hoagland (C). *: Significantly different from C; **: Significantly different from B1; ***: Significantly different from B2

Conclusions

Boron is a crucial micronutrient and plays an important role in plant growth and development, being mainly involved in carbohydrate metabolism and cell division. Although there are differences in B accumulation from plant to plant, analysis of plant parts, such as tissues in the leaf margins and in the rest of the leaf blade, gives 10 to 50 ppm of the dry weight as an accepted adequate range. According to van Goor and van Lune (1980), the B concentration is relatively higher than other micronutrients and differed in phloem (10 μg g^{-1}) and in leaf tissue (34 μg g^{-1}), but it is less than other elements while transported within veins. These findings agree with the present study, which indicated that B caused increase in leaf fresh and dry weights and leaf area in B1 treatments, but B content was found to be less in leaf stalks in B1 application compared to controls. The present study revealed that 15 ppm B application to parsley plants was an adequate amount that promoted plant growth and development in nearly all parameters except plant height and stalk weight, for which the effect of B was unclear. In contrast, 150 ppm B seemed to be an excessive amount that decreased all growth parameters. HA treatments had a very slight effect of reducing the effects of excess B absorption on growth parameters. Considering plant nutrient composition changes in the parsley leaves, HA affected absorption of Ca, Mn, Fe and Zn in HB1 and HB2 treatments when compared to the boron-only treatments i.e. B1 and B2 respectively. Boron content also increased after HA application. This finding of HA effect agrees with Goldberg (1997). Szabolcs (1989) stated that increased B accumulation in plant depends on nutrient solution and humus content, and is greater than the plant needs physiologically. Na and K concentration were decreased in both HA treatments. As a conclusion, under deficient, adequate or excessive conditions of B, synergistic or antagonistic interaction of B may affect plant nutrition composition. B content had a varied effect on the different growth parameters observed, with differing responses related to B concentrations (15 ppm and 150 ppm B) and HA additions. HA can contribute uptake of certain nutrients from soil, although the ratios absorbed did not seem to be adequate to fulfill requirements of parsley plants for ideal growth and development in the pot experiment.

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