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RESEARCH PAPER

The effects of humic acid and chelate applications on some morphophysiological properties and antioxidant enzyme activities of ornamental cabbage (*Brassica oleracea* var. *acephala*) under boron stress

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Abstract

The increase of industrialization, the overuse chemical fertilisers and mining activities are brought about heavy metal-led environment pollution, especially agricultural land. This leads to more boron (B) contamination and accumulation in the soil. This study was carried out to evaluate B uptake from the soil of ornamental cabbage grown as a hyperaccumulator plant under B stress conditions in a controlled greenhouse on plant morphology, physiology, antioxidant enzyme activity the effects of humic acid (50mg kg^{-1} B + 2% humic acid -HA) and chelate [0.5 g kg^{-1} chelate (EDTA)] applications. According to the results, especially chelate application significantly increased the B uptake of the plant, and B accumulation was higher in the plant shoot than in the root. However, HA and chelate applications brought out the negative effects of B stress on growth and physiological characteristics and reinforced the increases in malondialdehyde (MDA) content and superoxide dismutase (SOD) and catalase (CAT) enzyme activities. In conclusion, this study shows that HA and chelate additions increase the efficiency of the use of ornamental cabbage to remove excess boron from the soil. According to these results, it is possible to increase the use of ornamental cabbage for phytoremediation purposes, especially with chelate application.

Introduction

Boron (B) is an essential element for the vital activities of plants (Hussain et al., 2017). Boron element is included a great number of processes in plants such as: photosynthesis, the cell walls and the lignification process, the transport of sugars, the ascorbate/glutathione cycle, metabolism of phenolic compounds, pollen tube formation, the plasma membrane integrity and its function, the nitrogen metabolism. In addition, it can create a stress affect at higher concentrations than a certain dose range. In case of excess boron in the soil, many physiological and biochemical processes in the life cycle of plants are negatively affected and occurred significant losses in yield and quality (Princi et al., 2016; Garcia-Sanchez et al., 2020). B toxicity is an important problem on plants, especially in regions where boron mines are located

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and B contamination can cause severe damage to local ecosystems (Stiles et al., 2011). It is highly important for sustainability to clean and improve boron-contaminated areas through the use of B-tolerant plant species in the revitalization of ecosystems that are in danger of extinction (Rámila et al., 2016). At this point, phytoremediation technique emerges as a cost efficient and environmentally friendly application for on-site improvement in areas excess of B (Eman Gökseven & Kıran, 2021). The use of hyperaccumulator plants with high B tolerance is important for the success of this technique, especially in areas where pollution is intense. The use of agents such as chelate, and humic acid is also seen as an effective approach in order to support the accumulative properties of plants and increase their capacity. As a matter of fact, it is known that ethylene diamine tetra acetic acid (EDTA) as a synthetic chelator has an effect on the metal bioavailability potential of plants (Arshad et al., 2020; Konkolewska et al., 2020; Saffari & Saffari, 2020). Moreover, it is stated that humic acids, which are considered the most active components of soil and compost organic matter, demonstrate physiological, morphological, biochemical and genetic effects on plants by forming strong bonds with toxic heavy metal ions (Ferrara & Brunetti, 2008; Shehata et al., 2019), and are also effective in mitigating the negative effects of heavy metals on plants (Özkay et al., 2016). In addition, humic acid improves the morphological characteristics of plants grown under B stress conditions and increases the amount of nutrients (Karaman et al., 2017). In addition, it is observed that plants grown under heavy metal stress (Cu, Cd, Pb, and Zn) conditions help reduce the effect of oxidative stress by regulating antioxidant enzyme activities such as SOD, CAT, GR, and APX (Kıran et al., 2014). Furthermore, humic acid improves growth by reducing oxidative stress in plants due to its organic structure. Therefore, it can increase the effect of phytoremediation in soils contaminated with heavy metals (Canal et al., 2022).

Oxidative stress caused by heavy metals in plants may vary depending on the plant species, the type and concentration of the heavy metal (Olaniya et al., 1998). Many researchers have reported that some vegetables species have the capacity to accumulate heavy metals in the soil. Especially, certain *Brassica* spp. are mostly evaluated as hyperaccumulator plants because of their high accumulation of heavy metals in their tissues (Kusznierewicz et al., 2012; Ning et al., 2015; Haghighi et al., 2016; Eman Gökseven & Kıran, 2021). While it is not preferred to accumulate heavy metals, which can have negative effects on human health, in edible plant species, the use of species that are not consumed as food or feed is preferred for phytoremediation. Therefore, in our study, *B. oleracea* var. *acephala*, a subspecies of *Brassica oleracea* and considered as an ornamental plant, was used and evaluated as a potential hyperaccumulator plant in the improvement of B-contaminated areas. In this study, it was studied to increase the efficiency of use for phytoremediation in soils containing B by supporting the hyperaccumulator property of *B. oleracea* var. *acephala*. The specific aim of this study was to determine changes in some morphological and biochemical properties of ornamental cabbage by applying humic acid and chelate (EDTA) to plants grown in soil contaminated with boron.

Material and Methods

Plant materials and treatments

This study was carried out in the greenhouse of Soil, Fertilizer and Water Resources Central Research Institute with automatic temperature and relative humidity control (at 13-17°C temperature and 40-45% relative humidity) and laboratories of the Department of Horticulture in Ankara University and Soil, Fertilizer and Water Resources Central Research Institute in Ankara, Türkiye. Ornamental cabbage (*Brassica oleracea* var. *acephala*) was used as plant material. Table 1 shows physical and chemical properties of studies soil. Studied subjects were used 1. Control (only 50 mg kg^{-1} B dose), 2. 50 mg kg^{-1} B + Chelate (EDTA), 3. 50 mg kg^{-1} B + Humic Acid (HA) in this experiment. Ornamental cabbage (*Brassica oleracea* var. *acephala)* seedlings with 3-4 true leaves were planted in pots 15×17×22 cm (a plant per pot) in January 25, 2018. One week after planting, 50 mg l^{-1} B was applied to the pots except control group. Boron was applied as boric acid (H_3BO_3, 17.5% B) with irrigation water. In order to support B uptake in plants, chelate (0.5 g kg^{-1} EDTA by spraying) and humic acid (HA) (2% powdered humic acid (48.34% organic matter + 60.47% humic+fulvic acid)) were added to the potting soil one week after B application. The plants were irrigated at the level of field capacity with tap water. Plants were grown for eight weeks. In order to make observations and measurements, at the end of the 8th week, three plants from each subject were harvested and measurements were made and samples were taken for analysis.

Table 1. Physical and chemical properties of studies soil

Properties	
Texture class	Clay Loam (CL)
EC* (dS m^{-1})	0.92
pH*	7.49
$CaCO_3$(%)	37.6
Available P (P_2O_5) (kg ha^{-1})	11.0
Available K (K_2O) (kg ha^{-1})	78
Organic Matter(%)	1.86
Available Fe (mg kg^{-1})	15.44
Available Cu (mg kg^{-1})	2.59
Available Zn (mg kg^{-1})	2.42
Available Mn (mg kg^{-1})	16.68
Available B (mg kg^{-1})	0

Determination of plant growth characteristics

Each plant is divided into two as roots and shoots and weighed weekly in grams on a precision scale. After their fresh weights measured, the samples were dried in the oven set at 65°C until they reach constant weight then their dry weights were measured.

Measurements of physiological properties

Chlorophyll content

Before harvest, the chlorophyll amounts of the plants were measured each week by using Minolta Chlorophyll Meter (SPAD-502). Chlorophyll measurements (SPAD values) were taken as three readings on the 5 leaves of each plant, based on the central part of the leaf.

Stomatal conductance (gs)

Decagon SC-1 model porometer was used to determine stomatal conductance. It was determined by making measurements on the same leaf randomly determined each week before harvest between 13.00-14.00 p.m.

Relative water content (RWC)

Leaf samples taken before harvest were immediately weighed and their fresh weights (FW) were measured, the samples were kept in pure water for 4 hours and then their turgor weight (TW) was measured. Finally, the leaf samples were dried in an air circulation drying cabinet at 65°C for 24 hours and their dry weight (DW) was measures (Dhanda & Sethi, 1998). The relative water content of the leaves was calculated with the help of the equation below:

$$RWC (\%) = [(FW-DW)/(TW-DW)] \times 100$$

Boron analysis in plant

The shoots and roots of the plants to be sampled were washed first with tap water and then with pure water, then placed in a paper bag and dried until they reached a constant weight at 65°C. Plant shoots and roots were ground finely to pass through a 200 µm sieve for analysis. 0.25 g of shoots and root samples were first digested with nitric acid (HNO_3) in a microwave device, then these samples were transferred to 50 mL Erlenmeyer flasks and completed with deionized water and filtered through the blue tape filter paper. Total Boron in the plant solution obtained by method of wet decomposition was determined in Shimadzu UV-160 Spectrophotometer according to the vanadomolybdophosphoric yellow color method (Kacar & İnal, 2008). The boron content of the sieves obtained according to the method of wet decomposition was determined in Varian 720-ES ICP-OES (Kacar & İnal, 2008).

Lipid Peroxidation

Lipid peroxidation is called damage to cell membranes. The method developed by Lutts et al., (1996) was followed in order to determine the amount of malondialdehyde (MDA), which is a product of lipid peroxidation. Fresh tissues (0.5 g) were homogenized in 10 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 15.000 g for 5 min. Assay mixture containing 1 mL of the supernatant and 4 mL of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA was heated at 95°C for 30 min and rapidly cooled in an ice bath. After centrifugation (10.000 g for 10 min), the absorbance of the resulting supernatant was measured at 532 and 600 nm wavelengths. The concentration of MDA in the solution was calculated as MDA (nmol/ml) = [A532-A600)/155000] x 106 by Sairam & Saxena, 2000.

Assessment of the Antioxidant Enzymes Activity: Superoxide dismutase (SOD), Catalase (CAT)

To assess enzyme activity, approximately 1 g fresh leaf tissue was crushed in liquid nitrogen in a porcelain mortar and homogenized in extraction medium containing 5 mL of 0.1 M Na-phosphate, pH 7.5; 0.5 mM Na-EDTA and 1 mM ascorbic acid. The homogenate was centrifuged at 18.000 g for 30 min at 4 °C. The supernatant was used to assess catalase (CAT) and superoxide dismutase (SOD). Measurements were performed in an Analytical Jena 40 model spectrophotometer. The CAT activity was calculated as the rate of decomposition of H_2O_2 during at 240 nm (E=39.4 mM cm^{-1}). The reaction mixture (2.5 mL) contained 0.05 M phosphate buffer (pH 7.0), 1.5 mM H_2O_2 and 0.2 mL enzyme extract (Jebara et al., 2005). Superoxide dismutase (SOD) activity was adjusted by the nitroblue tetrazolium (NBT) method (Rahnama & Ebrahimzadeh, 2005).

Statistical analysis

Experiments were carried out randomized plots with a factorial design with 3 replications. All data was statistically analysed using the MSTAT-C (Freed et al., 1989). The significant differences were compared with LSD test at $P \leq 0.05$.

Results

Shoot -root fresh and dry weights

The effect of humic acid and chelate applications with B on plant shoot-root fresh and dry weights parameters are shown in Table 2. Differences between B applications was found statistically significant ($P \leq 0.05$) in terms of shoot-root fresh weights and root dry weight, while shoot dry weight was not significant ($P>0.05$). All application forms significantly reduced shoot fresh weight of the plants to the control plants. The highest shoot fresh weights in plant were obtained

Table 2. ANOVA for shoot-root fresh and dry weights, stomatal conductance (gc), chlorophyll, relative water content (RWC)

Source of variation	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	gc	Chlorophyll (SPAD value)	RWC
Application forms	**	NS	**	**	**	**	NS
CV (%)	2.85	17.88	6.94	16.29	5.12	6.00	3.75

CV: Coefficient of variation; **: P≤0.01 is significant at probability level; *: P≤0.05 is significant at probability level; NS: Not significant

Table 3. The effect of humic acid (HA) and chelate associated with B application on plant shoot-root fresh and dry weights, stomatal conductance (gc), chlorophyll and relative water content (RWC)

Application forms	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})	gc (mmol (m^{2}s)$^{-1}$)	Chlorophyll (SPAD value)	RWC (%)
Control	109.79±1.22 a	11.27±0.95	43.11±2.42 a	7.69±0.85 a	102.10±3.24a	36.72±1.62a	77.38±2.21
50 mg kg^{-1} B	80.56±2.00 b	12.06±1.85	38.03±2.28 b	5.44±0.47 b	86.92±7.39b	27.50±2.18b	74.43±2.14
50 mg kg^{-1}B+Chelate	76.83±2.57 b	9.00±2.00	20.88±1.64 d	4.34±0.62 b	63.43±5.36c	25.53±1.16b	76.37±1.71
50 mg k^{-1}g B+HA	70.33±2.52 c	9.45±1.65	27.87±1.80 c	3.74±0.98 b	59.93±4.58c	25.60±4.13b	74.00±3.46
LSD (5%)	4.80	-	4.50	1.73	7.99	3.46	-

*: Means with the different letter within same column are significantly different (P≤0.05)

from 'B' (80.56±2.00 g plant^{-1}) and 'B + Chelate' (76.83±2.57 g plant^{-1}), which were statistically same group (Table 3). The lowest shoot fresh weight value was determined in the 'B + HA' (70.33 ±2.52 g plant^{-1}) (Table 3). This application method draws attention as the application method in which plants most exploit the boron element from the soil, thus affecting the development most negatively compared to the control. It was determined that the root fresh weight of the plants decreased significantly compared to the control plants in all applications. The highest root fresh weight was obtained from 'B' (38.03±2.28 g plant^{-1}). After 'B' application, root fresh weights were obtained from 'B + HA' (27.87±1.80 g plant^{-1}) and 'B + Chelate' (20.88±1.64 g plant^{-1}), respectively (Table 3). The highest root dry weights were not statistically different between 'B' (5.44±0.47 g plant^{-1}), 'B + Chelate' (4.34±0.62 g plant^{-1}) and 'B + HA' (3.74±0.98 g plant^{-1}) applications, respectively (Table 3).

Stomatal conductance (gc), chlorophyll and relative water content (RWC)

The differences between the B applications were not statistically significant in terms of relative moisture content (P>0.05), while the differences between the applications were found to be important in terms of gc (P≤0.05) (Table 2). In ornamental cabbage, gc was significantly reduced in all B application forms compared to the control plant. While the highest gc was determined in the 'B' (86.92±7.39 mmol m^{2}s^{-1}) application, the lowest gc was found in the combinations of 'B + Chelate' (63.43±5.36 mmol m^{2}s^{-1}) and 'B + HA' (59.93±4.58 mmol m^{2}s^{-1}), which were statistically in the same group (Table 3). Chlorophyll content of the plants in all B application forms was decreased compared to control plants. The highest chlorophyll content was obtained from the 'B' (27.500±2.18 SPAD).

Boron contents

The differences between the application forms ('Control','B','B+ Chelate', 'B + HA') was found to be statistically significant the amounts of B accumulated in the shoot, root and whole plant (P≤0.05) (Table 4). The highest B accumulation occurred 'B + Chelate' in terms of the shoot, root and whole plant. B applications led to increase in the amount of B in shoot, root and the whole plant. The increases were found to be significant when compared to the control plants. It was determined that B accumulation in the shoot was higher that the accumulation in the root, in 'B', 'B + Chelate' and 'B+ HA applications. After 'B + Chelate' application, the highest B accumulations in shoot, root and whole plant were followed by 'B + HA' and 'B' applications, respectively (Figure 1, Figure 2 and Figure 3).

Table 4. ANOVA for boron content in shoot, root and whole plant

Source of variation	B in shoot	B in root	B in whole plant
Application forms	**	**	**
CV(%)	2.57	5.51	23.84

**: P≤0.01 is significant at probability level

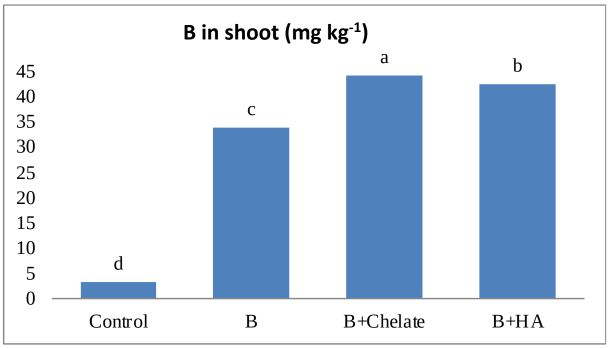

Figure 1. B content in shoot depending on application forms

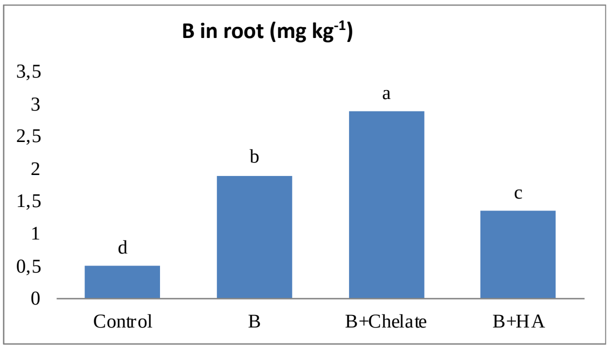

Figure 2. B content in root depending on application form

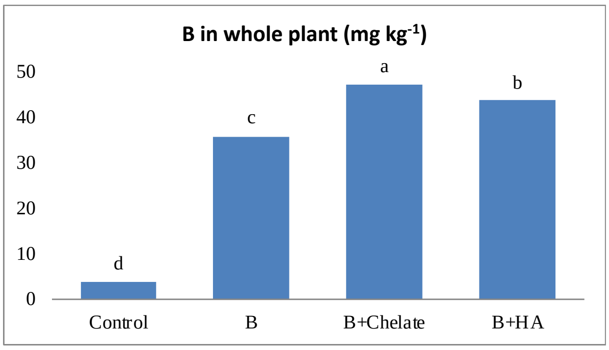

Figure 3. B content in the whole plant depending on application forms

Lipid peroxidation and activities of antioxidant enzymes

The amount of malondialdehyde (MDA), which is a product of lipid peroxidation, and antioxidative enzyme activities (SOD and CAT) were found to be statistically significant in terms of B application forms (P≤0.05) (Table 5). Compared to control, B application brought about an increase in MDA levels by creating oxidative stress in plants. The differences between all B applications were in the same statistical group. The highest MDA value was obtained from the 'B + Chelate'(12.96±0.88 µmol g^{-1}) and followed by 'B' (10.01±0.54 µmol g^{-1}) and 'B + HA' (9.48±0.58 µmol g^{-1}), respectively (Table 6).

Table 5. ANOVA for MDA contents and activities of antioxidant enzymes (SOD and CAT)

Source of variation	MDA	SOD	CAT
Application forms	**	**	**
CV (%)	7.18	6.71	1.33

**: P≤0.01 is significant at probability level. MDA: Malondialdehyde SOD: Superoxide dismutase, CAT: Catalase.

The highest SOD antioxidative enzyme activity was determined as 'B + Chelate' (77.90±6.06 U min^{-1} mg^{-1}) and this application was followed by 'B + HA' (71.07±7.79 U min^{-1} mg^{-1}), which was in the same statistical group. 'B' (60.15±2.03 U min^{-1} mg^{-1}) application was lower SOD enzyme activity compared to other B applications. In general, all B applications showed an increase compared to the control (Table 6). Compared to control, B applications led to increases in CAT activity. The highest CAT activity was determined as 'B + Chelate' (705.50±8.23 µmol min^{-1} mg^{-1}) and this application was followed by 'B + HA' (526.26±8.62 µmol min^{-1} mg^{-1}) and 'B' (520.00±5.00 µmol min^{-1} mg^{-1}), which were in the same statistical group (Table 6).

Table 6. The effect of chelate and humic acid (HA) associated with B application on MDA contents and activities of antioxidant enzymes SOD and CAT)

Application forms	MDA (μmol g^{-1})	SOD (U min^{-1} mg^{-1})	CAT (μmol min^{-1} mg^{-1})
Control	3.44±0.19b	11.61±0.62c	179.19±5.07c
50 mg kg^{-1} B	10.01±0.54a	60.15±2.03b	520.00±5.00b
50 mg kg^{-1} B+Chelate	12.96±0.88a	77.90±6.06a	705.50±8.23a
50 mg kg^{-1} B+HA	9.48±0.58a	71.07±7.79a	526.26±8.62b
LSD (5%)	5.14	7.77	13.51

*: Means with the different letter within same column are significantly different (P≤0.05)

Discussion

In this study, it was determined that the shoot fresh and dry weights of ornamental cabbage were higher in 50 mg kg^{-1} B application compared to '50 mg kg^{-1} B+HA''. However, chelate addition (B+chelate) had a similar effect to B application (50 mg kg^{-1} B) in terms of shoot fresh weight. On the other hand, HA supplementation caused significant reductions in trunk fresh weights compared to control and B application (50 mg kg^{-1} B) with the effect of toxic level of B accumulation. Similar results were obtained by Tursun (2014), on parsley, it was reported that '150 ppm B' and '150 ppm B + HA' application significantly reduced leaf fresh and dry weights. It has been reported that in boron pollution in rapeseed, HA and EDTA applications brought about decreases in root yield due to dose increase and repetitions (Esringü, 2012). In addition, it was stated that the effects of B toxicity and humic substances applications on biomass yield in cotton were positive, but this could not be observed clearly and there were differences between the application doses (Kaptan, 2013).

According to the findings obtained in this study, 'B+ Chelate' and 'B+ HA' applications significantly reduced root fresh and dry weights compared to the control. In terms of root fresh weight, these losses can be seen more clearly in HA applications. Root fresh weight losses, especially with chelate application, were revealed by B accumulated more in the root due to the addition of chelate. Moreover, EDTA, which is a chelate, has the potential to increase the phytoextraction of metal (Saffari & Saffari, 2020). Similar result was obtained by Göker (2019) who reported that EDTA application decreased root fresh and dry weight in corn plant compared to the control in chromium pollution. Moreover, our results were in line with the findings of Karabulut (2020), who notified that there was a decrease in chelate application root fresh weight and root dry weight of rosemary plant in pots contaminated with lead. Our results consistent with finding that 150 ppm B and 150 ppm B + HA applications reduced root fresh and dry weights compared to control on parsley plants. However, it has been also reported that HA has no effect on the prevention of boron toxicity, especially in terms of growth parameters (Tursun, 2014).

The stomatal conductance decreased during stress in all B applications compared to the control. In general, a decrease in stomatal conductance was determined in all B applications, while additional chelate and HA applications revealed losses in gc of plants more evident. There was a similar finding between chelate and HA applications on gc. It has been reported that HA increases stomatal activity and leaf K content in potato (Lopez, 1993), lettuce (Haghighi et al., 2012) and strawberry (Ameri & Tehranifar, 2012) plants grown in B-free conditions.

In our study, the findings obtained with stomatal conductivity differed, and B, which accumulated more on the leaves with HA application, prevented gas diffusion and led to a decrease in stomatal conductivity. Canal et al. (2022) reported that HA increases the metal accumulation in the plant. According to previous similar studies, reductions in stomatal conductivity were observed in plants exposed to excess B (Lovatt & Bates, 1984; Papadakis et al., 2004). Pereira et al. (2000) hypothesize that one of the possible causes of decreased photosynthesis by excess B is structural damage to thylakoids. Pereira et al. (2000) also explained this situation by changing the electron transport rate and affecting CO_2 photoassimilation, which can also be limited by stomatal reduction (Landi et al., 2012).

Chlorophyll content decreased in all B applications compared to control. There was no difference in the effects of additional chelate and HA applications in the decreases in the amount of chlorophyll content emerged by the effect of B toxicity. Due to the increase in the boron uptake capacity of the plant with HA and chelate applications, increased B concentrations in the shoot may have been effective in structural damage to chloroplasts. Güllüce et al. (2012) reported that different doses of HA led to a significant decrease in chlorophyll content in radish grown in areas contaminated with Pb and Cd. Additional EDTA application to As-contaminated soil significantly reduced the chlorophyll content of corn leaves (Abbas, 2013). A similar result was obtained by Wang et al. (2019), on the toxicity of Cd in lettuce plant, with a decrease in chlorophyll content with fulvic acid application.

Boron analysis in plants

Small amount of EDTA rates can be a helpful factor in exceeding the limits for metals uptake by plants. (Chen et al., 2004; Meers et al., 2005). Vanlı (2007), reported that increases in B uptake were observed in plants according to the chelate dose in canola, corn, and sunflower to which different amounts of chelate were added. Similar findings were obtained in our study, EDTA application led to more B accumulation in the shoot and root of ornamental cabbage, resulting in higher B concentrations. Although previous studies have shown that chelates applied to increase the uptake of B element by the plant have positive effects, it has also been noted that there are decreases according to the increase in the dose and number of repetitions of chelators (Esringü, 2012). Except EDTA, regulators such as citric, tartaric, and humic acids applied to the soil also caused an increase in heavy metal uptake in plants. (Eren, 2019a; Eren, 2019b). In our study, it was determined that HA application also had a positive effect on B uptake in the shoot and root, but this effect remained at lower levels compared to chelate application. Increasing the uptake of nutrients by plants may be associated with the chelating properties of humic substances on micronutrients and their hormone-like effects in the soil. It has been reported that the addition of different amounts of HA and different B doses in Vetiver (*Vetiveria zizanioides*) have a positive effect on B uptake in roots and shoots (Angin et al., 2008). Kaptan (2013), notified those humic substances increased the available B content in the soil, but its reflection on plant boron contents could not be determined clearly.

Lipid peroxidation and activities of antioxidant enzymes

Lipid peroxidation is an indicator of oxidative damage. Many researchers bring forward that there is a relationship between the occurence of reactive oxygen species as a result of oxidative stress and increased lipid peroxidation concentration. (Karabal et al., 2003; Han et al., 2009). Under stress conditions, MDA is generally used to evaluate lipid peroxidase or membrane damage. By detecting the MDA content, both the degree of lipid peroxidase and thus the degree of stress are determined (Onbaşı, 2017). In our study, B stress led to significant increases in the amount of MDA. Similar results have ben reported in vine (Güneş et al., 2006), apple rootstock (Molassiotis et al., 2006), tomato (Cervilla et al., 2007), patato (Ayvaz, 2009), barley plants (Onbaşı, 2017). Our results are consistent with also those of Barışık Kayın (2020), who notified that there are significant increases in MDA contents in plants under B toxicity. The use of chelate increased the B uptake of ornamental cabbage from the soil and brought about more B accumulation in the plant compared to '50 mg kg^{-1} B'. As a result of, MDA content increased because of oxidative damage in the plant.

Increases in SOD activity have been reported with an increase in reactive oxygen species (ROS) production due to stress. (Mittler, 2002; Ayvaz, 2009). The increased activity of SOD can be expressed as an increasing index of superoxide (O_2-) production in plants under B stress. It has been reported by many researchers that superoxides and toxic O_2 formation occur under B stress (Karabal et al., 2003; Kobayashi and Matoh 2004; Cervilla et al., 2007; Pandey and Archana, 2013). Increases in SOD enzyme activity in plants under boron toxicity were observed in chickpea (Ardıç, 2006), apple root shoots (Molassiotis et al., 2006; Sotiropoulos et al., 2006) and goldentop (Han et al., 2009), *Brassica juncea* (Giansoldati et al., 2012), pepper (Barışık Kayın, 2020). Varshney et al. (2015), it was determined that the highest increase in antioxidant enzyme activity was at the dose of 60 mg kg^{-1} B. In our study, the SOD activity increased with the administration of '50 mg kg^{-1}' B. In this regard, Varshney et al. (2015) and our findings are similar. The use of copper together with EDTA in rapeseed caused changes in antioxidant enzyme activity, and it was reported that EDTA application increased SOD activity (Habiba et al., 2015). In our study, chelate application increased B uptake from the soil to the plant. As a result, the SOD enzyme was activated more in order to eliminate the effect of oxidative damage due to excessive accumulation of B in the plant. It was reported that plants under boron toxicity increased CAT enzyme activity in chickpea (Ardıç, 2006), apple rootstocks (Sotiropoulos et al., 2006), *Citrus grandis* L. plants (Han ve ark., 2009) and rapeseed (*Brassica* sp.) (Pandey &Archana, 2013). Varshney et al. (2015), increases in CAT activity were reported at a dose of 60 mg kg^{-1} B. EDTA application activated CAT antioxidant enzyme more in Brassica napus L. plant under Cu stress (Habiba et al., 2015). Significant increases in CAT activity were also recorded by Barışık Kayın (2020) with the B application.

Conclusion

According to the results of this study, *B. oleracea* var. *acephala* as an ornamental plant, showed a significant potential against stress, toxicity, and accumulation of B. The application of HA and chelate as chelators negatively affected shoot fresh-dry weights and root fresh-dry weights. While the stomatal conductivity and chlorophyll content decreased in HA and chelate addition applications were added, there was an insignificant increase in the relative moisture content only in the 'B + Chelate' application. However, B applications caused an increase in MDA levels compared to the control by creating stress in plants and led to cell membrane damage. We found that chelate addition can be used to increase the efficiency of use of ornamental cabbage plant for removing excess boron from the soil, since it has an effect on increasing the B uptake of the plant. 'B + Chelate' application provided the highest MDA increase compared to other B applications during B stress. Antioxidative enzyme activities increased compared to control. On the other hand, the increase in the activities of antioxidative enzymes revealed that the tolerance mechanism in the cells was activated at a high level despite the damage. High B applications with HA added took place after the chelated applications, but the effect remained at lower levels. It can be said that testing different doses of both HA and chelate applications is likely to increase the effectiveness. It is considered that it would be beneficial to carry out this study, which was carried out in controlled conditions and in the form of pot trials, in field conditions in larger areas. It is thought that studies on the removal of element B by phytoremediation method and the application of different doses to understand the mechanisms of the toxic effects of element B in applications such as humic acid and chelate will eliminate the deficiencies in the literature on this subject.

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Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Author Contribution

Conceptualization: SK, SSE, Data Curation: SBEG, SK, SSE, Formal Analysis: SBEG, SK, SSE, Investigation: SBEG, SK, Methodology: SK, SSE, Project Administration: SK, SSE, Resources: SK, SSE, Supervision: SK, SSE, Visualization: SBEG, SK, SSE, Writing -original draft: SBEG, SK, SSE, Writing -review and editing: SBEG, SK, SSE.

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