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EFFECT OF ZINC AND BORON ON YIELD, QUALITY ATTRIBUTES AND PHYSIOLOGICAL TRAITS OF LETTUCE UNDER HYDROPONIC CULTURE

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Abstract

The micronutrient deficiency during the critical growth period of Lettuce (*Lactuca sativa* L.) by its controlling the plant intake of micronutrients can have a profound impact on yield, especially under hydroponic conditions and controlled environmental systems such as greenhouse. Boron (B) and Zinc (Zn) play a key role in plants as these are essential micronutrients of nutritional solutions for securing the crop quality and yield. The work was designed as a factorial experiment on based randomized complete block design with three replications for two factors of zinc ($ZnSO_4,7H_2O$) at three levels (0, 5, and 10mg/l) and boron (H_3BO_3) at three levels (0, 2 and 4mg/l) on quantitative and qualitative traits of lettuce under hydroponic conditions. The results demonstrated that as the concentration of zinc increased in the nutrient solution, the fresh and dry weight, chlorophyll index, chlorophyll *a* and *b*, total chlorophyll, carotenoids content, total protein concentration, and leaf zinc concentration significantly increased. Increasing in boron concentration significantly increased the proline content, malondialdehyde (MDA) content, guaiacol peroxidase (GPX), catalase (CAT), and ascorbate peroxidase (APX) activity levels and also leaf boron concentration. Adding zinc to the solution mitigated the adverse effects of high concentrations of boron on the mentioned parameters. According to the antagonistic relation between Zn and B, under high levels of B in nutrient solutions, supplementing the lettuce with adequate Zn is recommended.

Key words: Enzymatic activity, Chlorophyll, Microelements, Vegetable quality.

Introduction

Leafy vegetables that are consumed as fresh or minimally processed, are very important through the world (Brecht *et al*., 2010). Recently, consumer demand for these products has increased and many farmers have focused to produce lettuce, spinach, and leafy beets (Vernieri *et al*., 2006). The quality of vegetables is affected by pre- and post-harvest factors. Management of plant nutrition is one of the key factors in determining the quantity and quality of leafy vegetables. So, hydroponic system is an important tool to achieve the mentioned goal because it provides the precise control of plant nutrition during crop growth and development (Kang & Kim, 2007).

Due to the limitation of the root media and the high density of the plant, leafy greens require careful management of fertilizers. Also, hydroponic systems, the concentration of the essential elements for plants is sometimes insufficient to maintain continuous growth of the plant. Therefore, optimizing the concentration of nutrients is necessary to obtain the best performance and quality of leafy greens. The concentration of all the elements in the nutrient solution which is used in hydroponic system is one of the most important aspects for successful production of leafy vegetables since excessive levels of elements caused to osmotic stress, ion toxicity and ion imbalance and also extremely low levels of elements lead to nutrient deficiency (Soundy *et al*., 2001). So it is clear that to have an equilibrium growth, we have to adjust adequate amounts of each element in the solution.

Lactuca sativa L. is one of the leafy vegetables relating to Asteraceae which is cultivated in hydroponic method in most of the commercial greenhouses. In this method, all the essential nutrients needed by the plant must be solved in the nutrient solution at the disposal of the plant roots. Therefore, management of nutrient solution is an important key to success in many greenhouses. Some of the plants are more sensitive to certain elements more than others. The composition of the nutrient solution also affects plant growth and yield. Little is known about the effects of nutrient solution composition on lettuce yield and quality (Fallovo *et al*., 2009).

Zinc (Zn) is a major micro nutrient for the metabolism of plant, animal, and human which is involved in the activity of more than 200 enzymes. Regarding to the low mobility of zinc in the soils, zinc deficiency is an especially common difficulty in arid and semi-arid regions with calcareous soils, where plants cannot absorb enough amounts of this micro mineral (Broadley *et al*., 2007). Zinc binds to at least four enzymes: ADH, SOD, carbonate dehydratase, and RNA polymerase (Aravind & Prasad, 2003). The foliar spray of zinc led to increase in the performance of mung bean (Haider *et al*., 2020), chickpea (Pal *et al*., 2021), and broccoli (Rivera-Martin *et al*., 2020), among others. One of the adverse effects of excessive Zn in plant solution is reduction in Pi concentration in plants (Rouached *et al*., 2015). High levels of Zn in the soils and nutritional solutions can disturb physiological, biochemical, and metabolic processes leading to reduce in plant vegetative and reproductive phases and consequently, stunted growth. It could be because of the alteration in photoassimilation (Marschner, 2012), nutrient deficiency including Mg and Fe (Sagardoy *et al*., 2010), oxidative damage to the cell membrane, and interfering with DNA replication (Vassilev *et al*., 2007). Also, it is reported that plants grown in nutrient solution having ≥50 mg L^{-1} Zn displayed severe Zn toxicity symptoms (Coolong *et al*., 2004).

Boron (B) belongs to the group of metalloids, whose chemical behavior falls between that of metals and nonmetals. On the other hand, excess amount of B in the soil or nutrient solution can be affected plant growth and development so that represented as leaf necrosis (Alpaslan & Gunes, 2001) as it is reported that soils with 30 mg Kg^{-1} B had the most deleterious effects on plants and decreased the growth parameters (Choudhary *et al*., 2020). Also, boron uptake by plants is greatly reduced at pH > 8 and in calcareous soils. While calcareous soils have a higher concentration of boron than acidic soils, because of the interaction between calcium and boron, it is difficult for the plant to absorb the mentioned element from calcareous soils. In order to moderate the harmful effects of the disproportionate B concentration, using of other nutrients with corrective function is necessary.

However, little information is available about the optimal concentration of elements for many vegetables, especially leafy greens as optimum concentration of fertilizers and access to water for crops under hydroponic systems also depends on environmental conditions. For example, Kang & van Iersel (2001) reported that the optimal concentration of fertilizers for petunia potted plants decreases as well as increase in temperature. So, the present project was subjected to study the effect of zinc and boron on some physiological and biochemical aspects of lettuce under hydroponic culture to see if supplementing zinc to the nutrient solution, is able to mitigate the adverse effects of excess boron in the nutrient solution or not. We also measured the amounts of some essential minerals in lettuce.

Materials and Methods

The project was done to evaluate the combined effects of zinc and boron on growth and physiological traits of Lettuce cv. Parris Island. In this study, 12-liter plastic pots (30 cm height and 20 cm diameter) as planting containers and sand as the growing medium were used. In this experiment, pots with 5 Kg weight were filled with sand and washed with benomyl fungicide (500 ppm) and acetamiprid insecticide (250 ppm) .

Four seeds were planted in each pot. Backup seeds were also planted in extra pots. Temperature was 20±2°C during the day and 18±2°C during the night, and relative humidity was about 60%. The nutrient solution used in the experiment was the modified Hoagland solution (Coolong *et al*., 2004). The concentration and composition of salts in Hoagland solution are given in Table 1. The pH of the nutrient solution was adjusted to 6.5. Using the Aqualytic AL10con conductivity sensor, the electrical conductivity (EC) of the solution was recorded to 1.55 dS m^{-1}. After preparation, the nutrient solution was poured into 20-liter containers and transferred to the growing media.

The experiment was performed as a factorial on based randomized complete block design with 3 replications and two factors of zinc ($ZnSO_4$, $7H_2O$) at three levels (0, 5, and 10 mg/L) and boron (H_3BO_3) at three levels of (0, 2 and 4 mg/L). For the control treatment, the Hoagland solution was used alone. For the experimental treatments, the mentioned amounts of boron and zinc were added to the Hoagland solution.

Measurement of photosynthetic pigments: About 0.2 g of mixed of leaf samples were extracted using 10 mL of 80% acetone. After 24 hours incubation in dark place chlorophyll was extracted from completely blanched leaves. Then the amount of absorbance of extracted samples was recorded at 663, 645, and 470 nm by a spectrophotometer (Shimadzu UV-2550, Japan) for measuring Chl *a*, Chl *b* and carotenoids similar to Arnon (1949) and calculated as mg g^{-1} FW.

Measurement of proline concentration: About 0.2 g fresh weight of leaves were homogenized in 2 mL of 3% aqueous sulfosalicylic acid and centrifuged at 10,000 rpm for 30 min. After removing the supernatant, pellet was washed with 3% aqueous sulfosalicylic acid. The supernatant was pooled, and the content of proline was measured by use of ninhydrin reagent and toluene extraction (Bates *et al*., 1973). The standard solution of proline within the certain range of the method (0–39 mg mL^{-1}), was used for calibration.

Measurement of malondialdehyde concentration: Malondialdehyde (MDA) was measured as a product of lipid peroxidation. So, 0.5 g of leaf samples was ground with 1.5 ml of 0.1% trichloroacetic acid (TCA). The homogenate extraction was centrifuged at 10000 g at 4°C for 10 min. Then 0.5ml of supernatants, were mixed with 1ml of 0.5% thiobarbituric acid (TBA) solution and placed in a water bath at 95°C for 30 min. Immediately afterward, the mixture was placed in an ice bath to complete the reaction. After centrifuging at 10000 g for 10 min, MDA concentration was determined by spectrophotometry at 440, 532, and 600nm with the extinction coefficient considered to be $155 mM^{-1} cm^{-1}$ (Heath & Packer, 1968).

Table 1. Composition and concentration of salts in the modified Hoagland solution of Coolong *et al*., (2004).

Nutrients	Concentration (g/L)	Nutrients	Concentration (g/L)
$Ca (No_3)_2,2H_2O_2$	0.47	H_3BO_3	2.86
KNO_3	0.3	$Mncl_2$, $4H_2O$	1.81
$MgSO_4,7H_2O$	0.25	$ZnSO_4$, $7H_2O$	0.22
$NH_4H_2PO_4$	0.06	NaMOO4, $2H_2O$	0.02
FeEDTA	0.1	$CuSO_4$, $5H_2O$	0.08

Measurement of total soluble protein and oxidative enzyme activity: The 0.5 g fresh weight of leaves were mixed with 3ml of 50 mM potassium phosphate buffer (pH=7) having 1w/v% polyvinylpyrrolidone (PVP) and vortexed. The obtained extract, was centrifuged at 13000 g for 15 min. The supernatant was used directly to assay total protein, catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) enzymes.

The activity of GPX was evaluated by the protocol of Cakmak (2000), which involves preparing a reaction mixture containing of 750 µl of 100 mM potassium phosphate buffer (pH=7), 100µl of 70 mM H_2O_2, 750µl of 10 mM guaiacol and crude protein extract, and spectrophotometry at 470 nm with the extinction coefficient of 26.16 $mM^{-1}cm^{-1}$. The activity of APX was measured by the protocol of Chen & Asada (1989). For this purpose, a reaction mixture containing 200 µl of 2m M ascorbate, 750 µl of 100 mM phosphate-potassium buffer, 200 µl of 2 mM hydrogen peroxide, and plant extract was prepared. Then, the reaction was started by adding H_2O_2, and finally, the enzyme activity was measured by measuring the change in absorbance at 290 nm with the consumption of H_2O_2 based on the extinction coefficient of 2.8 $mM^{-1}cm^{-1}$.

Measurement of catalase (CAT) activity: The activity of CAT was measured by the protocol introduced by Mishra *et al*., (1993). The reaction mixture for this measurement consisted of 700 µl of 100 mM potassium phosphate buffer (pH=7), 750µl of 70 mM hydrogen peroxide (H_2O_2), and plant extract was prepared. The reaction was started by adding H_2O_2 to the mixture and the enzyme activity was measured measuring the change in absorbance at 240nm with the consumption of H_2O_2. For this measurement, the extinction coefficient was 39 $mM^{-1}cm^{-1}$.

Measurement of total protein concentration: Total protein content was measured by the protocol of Bradford (1976), which involves spectrophotometry at 595 nm. Bovine serum albumin (BSA) was used to plot the standard curve.

Measurement of leaf chlorophyll index (SPAD readings): Chlorophyll content of leaves, as an indicator of health, was measured by SPAD (SPAD-502 Chlorophyll meter) on three fully expanded leaves.

Measurement of zinc concentration through digestion: Zinc concentration was measured by the wet digestion (Dong *et al*., 2006). First, 1g of dry plant matter was measured. The plant matter was carefully transferred to the digestion tubes and 10 ml of concentrated nitric acid (65%) was added to each tube. The tubes were placed in the compartments of the digestion unit and covered with a special lid. The samples were left until the next day without applying any temperature. The next day, the samples were heated at 100°C for 5 hours. In this stage, the appearance of a clear yellow liquid was considered as the sign of the end of digestion. Afterward, the tubes were removed from the digestion unit and placed in a holder to cool. The resulting extract was filtered through Whatman grade 42 paper and brought up to a volume of 100ml with distilled water. The product was transferred to volumetric flasks of an atomic absorption spectrophotometer (Shimadzu, AA6300, Japan), which was then used to evaluate zinc concentration.

Measurement of boron concentration through digestion: For this measurement, first, 1g of dry plant matter powder was placed in a crucible and heated in an electric oven at 550°C for 6 hours. Once the samples turned into ash, first 5 or 6 drops of distilled water and then 10ml of 0.36N sulfuric acid were added. After leaving the solution at laboratory temperature for one hour, it was stirred several times with a rod and then filtered.

Statistical analysis

Statistical analyses were performed using MSTATC and SPSS software. Duncan's Multiple Range Test was used to compare the mean results of the treatments. All statistical reports of this study are at the 1% significance level.

Results and Discussion

According to the results of Table 2, it is demonstrated that interaction effect of zinc and boron was significant on growth traits including fresh weight, dry weight, Chl *a* and Chl *b*, total Chl and carotenoids content. However, zinc and boron had significant effect on chlorophyll index (SPAD reading).

Table 2. Analysis of variance of the effects of zinc and boron on fresh and dry weight, chlorophyll Index, Chl *a*, Chl *b*, Total Chl and carotenoids of lettuce.

Source of variation	df	MS						
		Fresh weight	Dry weight	SPAD reading	Chl *a*	Chl *b*	Total Chl	Carotenoid
Zinc	2	2781.8**	19.9**	153.8**	204.1**	42**	469.5**	107.4**
Boron	2	6555.2**	46.2**	144.9**	167.1**	11**	239**	54.3**
Zinc × Boron	4	940.9**	6.5**	2.7ns	9.5**	2.8**	28.3**	2.8**
Error	16	4.7	0.03	3	1	0.1	2.5	0.4
CV (%)		4.8	4.6	4.5	3.7	3.8	4.2	6.7

ns, * and **: Not significant, Significant at 5 and 1 % level of probability, respectively

The comparison of means showed that along with increase in boron concentration in the nutrient solution, fresh and dry weight of lettuce significantly decreased compared to the control as at levels of 5 and 10 mg/L of Zn (Figs. 1a and b), the lettuce grown at higher levels of boron, had lower fresh and dry weight. Similarly, along with increasing in zinc concentration, a significant increase was observed in lettuce fresh and dry weights compared to control. The same effect, was observed in the interaction treatments, too, indicating that adding zinc improves the growth conditions that are adversely affected by high boron concentrations. The highest fresh and dry weights were obtained at 10 mg/L zinc concentration without adding boron. Also, the lowest fresh and dry weight was observed at 4 mg/L concentration of boron which was in agreement with Güneş *et al.*, (2000) findings in tomato. At the mentioned level of boron, even adding zinc could not compensate the toxicity of boron (Figs. 1a and b).

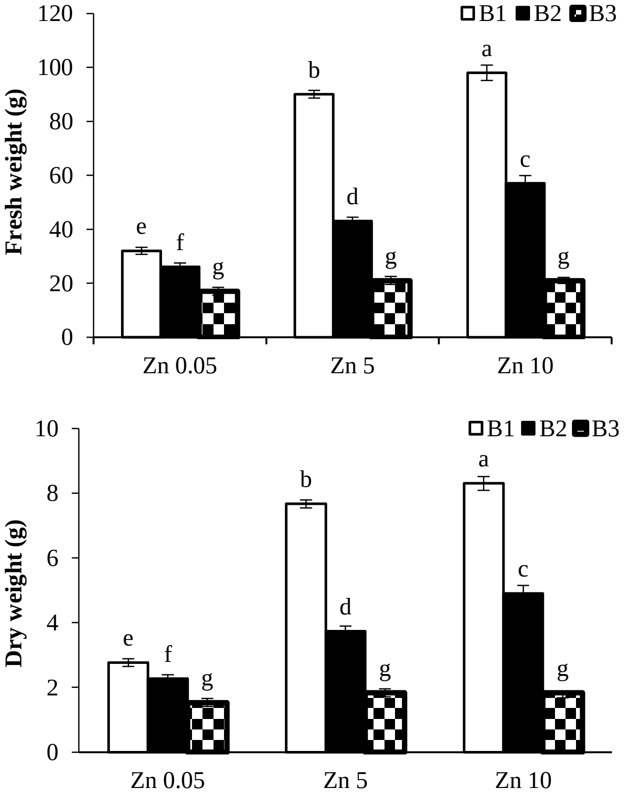

Fig. 1. Effect of zinc and boron on the a) fresh and b) dry weight of lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05

The results showed that zinc increased the fresh and dry weights of the plant via increasing in the leaf chlorophyll content and thus it acts as enhancing of photosynthesis. Furthermore, it can also reduce the adverse effects of high amounts of boron in the soil. Increasing in concentration of boron in the nutritional solution decreased the amount of Chl *a*, Chl *b*, and total Chl. The plants grown at higher levels of boron, had lower Chl *a*, Chl *b* and total chlorophyll. This difference was not significant at levels of 0 and 2mg/L boron across all levels of zinc. With the increasing in zinc concentration, Chl *a*, Chl *b*, and total Chl content significantly increased compared to the control (Figs. 2a, b and c). This indicated that adding zinc, improves the growth and development of lettuce at the presence of high boron concentrations, mitigating the negative effect of the mentioned element.

The highest content of Chl *a* was observed at level of 10 mg/L zinc without boron (Fig. 2a). However, the difference between treatments was not significant in terms of Chl *b* and total Chl (Figs. 2b and c). The lowest level of Chl *a*, Chl *b*, and total Chl was observed at level of 4 mg/L boron. At 4 mg/L boron, adding zinc could reduce and even neutralize the adverse effect of this element, while there was no significant difference between the amounts of Chl *b* at various concentrations of boron when 10 mg/L zinc was added to the nutrient (Fig. 2b). Another reason for the reduction in total chlorophyll could be the change in nitrogen metabolism and the greater use of glutamate (the main substance used in the synthesis of proline and chlorophyll) in the proline synthesis as opposed to the chlorophyll synthesis process (Franzoni *et al.*, 2021).

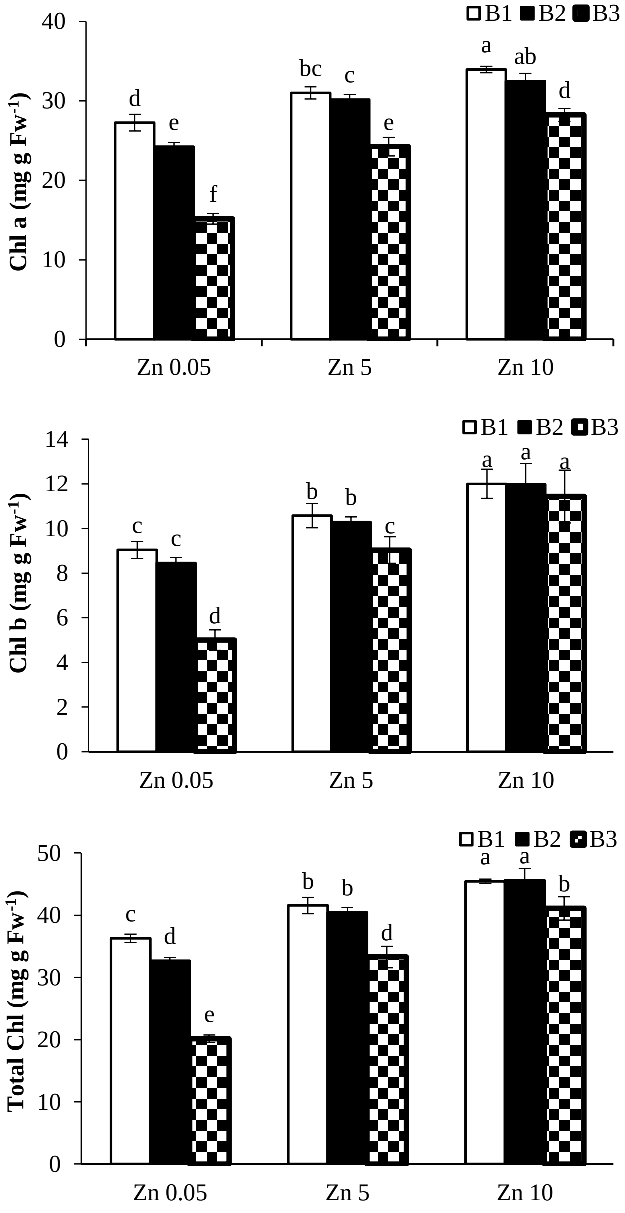

Fig. 2. Effect of zinc and boron on a) Chl *a*, b) Chl *b* and c) Total Chl in lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05

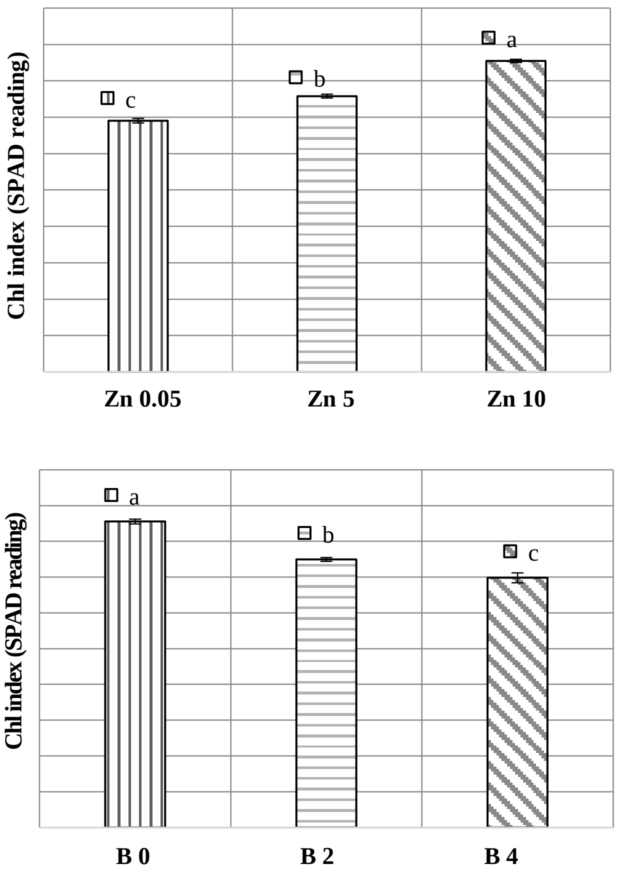

Fig. 3. a) Effect of zinc and b) B on the chlorophyll index (SPAD) in lettuce; Means not sharing the same letter do not differ significantly at p≤0.05.

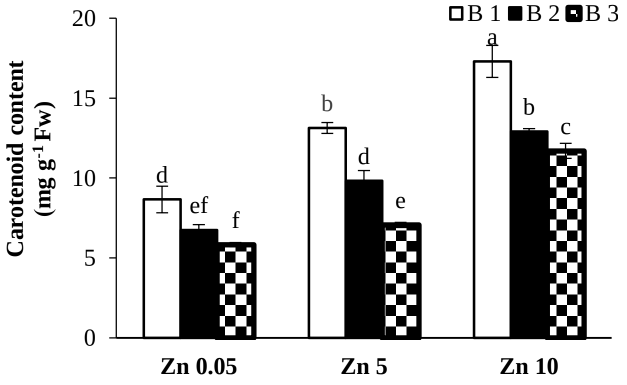

Fig. 4. Effect of zinc and boron on carotenoid content in lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05.

In the analysis of the main effects of zinc (Fig. 3a), the chlorophyll index increased significantly compared to the control with increasing in zinc concentration as the highest and lowest chlorophyll index was observed at 10 mg/ L zinc and control, respectively. The findings indicated that the main effect of boron on the chlorophyll index was significant, too. The highest chlorophyll index was observed at the control level of boron and the lowest amount was at the level of 4mg/L boron (Fig. 3b).

Zare *et al.*, (2007) reported that adding zinc at concentrations of 5 and 10 µM led to a slight increasing in the chlorophyll content. This fact may indicate the importance of zinc in the activation of protein synthetase enzyme in the biosynthesis pathway of chlorophyll as well as some enzymatic antioxidants such as APX and GR, which provide protection against chlorophyll degradation by reactive oxygen radicals. Some studies had suggested that Zn has a major effect in triggering of certain enzymes involved in the biosynthesis of chlorophyll. Considering the mentioned role of zinc, it can be concluded that the zinc-treated plants have a higher chlorophyll synthesis capability. Zinc also has a major effect on plant cell defense against oxidative damage, effectively protecting these cells and their compounds such as lipid and cell membrane protein, chlorophyll, enzymes containing -SH group, and DNA against this stress (Cakmak, 2000). It is demonstrated that Zn deficiency disrupts the chlorophyll synthesis (Hisamitsu *et al.*, 2001). Increased levels of chlorophyll in the presence of Zn can be due to the structural and catalytic role of Zn in proteins and enzymes and also Zn acts as a co-factor in biosynthesis of photosynthetic pigments (Samreen *et al.*, 2017).

Carotenoids protect photosynthetic tissues, especially chlorophylls, against oxidative stress (Candan & Tarhan, 2003). Results showed that increase in the concentration of boron in the nutritional solution cause to decrease in the level of carotenoids significantly. Increasing in boron concentration reduced the carotenoid level at both zinc levels (5 and 10 mg/L). The main effects of zinc also showed that as zinc concentration increased, the level of carotenoids in plants showed a significant increase in compared to the controls. This increasing was also observed in the interaction effects of both boron levels, meaning that adding zinc decreased the effect of high concentrations of boron on the carotenoid level. The highest carotenoid content was observed at 10 mg/L concentration of zinc without adding boron and the lowest was at the 4 mg/L boron without application of zinc. Overall, these results proposed that adding zinc could mitigate the toxic effect of high boron levels as illustrated in Fig. 4.

Results showed that along with increasing in the boron concentration in nutritional solution, the protein content significantly decreased. Increasing in the Zn concentration, increased the protein content as increasing in the level of zinc (5 and 10 mg/L), which indicated that supplementing nutrient solution with zinc mitigates the harmful effect of high boron concentrations on plant biochemical traits. Also, main effects of zinc showed that as the concentration of zinc increased, the protein level increased significantly in comparison with the controls. The highest protein content was observed at the level of 5 mg/L zinc along with 2 mg/L boron and also at the level of 10 mg/L zinc without boron, which effects were not significantly different. The lowest protein content was observed at the concentration of 4 mg/L boron without zinc, which shows the adverse effect of boron on protein content and the fact that zinc could reduce this effect (Fig. 5).

According to the Table 3, the effect of zinc and boron on total soluble protein, proline, and MDA concentration were significant.

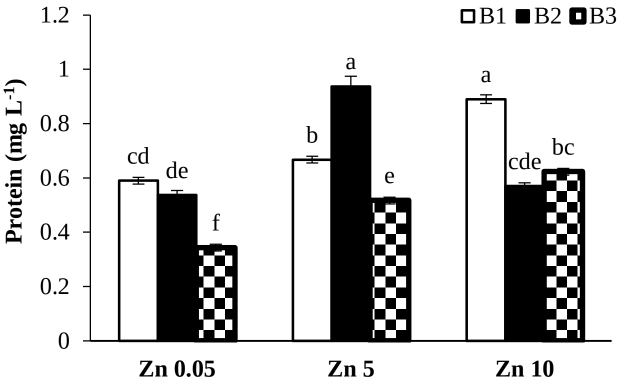

Fig. 5. Effect of zinc and boron on the protein content in lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05.

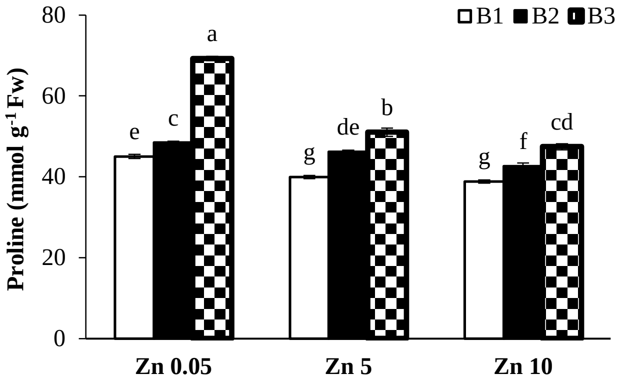

Fig. 6. Effect of zinc and boron treatments on the proline content in lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05.

Table 3. Analysis of variance of the effects of zinc and boron treatments on the total soluble protein, proline, and MDA concentrations in lettuce.

Source of variation	MS			
	df	Total soluble protein	Proline	MDA
Zinc	2	0.136**	310.4**	0.002**
Boron	2	0.130**	505.8**	0.008**
Zinc×oron	4	0.072**	78.7**	0.006**
Error	16	0.001	0.59	2.231×10^{-5}
CV (%)		4.2	1.6	16.6

ns, * and **: Not significant, Significant at 5 and 1 % level of probability, respectively

Table 4. Analysis of variance of the effect of zinc and boron on the activity of GPX, CAT, and APX.

Source of variation	df	MS		
		GPX	CAT	APX
Zinc	2	0.115**	0.018**	0.003**
Boron	2	0.390**	0.068**	0.017**
Zinc × Boron	4	0.003**	0.016**	0.001**
Error	16	0.0001	0.0001	4.028×10^{-5}
CV (%)		3.25	5.18	16

ns, * and **: Not significant, Significant at 5 and 1 % level of probability, respectively

Zinc is an essential element in the activation of different enzymes such as dehydrogenase, aldolase, isomerase, transphosphorylase, RNA polymerase and DNA polymerase, and has a major effect in sugar metabolism and protein biosynthesis. Therefore, zinc deficiency can disrupt sugar metabolism and protein production (Candan & Tarhan, 2003). The decreased protein level in plants with zinc deficiency is may be due to increased RNA degradation.

According to the figure 6 as increasing at boron concentration in nutrient solution, the proline content significantly increased in lettuce leaves. But the mean comparisons between zinc concentrations (5 and 10 mg/L) showed that, adding boron levels reduced the proline content. Analyzing the main effects showed that as the zinc concentration increased, the proline level decreased significantly in comparison with the controls as the highest level of proline was related to the 4 mg/L levels of boron without zinc while the lowest level of proline was recorded at 5 mg/L and 10 mg/L concentration of zinc without boron, which were not significantly different.

In a study by Rékási *et al.*, (2021) on some vegetables, adding zinc under boron-induced stress conditions significantly changed the plant growth traits. Also, increasing in the amount of boron in irrigation water had a significant negative effect on the proline content.

Analysis of the main effect of boron showed that as the boron level increased, MDA content increased but in interaction with zinc concentration (5 And 10 mg/L), increasing the boron level decreased the MDA content. It is important to consider that the highest MDA content, observed in the treatment with 5 mg/L zinc along with 2mg/L boron, was even higher than the its amount in the 4 mg/L boron (Fig. 7). The main effects of zinc showed that MDA content decreased with increasing in zinc concentration. The lowest MDA level was recorded at the levels of 5 mg/L and 10 mg/L Zn, which were not significantly different. This effect can be attributed to lower lipid oxidation.

The production of malondialdehyde, is an indicator for peroxidation of membrane lipids (Marschner, 1995). Lipid peroxidation can be caused by damage to the cell wall by stresses. The lipid peroxidation can result from the effect of free radicals and be a reflection of the damage caused by stresses at the cellular level, the amount of MDA produced during lipid peroxidation could be an indicator of oxidative damage. MDA can damage membrane structure and function by binding to membrane proteins and enzymes (Marschner, 1995). Zinc increases enzyme activity and decreases the MDA level. Therefore, it can be concluded that using of zinc at the accurate concentrations, can prevent lipid peroxidation by the increase in the enzyme activity of antioxidants.

Regarding the antioxidant enzymes, the interaction between zinc and boron showed a significant difference in antioxidative enzyme activities such as GPX, CAT, and APX (Table 4).

Regarding to the figure 8, as well as increasing in the concentration of boron in the nutritional solution, the activity of GPX also significantly increased. However, in interaction with increased zinc concentration (5 and 10 mg/L), increasing the boron level decreased the GPX activity. The main effects of zinc showed as zinc concentration increased, GPX activity significantly decreased in comparison with the controls. The highest GPX activity was observed in the 4 mg/L boron concentration without application of zinc and the lowest was recorded at the 10 mg/L zinc concentration without boron supplementing (Fig. 8).

The activity of these enzymes can be increased by the production of free radicals and decreased by the reduction of them. GPX is one of the most important enzymes that control hydrogen peroxide in plants and can serve as a biomarker to assess the damage caused by toxic concentrations of various heavy metals (Packr *et al*., 2003).

According to the figure 9 the main effects of boron showed that the as well as increasing in the boron level in the nutritional solution, the activity of catalase increased significantly. The mentioned elevated CAT activity was especially exposed in the treatment with 4 mg/L boron along with 5 mg/l zinc, which indicates an increase in CAT activity because of the toxic effect of boron and the insufficient ability of this concentration of zinc to mitigate this effect. However, a higher zinc concentration (10 mg/L) controlled the effect of the same amount of boron on CAT activity. The analysis of the main effects of zinc showed that as zinc concentration increased, the activity of CAT did not significantly change from the control levels. The lowest CAT activity, observed in the 10 mg/L zinc without boron, was not significantly different from the CAT activity observed with the same amount of zinc but with high amounts of boron, which shows the good effect of zinc (at the concentration of 10 mg/L) in neutralizing adverse effects of boron.

CAT and SOD are important antioxidant enzymes that sweep active oxygen species and prevent lipid oxidation, chlorophyll degradation, and cell wall damage (Singh, 2000). A catalase molecule can turn millions of H_2O_2 molecules into water and oxygen. The suitable pH for good CAT activity is 7, but the suitable temperature for this purpose varies with species. The presence of this enzyme is essential for discarding the H_2O_2 resulting from light respiration. In most of literatures on the interaction between plant and zinc, it has been reported that catalase activity decreases with increasing zinc concentration (Saeidi-Sar, 2007).

Regarding the mean comparisons of ascorbate peroxidase (APX) data, showed that as boron in the nutrient solution increased, the activity of APX also significantly increased. However, at all concentration of zinc (5 And 10 mg/L), increasing the boron level decreased the APX activity. When analyzing the main effects of zinc, it was found that increasing the concentration of zinc by 5 mg/L significantly decreased the activity of ascorbate peroxidase compared to the controls, but the change made by using higher concentrations of zinc (10 mg/L) was not significant. The highest activity of ascorbate peroxidase was belonged to the level of 4 mg/L boron without application of zinc, while the lowest activity of ascorbate peroxidase was recorded at concentrations of 5 and 10 mg/L zinc without added boron, which were not significantly different from each other in this respect. These results showed that the adverse effects of boron on APX activity started at the level of 2 mg/L and enhanced to the peak level at concentration of 4 mg/L, but zinc acted to mitigate this effect (Fig. 10).

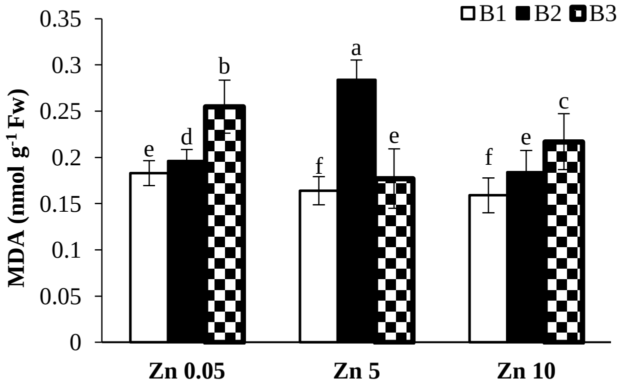

Fig. 7. Effect of zinc and boron treatments on the MDA content in lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05.

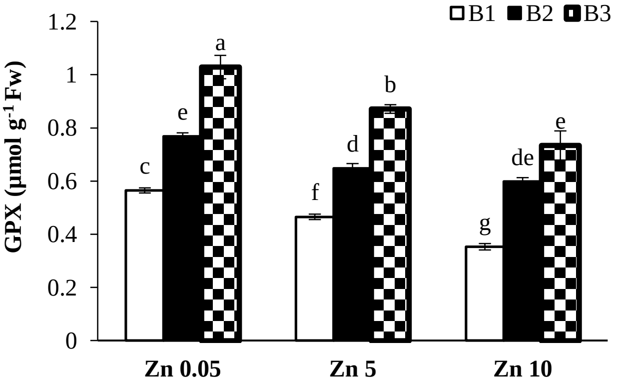

Fig. 8. Effect of zinc and boron on the guaiacol peroxidase (GPX) activity in lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05.

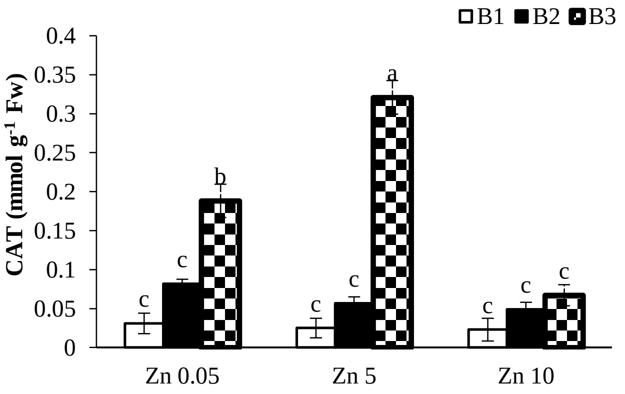

Fig. 9. Effect of zinc and boron treatments on the catalase activity (CAT) in lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05.

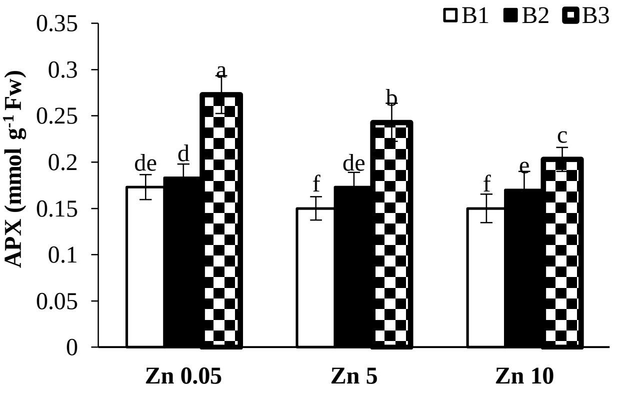

Fig. 10. Effect of zinc and boron on the ascorbate peroxidase (APX) activity in lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05.

Table 5. Analysis of variance of the effect of zinc and boron on the leaf zinc and boron concentrations in lettuce.

Source of variation	MS		
	df	Leaf zinc concentration	leaf boron concentration
Zinc	2	1886.8**	1934.9**
Boron	2	273.7**	14259.6**
Zinc × Boron	4	363.9**	322**
Error	16	0.5	2.5
CV (%)		1.9	2.9

ns, * and **: Not significant, Significant at 5 and 1 % level of probability, respectively

APX acts as defend mechanism against H_2O_2 by converting it to water. In this enzyme, ascorbic acid acts as an electron donor in the first phase of the glutathione-ascorbate cycle, where two molecules of this acid are consumed to convert H_2O_2 into water. APX has an important effect on the clearance of H_2O_2 in intracellular organs, thereby helping to maintain the redox state in cells (Costa *et al*., 2010). Results in Arabidopsis had shown that increased H_2O_2 level under stress conditions alters the electron transfer of plastoquinone, which is likely to induce APX (Foyer, 2018).

The ANOVA for zinc and boron concentrations of the nutrient content in plant (Table 5) revealed that the interaction between zinc and boron had a significantly difference on the amount of zinc and boron in lettuce leaves.

According to the figure 11 the interaction between boron and zinc concentration in the leaves, showed that increase in boron concentration in the nutritional solution caused to decrease in the amount of zinc in lettuce significantly. Also, as the concentration of zinc increased in the nutrient solution, so did the amount of zinc in the leaves of the plant in comparison with the control. A similar trend was also observed in the interaction effects of 2 mg/L boron with different zinc levels. However, in the interaction with 10 mg/L zinc, increasing the boron level decreased the concentration of zinc in the leaves. The leaf zinc concentration resulting from the combination of the 4 mg/L boron treatment with the 5mg/L zinc was lower than the levels observed with the 2 mg/L boron. The highest leaf zinc concentration was observed in 5 mg/L zinc along with 2 mg/L boron (Fig. 11)., which is probably because of the onset of the toxic effect of boron at this concentration followed by substantial zinc absorption from the root as a defensive reaction. The lowest leaf zinc concentration was observed at the control level of zinc, where even adding high amounts of boron to the nutrient solution made no significant change in the leaf zinc concentration.

According to the results obtained from this experiment, zinc can be a good way to increase the amount of zinc in lettuce leaves. O'Dell (2000) concluded that zinc by creating a protective role on the external surface cell membrane, the root counteracts the stress caused by boron toxicity.

The main effects of boron revealed that the amount of boron in the lettuce leaf increased significantly with the amount of boron in the nutrient solution. But this effect disappeared when boron was combined with zinc (5 and 10 mg/L). It should be noted that in each level of zinc, leaf boron concentration increased as the level of boron increased in the nutritional solution. Analyzing the interaction effects of zinc and boron also showed that as zinc concentration increased, leaf boron concentration decreased, which indicates the effectiveness of zinc in reducing the boron concentration in the lettuce leaf. The highest amount of leaf boron was recorded in the control plants and also lettuce fed with 4 mg/L boron. The lowest concentration of boron was recorded at the level of 10 mg/L zinc, though it was not significantly different from the concentration of 5 mg/L zinc (Fig. 12). It seems that the major role of zinc in cell membranes are related to the protection of membrane lipids and proteins against oxidation and decay as Zn^{2+} interfere generation of ROS and their oxidative damage in cell membranes (Güneş *et al., 2000*). However, adding zinc mitigated the effects of having high concentrations of boron in the nutrient solution. This is probably because zinc neutralizes the stress induced by boron toxicity and creating a protective layer on the root's outer surface or cell membranes. However, regarding the interaction effect of zinc and boron in oranges, this protective role of zinc disappears when the concentration of boron in the nutrient solution rises above a certain level, in which case, large amounts of boron will be accumulated in the plant organs. Therefore, it is important to choose the right concentration of zinc to avoid this problem.

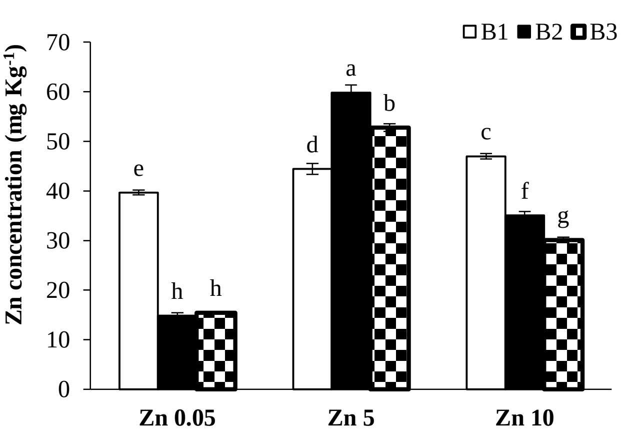

Fig. 11. Effect of zinc and boron treatments on the leaf zinc concentration in lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05.

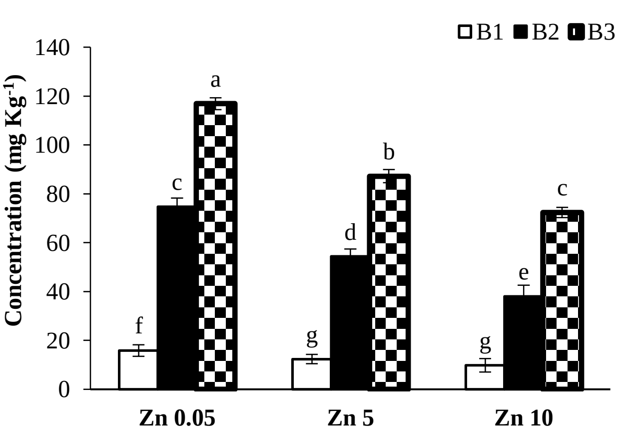

Fig. 12. Effect of zinc and boron on the leaf boron concentration in lettuce; B1: 0, B2: 2 and B3: 4mg/L; Means not sharing the same letter do not differ significantly at p≤0.05.

Conclusions

Boron and zinc are both important elements for plant physiological reactions such as improvement in leaf relative water content, chlorophyll index, leaf area, plant growth rate, and etc by means of improve in the entire yield related components in lettuce. The results of the present work demonstrated that when the level of zinc increased in the nutrient solution of lettuce, its growth traits such as fresh and dry weight, greenness, Chl *a* and *b*, total Chl, and carotenoids content, total protein, and leaf zinc concentration significantly increased. When boron increased in the nutrient solution of lettuce, its proline content, MDA content, the antioxidative enzyme activities such as GPX, CAT, and APX in the lettuce, and also its leaf boron concentration significantly increased. The results of this study indicated that adding zinc to the nutrient solution is of potential practical importance in the control of B absorption and toxicity where the plants are grown under Zn deficiency and/or boron toxicity conditions.

Author contributions: F. B. main idea and data analyzing; S.H.F.G. do experiment; H.S.H. manuscript writing and data analayzing; A. A. design experiment and A.P. data analyzing. H.S.H. wrote and proof the final paper.

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