JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2020, VOL. 3 (SPECIAL ISSUE: ABIOTIC AND BIOTIC STRESSES IN HORTICULTURAL CROPS), 43-52

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Physiological, chemical and growth responses of summer savory (*Satureja hortensis* L.) to boron under greenhouse conditions

Farid Moradinezhad[1], Soheyla Hajizadegan[1], Mohammad Hassan Sayyari[2] and Mehdi Khayyat[1*]

1, *Department of Horticultural Science, College of Agriculture, University of Birjand, Iran*
2, *Department of Soil Science, College of Agriculture, University of Birjand, Iran*

ARTICLE INFO

Original Article

Article history:
Received 7 June 2019
Revised 18 August 2019
Accepted 27 September 2019
Available online 15 February 2020

Keywords:
Boron toxicity
Chlorophyll fluorescence
Phenolic
Savory
Secondary metabolites

DOI: 10.22077/jhpr.2019.2464.1057
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**

Department of Horticultural Science, College of Agriculture, University of Birjand, Iran.
Email: mhdkhayat@birjand.ac.ir

ABSTRACT

Purpose: Boron toxicity is an important factor, which reduces crop productivity, quality and medicinal characteristics around the world. **Research method:** This research was conducted to investigate the effect of different boron (B) concentrations on growth, chemical and physiological characteristics of *Satureja hortensis* plants under greenhouse conditions in 2016. The experiment was conducted in a glasshouse with 25±3 °C and 15±3 °C (day-night) temperatures, 60% relative humidity and 340 ppm CO_2 concentration. Treatments were different B concentrations as H_3BO_3 that used via irrigation water including 0, 5, 10, 25 and 50 mg L^{-1}. **Main findings:** Results indicated that total nitrogen, Fm, Fv, Fv/Fm, chlorophyll a, b and total, leaf number, relative water content (RWC), specific leaf weight (SLW), areal fresh and dry weights significantly decreased by B increase. On the other hand, increased B concentration in leaf was led to increasing of F0, ΦN0, proline, total carbohydrate and phenolics, specific leaf area and leaf electrolyte leakage. **Limitations:** No significant limitation to report. **Originality/Value:** It is concluded that summer savory can tolerate B concentrations up to 10 mg L^{-1}, and leaf boron accumulation significantly inhibited, however, increased with more B concentration of root medium, which affected all physiological aspects mentioned above.

INTRODUCTION

Aromatic and medicinal plants are known since long time ago because of aromatic, antiseptic and preservative properties and they are used as spices, natural foods and in perfume industries. *Satureja hortensis* is the annual species, belongs to Lamiaceae family and distributed in southern and south-eastern of Europe, Asia Minor and northern Africa (Mihajilov–Krstev et al., 2010). This plant is used in cookery, food preservations and for treating many diseases and under normal growth or stress conditions, it produces essential oils and antimicrobial secondary metabolites (Šilić, 1979).

B may be excess in soil naturally, or as a result of high fertilization and/ or applying water rich in B. In general, B toxicity occurs in soils of arid and semiarid regions either as a result of irrigation water with high B concentration or high soil B level, whereas B deficiency appears in sandy soils or humid regions (Feigin et al., 2012). Supplying some fertilizers including potassium chloride and some animal manure can add a significant amount of B to soils where applied (Muntean, 2015). On the other hand, B toxicity is an important cause, which reduces crop productivity around the world (Nable et al., 1997) and affects plant growth and some morpho-physiological processes (Shah et al., 2017). Conversely, it may be insufficient in some areas, since it is present in soil as boric acid, which is easily leached out (Tanaka & Fujiwara, 2008). Boron is a micronutrient that its mobility within plant organs is species dependent, which determines visual symptoms of B excess (Brown & Shelp, 1997; Nable et al., 1997). In plants with low B mobility, the toxicity symptoms are chlorosis and/ or necrotic patches in older leaves where B tends to accumulate (Nable et al., 1997). Conversely, in plants with higher mobility, the first symptom of B toxicity is seen in meristematic regions and fruits, but not in mature leaves (Brown & Hu, 1996). It was cleared that via reduction of leaf expansion, photosynthetic efficiency, fruit set and crop production significantly decreased under boron toxicity (Nable et al., 1997; Simón-Grao et al., 2018). There are many reports indicating that B toxicity changes physiological activity in plants. Landi et al. (2013) evaluated the resistance of two basil varieties under B excess and showed negative effects of this stress on growth and photosynthesis, depending on variety. Guidi et al. (2011) in *Solanum lycopersicum* and Han et al. (2009) in citrus found a reduction of the maximum quantum yield of PSII (F*v*/Fm) in leaf under B toxicity. Goldberg et al. (2003) and Sonmez et al. (2009) showed that increment of boron around root area may increase its concentration within plant tissues. Nalini Pandey (2013) stated that boron toxicity (0.33 and 33 mg L^{-1}) significantly decreased photosynthetic pigments in *Brassica*. However, some plants do not show any toxicity symptoms despite having B in their tissue at high concentrations. Nable et al. (1997) stated that B–toxicity is getting more attention in arid and semi-arid regions, which severely reduces plant growth and yield. To our knowledge, there is no report about summer savory response to boron toxicity. Thus, the present work was conducted to find physiological responses of this medicinal plant to boron toxicity.

MATERIALS AND METHODS

Plant materials and growth conditions

A pot experiment was conducted in a glasshouse with 25±3 and 15±3 °C (day-night) temperatures, 60% relative humidity and 340 ppm CO_2 concentration in College of Agriculture, University of Birjand, Iran. The conditions were controlled using split greenhouse instruments by MOELLER, China. Certificated seed of summer savory (PakanBazr CO., Isfahan, Iran) was sown in 3 Kg pots containing sandy–loamy soil (pH=7.4;

ECe=1.3dS m^{-1}; N=0.032%; P=8mg Kg^{-1} Soil; K=210mg Kg^{-1} Soil; Ca=2.60 meq L^{-1}; Mg=2.60 meq L^{-1}; B=0.89mg Kg^{-1} Soil; Fe=4.79mg Kg^{-1} Soil; Cu=0.65mg Kg^{-1} Soil; Zn=0.74 mgKg^{-1} Soil; Mn=1.96 mg Kg^{-1} Soil; Cl= 0.50 meq L^{-1}; and Na= 4 meq L^{-1}). Irrigation was done using distilled water until 6th leaf was presented. Thereafter, seedlings submitted to different boron concentrations at 0, 5, 10, 25 and 50 mg $L^{-1}$by adding to irrigation water. To avoid osmotic shock to the plants, treatments were applied incrementally over three-days to achieve the highest level of boron level. For each irrigation the soil moisture was brought to field capacity by weighing the pots with no drainage.

Nitrogen and boron determination

Nitrogen was determined by the Kjeldahl method, and azomethine-H method (Wolf, 1974) was used for boron. Each sample was dried at 50 °C for 48 h. The oven-dried samples were then ground and ashed at 550°C in a porcelain crucible for 90 min. The ash was taken up in sulfuric acid and filtered for future use. The B concentration was colorimetrically determined at 420 nm using spectrophotometer (Shimadzu AA–670, Japan).

Chlorophyll (Chl) fluorescence and chlorophyll content

Chl fluorescence was measured on the top attached and dark-adapted leaf of each plant using a Mini Pam Fluorometer (Walz, Effeltrich, Germany) using the protocol described by Genty et al. (1989). For assessment of Chl fluorescence parameters, leaves were put in the dark-adapted state for 30 min (Genty et al., 1989) using light exclusion clips. Under the dark state, all reaction centers and electron carriers of PSII are re-oxidized that is necessary for rapid induction of fluorescence. Roháček (2002) and Zhang and Xu (2003) stated that under this condition qN is relaxed to its minimum value. Low intensity modulated light (<0.1 µmol m^{-2} s^{-1}) that not to induce any effect in the fluorescence variable, was used to measure F_0. The F_m was obtained by 0.3-second pulses of 20000 Hz saturating light. From F_0 and F_m, ΦN0 ($\frac{F0}{Fm}$) (Roháček, 2002) were calculated as followed (1):

$$\Phi N0 \text{ (Basal quantum yield of non} - \text{photochemical processes in PS2)} = \frac{F0}{Fm}: \quad (1)$$

Where F_0 is the minimal fluorescence in the dark – adapted state, F_m is the maximal fluorescence in the dark – adapted state; F_v– the variable fluorescence; F_v/F_m– The maximum quantum yield of PSII or maximum PSII photochemical efficiency.

Chl content (mg g^{-1} FM) was determined using 80% acetone and also hand-held SPAD 502 meter (Minolta, Osaka, Japan). 10 mL acetone (80%) was added to 0.25 g leaf disks for extraction, then centrifuged in 8000 g for 10 min and the supernatant was used to make a final volume of 50 mL of leaf extract. Homogenization of leaf tissue with the buffer extraction was continued until colorless. The absorbance of the extract was read in 645 and 663 nm with a spectrophotometer (Shimadzu AA–670, Japan). For the blank, 80% acetone was used. Then, chlorophylls a, b and total content was calculated based on the method of Saini et al. (2001).

Proline, total soluble carbohydrate, and phenolics

Proline was determined by the ninhydrin method described by Bates et al. (1973), using L–proline as a standard (0–500 µmol, *MERCK*). 0.5 g leaf samples (FM) were homogenized in 10 ml of 3% (w/v) aqueous sulfosalicylic acid and centrifuged for 30 min at 14,000 × *g*. Ninhydrin (2 ml) and glacial acetic acid (2 ml) were added to the supernatant and the mixture was boiled at 100°C for 1 h and then placed in an ice bath to stop the reaction. After extraction with toluene, free proline was quantified at 520 nm using a spectrophotometer

(Shimadzu AA–670, Japan). The equation used for standard curve preparation was as followed (2):

$$y = 252.38\ x - 8.25\ (R^2 = 0.90) \quad (2)$$

Total leaf soluble carbohydrates were determined according to Irigoyen et al. (1992) and glucose (0–100 mg l^{-1}, from *MERCK*) was used as a standard. Leaf samples of 0.5 g (FM) were homogenized in 5 ml ethanol (95%) and centrifuged at 4,500 × *g* for 15 min, the supernatant was removed from the sample and the residue was resuspended in 5 ml of 70% ethanol. Then the supernatant was centrifuged again for final extraction. Both supernatants were combined. Anthrone-sulfuric acid assay was used for determination. An aliquot of 100 µl was added to 3 ml of anthrone-sulfuric acid solution and the mixture was shaken, heated in a boiling water bath for 10 min and cooled at 4°C. The absorption was determined at 625 nm by spectrophotometer (SHIMADZU AA-670, Japan). Equation used for standard curve preparation was as followed: y = 545.04 x – 29.973 (R^2 = 0.94).

One g of dried leaves were extracted by the addition of 10 ml of 70% (v/v) aqueous methanol (Merck KGaA, Darmstadt, Germany), after being shaken for 2 h at room temperature, and centrifuged at 4.000 × *g* for 10 min. Consequently, the supernatant was separated from the solid particles and was analyzed for total phenolic content (TPC). Total phenolic content (TPC) was estimated by Folin-Ciocalteau's (FC) assay with some modifications (Makkar et al., 1993); 450 µl of distilled water was added to 50 µl of prepared extract. Then 250 µl of FC reagent (FC, Merck KGaA, Darmstadt, Germany) (1 N) was added, vortex-mixed and left to stand for 5 min. Next, 1.25 ml of $Na_2CO_3.10H_2O$ (20%, Merck KGaA, Darmstadt, Germany) was added to the mixture and incubated for 40 min under dark conditions at room temperature. Finally, the absorbance was measured at 725 nm using a spectrophotometer (Shimadzu AA–670, Japan). A calibration curve was also prepared using gallic acid (Merck KGaA, Darmstadt, Germany) and results were expressed as mg gallic acid per g dry matter.

Leaf number, relative water content (RWC), specific leaf area (SLA), specific leaf weight (SLW) and electrolyte leakage (EL)

After treating the plants with boron treatments, leaf numbers was evaluated in different times randomly. Leaf samples were weighed to determine the fresh mass (FM), soaked in distilled water at 25°C for 4 h to determine the turgid mass (TM), then oven-dried at 80°C for 24 h to determine the dry mass (DM). Finally, RWC was calculated based on the method by Barrs and Weatherley (1962). For SLA and SLW, leaf segments were dried in an oven at 100°C for 48 h and then calculated based on Hunt (1990). Large leaf segments were cut out at random, washed 3 times with distilled water in order to remove surface contaminants, and then placed individually in stoppered vials containing 10 ml of distilled water. Consequently, they were incubated at room temperature (25ºC) on a shaker (100 × *g*) for 24 h to measure EC of the solution (EC1). Then the same vials with leaf samples were placed in an autoclave at 120ºC for 20 min and the 2nd measurement of conductivity (EC2) was done after cooling the solution to room temperature. The ion leakage was calculated using a method by Lutts et al. (1995).

Biomass production

In regular intervals, some plants were removed from each pot, and used to weight for fresh weight (F.W.). Then, oven dried at 60 °C and weighted using a balance (with 0.0001 accuracy) to calculate dry weight (D.W.).

Statistical analysis

This experiment was set up as split-plot in time for leaf number, SLA, SLW, RWC and biomass production, and based on completely randomized design for other traits, with 5 treatments and 6 replications, and 5 pots in each replication. Some traits were evaluated once, and some were assessed two or three times. Statistical analysis of data was carried out using analysis of variance (ANOVA) procedure on GenStat program (12th edition). The averages were compared with LSD at 1% level.

RESULTS AND DISCUSSION

Nutrient concentrations

Boron toxicity was led to the reduction of leaf nitrogen content significantly and the lowest N concentration (2.56 and 2.31%, respectively) obtained in 25 and 50 mg L^{-1}, although there was no significant difference among 0, 5 and 10 mg L^{-1}. Princi et al. (2013) suggested that B excess can also affected nitrate uptake by roots, affecting the PM H^+-ATPase activity. The boron content of leaf significantly affected by treatments of 25 and 50 mg $L^{-1}$and the highest level (135.05 mg Kg^{-1}) observed in 50 mg L^{-1}, however, no sign of toxicity was seen in aerial plant tissues. These results were in agreement with findings of Landi et al. (2013) in basil plants. Moreover, results showed that there is no significant difference among 0, 5 and 10 mg L^{-1}about this variable (Table 1). It is clear that accumulation of B within areal tissues inhibited by boron concentrations up to 10 mg L^{-1}. Despite having high B concentrations in leaves, which was in agreement with Sonmez et al. (2009), who stated that some plant does not show toxicity symptoms under toxicity concentrations. Powell et al. (1997) reported that B moves into plants via active transpiration across a concentration gradient and then moves readily through the xylem in the transpiration stream and accumulates at the point where water is lost through stomata in the leaf. Data from different sampling times showed no significant difference between all B levels at two sampling times (data not shown).

Table 1. Effect of boron levels on leaf nitrogen (N) and boron (B) concentrations

Treatment (mg L^{-1} H_3BO_3)	N (%)	B (mg Kg^{-1})
0	3.96a	90.95c
5	3.61a	92.00c
10	3.56a	97.75c
25	2.56b	113.35b
50	2.31b	135.05a

Within each column, the same letter indicates no significant difference between treatments at 1% level of FLSD.

Table 2. Simple effect of boron levels and evaluation time on chlorophyll fluorescence indices

Treatment (mg L^{-1} H_3BO_3)	F0	Fm	Fv	ΦN0	Fv/Fm
0	188.33d	910.55a	722.22a	0.207d	0.79a
5	204.44cd	889.72b	685.27b	0.230c	0.76b
10	220.27c	882.22b	661.94c	0.250c	0.75b
25	283.05b	847.50c	564.44d	0.334b	0.66c
50	319.72a	814.16d	494.44e	0.394a	0.60d
Time (day)					
7	271.66a	859.33b	587.66c	0.320a	0.67c
14	244.16b	865.50b	621.33b	0.285b	0.71b
21	213.66c	881.66a	668.00a	0.244c	0.75a

Within each column, the same letter indicates no significant difference between treatments at 1% level of FLSD.

Chl fluorescence and chl concentrations

F0 significantly increased with the increase of B concentration from 0 to 50 and the highest level of F0 was observed in 50 mg L^{-1}. Fm significantly decreased with B increment, which the lowest rate was observed in 50 mg L^{-1}. Variable fluorescence (Fv) also affected by boron treatments and the highest and lowest rates were observed in control and 50 mg L^{-1}, respectively (Table 2). The reduction of Fv along an increase in F0 is considered to be the characteristic of inhibition of the acceptor side of PSII (Šetlík et al., 1990). The basal quantum yield of non–photochemical process in PSII also significantly increased with toxicity increment. The highest and lowest Fv/Fm was shown in control (0) and 50 mg L^{-1} boron concentration, respectively (Table 2) that was in agreement with findings of Han et al. (2009) and Guidi et al. (2011). Any decline in Fv/Fm caused by excess boron may result from produced reactive oxygen species (ROS). In fact, reduction of ATP and NADPH under stress condition induces excitation energy needed by ROS producing process. Decreasing carbon assimilation processes are related to photosystem II behavior into environmental stresses and may be caused by a combination of factors such as oxidative damage, reduced photosynthetic enzyme activities and impaired electron transport capacity (Han et al., 2009). Different sampling times including day 7th, 14th and 20th showed a significant effect of boron toxicity on fluorescence indices. Results revealed a reducing manner in F0 and ΦN0 ($\frac{F0}{Fm}$), and increasing trend for Fm, Fv and Fv/Fm with time (Table 2). The lowest and highest chlorophyll a, b and total was observed in 50 mg L^{-1} boron and control, respectively, which was in agreement with findings of Landi et al. (2013) and Nalini Pandey (2013). Total chl unaffected by concentrations up to 5 mg L^{-1}, but, both chlorophylls a and b significantly decreased when boron concentration increased. This decline was probably resulted from damages that occur in the chloroplast structure, specifically in the thylakoid membrane, which influences due to lipid peroxidation by ROS under B toxicity (Landi et al., 2013). The both ratio of a/b and b/a chlorophyll significantly responded to boron toxicity, and converse trend was observed. An increasing trend for a/b and reducing manner for b/a ratio were indicated with time (Table 3). Cave et al. (1981) on *Trifolium* stated that excessive starch accumulation physically distorted the chloroplast structure, leading to lower CO_2 assimilation and Chl content. Both sampling times showed a reducing trend for all chlorophyll indices, except by chl ratio (Table 3).

Table 3. Simple effect of boron levels and evaluation time on leaf chlorophylls a, b and total

Treatment	Chl a	Chl b	Total Chl	Chl a/b	Chl b/a
(mg L^{-1} H_3BO_3)	(mg g^{-1} Leaf F. W.)			Ratio	
0	11.25a	5.47a	9.34a	2.06bc	0.49b
5	9.60b	4.27b	9.20a	2.25ab	0.45b
10	8.43c	3.43c	8.76b	2.46a	0.43b
25	5.85d	3.10d	4.08c	1.87c	0.58b
50	2.70e	2.45e	3.32c	1.13d	0.97a
Time (day)					
14	8.39a	3.92a	7.38a	2.09a	0.56a
21	6.74b	3.56b	4.32b	1.81a	0.60a

Within each column, the same letter indicates no significant difference between treatments at 1% level of FLSD.

Table 4. Effect of boron levels on leaf proline, total carbohydrates, and phenolic contents

Treatment (mg L^{-1} H_3BO_3)	Proline (µg g^{-1} Leaf F.W.)	Total carbohydrates (mg g^{-1} Leaf F. W.)	Total phenolic (mg g^{-1} Leaf D. W.)
0	3.00c	24.33b	1.54c
5	3.36c	23.85b	1.54c
10	3.19c	26.31b	1.79bc
25	4.13b	33.97a	1.96b
50	5.05a	39.07a	2.28a

Within each column, the same letter indicates no significant difference between treatments at 1% level of FLSD.

Table 5. Simple effect of boron levels and evaluation time on leaf number, RWC, SLA, SLW, EL, fresh weight and dry weight

Treatment	Leaf no. (Plant^{-1})	RWC (%)	SLA (cm^2 g^{-1})	SLW (g cm^{-2})	EL (%)	F.W. (g Plant^{-1})	D.W. (g Plant^{-1})
0	25.33a	77.45a	20.60c	0.05a	30.08d	1.29a	0.39a
5	22.56b	74.09ab	19.78c	0.05a	40.95c	1.10b	0.27b
10	20.44c	75.17ab	19.16c	0.05a	41.17c	0.98bc	0.19c
25	18.67d	65.81c	26.54b	0.04b	58.43b	0.89cd	0.19c
50	16.78e	63.09c	31.16a	0.03b	61.00a	0.75d	0.10d
Time (day)							
7	17.33c	73.37a	26.24a	0.04b	46.10a	0.67c	0.20b
14	21.10b	71.36a	21.81b	0.05a	50.95a	1.07b	0.22b
21	23.83a	68.64b	22.29b	0.05a	53.24a	1.26a	0.26a

Within each column, the same letter indicates no significant difference between treatments at 1% level of FLSD.

Proline, total carbohydrates and phenolic content

The highest amounts of proline, total carbohydrate, and phenolic contents were obtained in 50 mg L^{-1} that was in agreement with findings of Nalini Pandey (2013) and Sarafi et al. (2017), although there was no significant difference between 25 and 50 mg L^{-1} about total carbohydrate (Table 4). Data showed that parameters including proline, total carbohydrate, and total phenolic content unaffected by B treatments up to 10 mg L^{-1} (Table 4). The increment of proline (Nalini Pandey, 2013) and total carbohydrates may be related to water deficit condition in plant tissues. It is likely that high boron concentration induces carbohydrate demand that may be a reason why this trait increased in our plant tissues. The antioxidant system in plants induces under biotic and abiotic stresses to respond to the generated oxidative damages (Mittler, 2002). There are some reports indicating that phenols, anthocyanins, and flavonoids raise anti oxidative ability in plants (Surveswaran et al., 2007; Lee & Scagel, 2009). Polyphenols are typically produced by plants for different reasons including defense mechanisms, nutrient availability (excess or deficiency) and UV radiation (Herms & Mattson, 1992; Rozema et al., 1997; Mullen et al., 2007). The antioxidant ability of phenols is mainly attributable to their redox properties, which induces their ability to act as reducing agents, hydrogen donators and singlet oxygen quenchers. Moreover, they have a metal chelation potential (Rice-Evans et al., 1997). Han et al. (2009) suggested that boron toxicity decreases plant CO_2 assimilation, so fewer energized-photons are captured by the light harvesting system and is used in electron transport. As a result and particularly under high PF (photon flux), excess absorbed photon flux can potentially lead to the production of $^1O_2$ and other reactive oxygen species (ROS), causing damage to photosynthetic apparatus. Under this condition and to minimize cellular damage caused by ROS, plants have evolved a scavenging system composed of antioxidants including phenolic, which may be the reason why total phenolic contents increased in our experiment.

Vegetative responses

Leaf number significantly decreased with increasing boron concentration, and the lowest number was obtained in 50 mg L^{-1}. It is probable that B could reduce the available concentration of important metabolic intermediates, particularly NAD^+ and to a lesser extent NADH and NADPH (Reid et al., 2004), which are important in assimilation and growth pre-requisites. Sharp and LeNoble (2002) suggested that increased internal ABA concentration may be the cause of reduced growth and development under mineral nutrient stress. B toxicity reduces growth and causes chlorosis starting at the leaf tip and the margin of mature leaves (Paparnakis et al., 2013; Sarafi et al., 2017). RWC unaffected by different concentrations up to 10 mg L^{-1}, however, significantly decreased in 25 and 50 mg L^{-1} that was in agreement with Nalini Pandey (2013) in *Brassica* seedling and may be related to the water deficit

condition in leaves. The highest SLA was observed in 50 mg L^{-1}, and this trait unaffected by 0 to 10 mg L^{-1}. SLW unaffected by concentrations up to 10, however, significantly decreased in 25 and 50 mg L^{-1}. SLA is directly related to leaf area, and this plant tried to increase leaf area under stress status. An increasing trend indicated about EL with an increment of boron concentration, and the highest value achieved in 50 mg L^{-1}. The highest fresh and dry weight was observed in control plants and increasing B concentration decreased these variables (was about by 58% in comparison to the control) (Table 5) that was in agreement with Yermiyahu et al. (2008) and Landi et al. (2013). Our findings indicated that high B concentration in the growth medium promoted B uptake and accumulation and then, a reducing biomass production was observed. All sampling times showed increasing trend about leaf number, SLW, fresh and dry weight and a reducing manner about RWC and SLA (Table 5). In control plants, leaf number significantly increased with time, but this trait showed the different trend at different B levels. Results indicated that there were no differences among 5, 10, 25 and 50 mg L^{-1} at first sampling time about leaf number, but, an improvement can be seen in next times, where the lowest leaf number at final sampling was observed just in 50 mg L^{-1} (data not shown), which may be related to proline, total carbohydrates and phenolics accumulation in plant tissues. In all sampling, the highest areal dry weight was observed in control plants and increasing B level significantly decreased this variable (data not shown).

CONCLUSION

Boron toxicity significantly affected all physiological aspects of *Satureja hortensis* plants. B accumulation within plant tissues significantly increased as external B levels increased. The maximum PSII photochemical efficiency and chl content decreased with increasing B levels. Osmotic adjustment using proline and carbohydrate induced with B accumulation and antioxidant activities also increased under this condition. Moreover, Leaf number, RWC, SLW and EL significantly decreased with boron concentration increment. Our results indicated that this plant can tolerate B concentrations up to 10 mg L^{-1}, and concentrations more than this levels may induce stress conditions and yield reduction.

Acknowledgements
We would like to thank University of Birjand for financial support of this research.

Conflict of interest
The authors declare no conflict of interest.

REFERENCES

Barrs, H. D., & Weatherley, P. E. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences*, *15*(3), 413-428. https://doi.org/10.1071/BI9620413

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, *39*(1), 205-207. https://doi.org/10.1007/BF00018060

Brown, P. H., & Hu, H. (1996). Phloem mobility of boron is species dependent: evidence for phloem mobility in sorbitol-rich species. *Annals of Botany*, *77*(5), 497-506. https://doi.org/10.1006/anbo.1996.0060

Brown, P. H., & Shelp, B. J. (1997). Boron mobility in plants. *Plant and Soil*, *193*(1-2), 85-101. https://doi.org/10.1023/A:1004211925160

Cave, G., Tolley, L. C., & Strain, B. R. (1981). Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. *Physiologia Plantarum*, *51*(2), 171-174. https://doi.org/10.1111/j.1399-3054.1981.tb02694.x

Feigin, A., Ravina, I., & Shalhevet, J. (2012). *Irrigation with treated sewage effluent: Management for Environmental Protection* (Vol. 17). Springer Science & Business Media.

Genty, B., Briantais, J. M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *990*(1), 87-92. https://doi.org/10.1016/S0304-4165(89)80016-9

Goldberg, S., Shouse, P. J., Lesch, S. M., Grieve, C. M., Poss, J. A., Forster, H. S., & Suarez, D. L. (2003). Effect of high boron application on boron content and growth of melons. *Plant and Soil*, *256*(2), 403-411. https://doi.org/10.1023/A:1026186311974

Guidi, L., Degl'Innocenti, E., Carmassi, G., Massa, D., & Pardossi, A. (2011). Effects of boron on leaf chlorophyll fluorescence of greenhouse tomato grown with saline water. *Environmental and Experimental Botany*, *73*, 57-63. https://doi.org/10.1016/j.envexpbot.2010.09.017

Han, S., Tang, N., Jiang, H. X., Yang, L. T., Li, Y., & Chen, L. S. (2009). CO2 assimilation, photosystem II photochemistry, carbohydrate metabolism and antioxidant system of citrus leaves in response to boron stress. *Plant Science*, *176*(1), 143-153. https://doi.org/10.1016/j.plantsci.2008.10.004

Herms, D. A., & Mattson, W. J. (1992). The dilemma of plants: to grow or defend. *The Quarterly Review of Biology*, *67*(3), 283-335. https://doi.org/10.1086/417659

Hunt, R. (1990). Basic growth analysis. *In: Plant Growth Analysis for Beginners*, London: Unwin Hyman, 110p. https://doi.org/10.1007/978-94-010-9117-6

Irigoyen, J. J., Einerich, D. W., & Sánchez-Díaz, M. (1992). Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativd*) plants. *Physiologia Plantarum*, *84*(1), 55-60. https://doi.org/10.1111/j.1399-3054.1992.tb08764.x

Landi, M., Pardossi, A., Remorini, D., & Guidi, L. (2013). Antioxidant and photosynthetic response of a purple-leaved and a green-leaved cultivar of sweet basil (*Ocimum basilicum*) to boron excess. *Environmental and Experimental Botany*, *85*, 64-75. https://doi.org/10.1016/j.envexpbot.2012.08.008

Lee, J., & Scagel, C. F. (2009). Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. *Food Chemistry*, *115*(2), 650-656. https://doi.org/10.1016/j.foodchem.2008.12.075

Lutts, S., Kinet, J. M., & Bouharmont, J. (1995). Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *Journal of Experimental Botany*, *46*(12), 1843-1852. https://doi.org/10.1093/jxb/46.12.1843

Makkar, H. P., Blümmel, M., Borowy, N. K., & Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of the Science of Food and Agriculture*, *61*(2), 161-165. https://doi.org/10.1002/jsfa.2740610205

Mihajilov-Krstev, T., Radnović, D., Kitić, D., Stojanović-Radić, Z., & Zlatković, B. (2010). Antimicrobial activity of *Satureja hortensis* L. essential oil against pathogenic microbial strains. *Archives of Biological Sciences*, *62*(1), 159-166. https://doi.org/10.2298/ABS1001159M

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, *7*(9), 405-410. https://doi.org/10.1016/S1360-1385(02)02312-9

Mullen, W., Marks, S. C., & Crozier, A. (2007). Evaluation of phenolic compounds in commercial fruit juices and fruit drinks. *Journal of Agricultural and Food Chemistry*, *55*(8), 3148-3157. https://doi.org/10.1021/jf062970x

Muntean, D. W. (2015). Boron, the overlooked essential element. *Bellevue: Soil and Plant Laboratory Inc*.

Nable, R. O., Ba-uelos, G. S. & Paull, J. G. (1997). Boron toxicity. *Plant Soil*, *193*, 181-198. https://doi.org/10.1023/A:1004272227886

Nalini Pandey, A. (2013). Antioxidant responses and water status in Brassica seedlings subjected to boron stress. *Acta Physiologiae Plantarum*, *35*(3), 697-706.

Paparnakis, A., Chatzissavvidis, C., & Antoniadis, V. (2013). How apple responds to boron excess in acidic and limed soil. *Journal of Soil Science and Plant Nutrition, 7*, 787-796.

Powell, R. L., Kimerle, R. A., Coyle, G. T., & Best, G. R. (1997). Ecological risk assessment of a wetland exposed to boron. *Environmental Toxicology and Chemistry*, *16*(11), 2409-2414.

Princi, M. P., Lupini, A., Araniti, F., Sunseri, F., & Abenavoli, M. R. (2013). Short-term effects of boron excess on root morphological and functional traits in tomato. In: XVII International Plant Nutrition Colloquium- Boron Satellite Meeting–Proceedings Book–17–18 August 2013. Istanbul, Turkey, pp. 1150-1151.

Reid, R. J., Hayes, J. E., Post, A., Stangoulis, J. C. R., & Graham, R. D. (2004). A critical analysis of the causes of boron toxicity in plants. *Plant, Cell & Environment*, *27*(11), 1405-1414. https://doi.org/10.1111/j.1365-3040.2004.01243.x

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, *2*(4), 152-159. https://doi.org/10.1016/S1360-1385(97)01018-2

Roháček, K. (2002). Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica*, *40*, 13-29. https://doi.org/10.1023/A:1020125719386

Rozema, J., van de Staaij, J., Björn, L. O., & Caldwell, M. (1997). UV-B as an environmental factor in plant life: stress and regulation. *Trends in Ecology & Evolution*, *12*(1), 22-28. https://doi.org/10.1016/S0169-5347(96)10062-8

Saini, R. S., Sharme, K. D., Dhankhar, O. P., & Kaushik, R. A. (2001). *Laboratory Manual of Analytical Techniques in Horticulture*. Published by Agrobois: India, pp. 49-50.

Sarafi, S., Siomos, A., Tsouvaltzis, P., Chatzissavvidis, Ch., & Therios, I. 2017. Boron toxicity effects on grafted and non-grafted pepper (*Capsicum annuum*) plants. *Journal of Soil Science and Plant Nutrition*, *17*(2), 441-460.

Šetlík, I., Allakhverdiev, S. I., Nedbal, L., Šetlíková, E., & Klimov, V. V. (1990). Three types of photosystem II photoinactivation. *Photosynthesis Research*, *23*(1), 39-48. https://doi.org/10.1007/BF00030061

Shah, A., Wu, X., Ullah, A., Fahad, S., Muhammad, R., Yan, L., & Jiang, C. 2017. Deficiency and toxicity of boron: Alterations in growth, oxidative damage and uptake by citrange orange plants. *Ecotoxicology and Environmental Safety*, *145*, 575-582.

Sharp, R. E., & LeNoble, M. E. (2002). ABA, ethylene and the control of shoot and root growth under water stress. *Journal of Experimental Botany*, *53*(366), 33-37. https://doi.org/10.1093/jexbot/53.366.33

Šilić, Č. (1979). Monografija rodova *Satureja* L., *Calamintha miller*, *Micromeria bentham*, *Acinos miller* I *clinopodium* L. Flora Jugoslavije. Zemaljski Muzej BiH, Sarajevo.

Simón-Grao, S., Nieves, M., Martínez-Nicolás, J. J., Cámara-Zapata, J.M., Alfosea-Simón, M., & García-Sánchez, F. (2018). Response of three citrus genotypes used as rootstocks grown under boron excess conditions. *Ecotoxicology and Environmental Safety, 15*, 159:10-19

Sonmez, O., Aydemir, S., & Kaya, C. (2009). Mitigation effects of mycorrhiza on boron toxicity in wheat (*Triticum durum*) plants. *New Zealand Journal of Crop and Horticultural Science*, *37*(2), 99-104. https://doi.org/10.1080/01140670909510254

Surveswaran, S., Cai, Y. Z., Corke, H., & Sun, M. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chemistry*, *102*(3), 938-953. https://doi.org/10.1016/j.foodchem.2006.06.033

Tanaka, M., & Fujiwara, T. (2008). Physiological roles and transport mechanisms of boron: perspectives from plants. *Archiv-European Journal of Physiology*, *456*(4), 671-677. https://doi.org/10.1007/s00424-007-0370-8

Yermiyahu, U., Ben-Gal, A., Keren, R., & Reid, R. J. (2008). Combined effect of salinity and excess boron on plant growth and yield. *Plant and Soil*, *304*(1-2), 73-87. https://doi.org/10.1007/s11104-007-9522-z

Zhang, H. B., & Xu, D. Q. (2003). Role of light-harvesting complex 2 dissociation in protecting the photosystem 2 reaction centres against photodamage in soybean leaves and thylakoids. *Photosynthetica*, *41*(3), 383-391. https://doi.org/10.1023/B:PHOT.0000015462.71601.d7