

IMPROVING GROWTH AND PRODUCTIVITY OF POTATO (*Solanum tuberosum* L.) BY SOME BIOSTIMULANTS AND LITHOVIT WITH OR WITHOUT BORON

Farouk, S.

Agricultural Botany Department, Faculty of Agriculture,
Mansoura University, Egypt.

Email: gadalla@mans.edu.eg



ABSTRACT

Two field experiments were conducted during the two growing seasons of 2014/2015 and 2015/2016 at private farm, Sherbeen region, Dakahlia Governorate, Egypt, to evaluate the role of some biostimulants and lithovit with or without boron foliar application on potato growth and yield as well as some physiological and anatomical characters.

Results revealed that foliar application of biostimulants and lithovit with or without boron application significantly increased potato growth parameters (i.e. Plant height, branch number per plant, shoot fresh and dry weights, and leaf area per plant), as well as the potato tuber number and total tuber yield per plant. Quality of potato tuber significantly increased with biostimulants and lithovit with boron application. The most effective treatment in this regard was seaweed extract plus boron fertilizer.

It is recommended to spray potato plants twice at 50 and 60 days from planting with 500 mg/l seaweed extract plus 1000 mg/l chelated boron in order to attain the highest yield and quality.

Keywords: Anatomy, biostimulants, lithovit, potato, quality

INTRODUCTION

Potato (*Solanum tuberosum* L.) as a member of the family Solanaceae is the fourth important crop in the world in production volume (Fabeiro *et al.*, 2001). Potato tubers are excellent sources of carbohydrates, protein, mineral, vitamins B and C (Muthoni and Nyamango, 2009). In Egypt, the policy of the country aims to improve potato production so as to meet the increasing demand of the local consumption and to increase the amount of potato for exporting. So we had to be searching for new means which enable us to increase the productivity of the land, which is made by using biostimulants and fertilizers.

Soils of arid and semi-arid regions, i.e. Egypt, are generally poor in organic matter and moisture contents, high in lime content and generally fine-textured, which decreases the boron uptake of the plants (Dursun *et al.*, 2010). Because of these reasons, plants cannot benefit from the boron element that exists in insufficient and sufficient levels in the soil. In order to provide normal growth of plants which are especially reactive to boron deficiency, it is necessary to apply additional boron fertilization as a non-toxic level for deficiencies. Boron (B) is an essential micronutrient necessary for plant growth and productivity. It plays an important role in physiological and biochemical events that occur in plants (Saleem *et al.*, 2011), like sugar transmission, cell wall synthesis and metabolism of carbohydrate, RNA, indole acetic acid and phenol (Camacho-Cristóbal *et al.*, 2008). In this regard,

El-Dissoky and Abdel-Kadar (2013) and Jafari-Jood *et al.*, (2013) found that spraying of boron significantly improved potato plant growth, yield and its components as well as tuber quality in special dry matter, protein and starch content.

Using biostimulants to promote plant growth has recently gained increasing attention worldwide (Farouk, 2012, Calvo *et al.*, 2014). Several products have been categorized as biostimulants, including humic substances, seaweed and plant extracts as well as nano-particles (Isaac, 2000). Seaweed extracts (SWE) (*Ascophyllum nodosum* Jol.) as organic biostimulants is fast becoming accepted practice in modern agriculture for sustainable production (Cassan *et al.*, 1992). According to the report by FAO (2006), a substantial amount of seaweeds (15 million metric tons annually) is used as supplementary for nutrients and biostimulants for the crop production. The beneficial effect of SWE is as a result of many components that work synergistically at different concentrations, although the modes of action still remain unknown. It is well known that SWE contains phytohormones (Kurepin *et al.*, 2014), certain micro and macronutrients (Zhang and Ervin, 2008), and secondary metabolites as Quaternary ammonium molecules, such as betaines and proline (Mackinnon *et al.*, 2010). SWE has been used as a foliar spray to increase growth, yield and quality, nutrient uptake, photosynthetic pigments and resistance to stress factors of many crops including potato (Arafa *et al.*, 2011, 2012, 2013, Calvo *et al.*, 2014).

Humic substances (HS) are an organic substance having bioregulatory effects due to its contents of some plant hormones-like substances (Pizzeghello *et al.*, 2002). HS may improve plant nutrient uptake (Bryan and Stark, 2003), increase root growth and enhance enzyme activity (Mikkelsen 2005, Mart, 2007), and increase yield (Farouk *et al.*, 2012, Rafeii and Pakkish, 2014). In this concern, Sanli *et al.* (2013) found that exogenous application of leonardite (a concentrated form of humic and fulvic acids) increased significantly plant height, total number of tubers per plant, and total tuber yield per hectare well as some quality parameter of tuber i.e, protein content, ascorbic acid content and specific gravity. Recently, Arafa *et al.*, (2011, 2012, 2013) found the application of humic acid increased significantly lettuce plant growth parameters as well as some chemical composition of plants like total chlorophyll, total carbohydrates, ion percentage, as well as yield and its quality.

Horseradish Tree or Malunggay leave extract (MLE) (*Moringa oleifera*. Lam., Family Moringaceae) accelerate the growth and yield by 20-35% of many crop plants (Ozobia, 2014, El-Sayed *et al.*, 2014) due to its posse's high antioxidant activity and its high content of some secondary metabolites as amino acids, essential minerals, vitamins as well as it is also a source of plant growth substances in special zeatin (5-200 mcg/g) and gibberellins (Rady *et al.*, 2013, Rady and Mohamed, 2015). In this concern, Abdalla (2015) found the application of MLE on rocket plants increased all growth parameters and photosynthetic pigments as well as some physiological characteristics like sugars, protein, phenol and ions.

Lithovit® a natural CO₂ Nano-fertilizers, is 100% organic calcite carbonate from natural limestone deposits, suitable and recommended for use in organic farming in the European Community, harmless to humans and animals and not hazardous to water according to EWG 2092/91 (Bilal, 2010). To our knowledge, there is a very few report indicated the role of lithovit for improving plant growth and yield. In this concern, Maswada and Abd EL-Rahman (2014) on wheat proved that under normal or salinity stress, application of lithovit significantly increased growth parameters and photosynthetic pigments, ion contents and yield and its components

The present investigation aimed to evaluate the effect of various biostimulants (seaweed extract, potassium humate, Horseradish Tree or Malunggay leaf extract "*Morenga oleifera* Lam.", lithovite) with or without boron fertilization on potato growth, and yield as well as some physiological and anatomical characteristics. It was intended to find out the most favorable treatments producing more quality and quantity of potato yield.

MATERIALS AND METHODS

Two field experiments were conducted during the two successive winter seasons of 2014/2015 and 2015/2016 at a private farm in Sherbeen, Dakahlia Governorate, Egypt under drip irrigation system to evaluate the effect of biostimulants i.e. seaweed extract (SWE), potassium humate (KH), Horseradish tree (moringa leaf extract "MLE"), and lithovite (Lith) with or without boron fertilizer (B) on potato growth, and yield as well as some related physiological and anatomical characteristics.

Before planting, random soil samples of the experimental site were collected (0-30 cm depth), air dried, grounded, mixed and kept in plastic bags for the analysis. The representative sample was subjected to mechanical and chemical analysis as described by Page *et al.*(1982) and presented in Table 1.

Table 1: Some physical and chemical properties of the experimental soil during 2014/2015 and 2015/2016 seasons.

Properties	Sand %	Silt %	Clay %	Texture	O.M. %	CaCO ₃	pH (Soil paste)	EC "dSm-1" (in 1:5 soil water extract)	Soluble cations (meq L ⁻¹)				Soluble anions (meq L ⁻¹)			
									Ca	Mg	Na	K	CO ₃	HCO ₃	CL	SO ₄
1 st season	25.82	32.66	41.52	Clay loam	1.22	3.55	7.82	1.12	5.36	3.32	5.28	0.28	0	4.21	4.74	3.20
2 nd season	25.95	32.45	41.60	Clay loam	1.65	3.58	7.62	1.16	5.36	3.19	5.30	0.26	0	4.20	4.70	3.16

Experimental design:

Farmyard manure has been added during soil preparation in organic fertilization in a dose of 40 m³/fed. A randomized complete block design in factorial arrangement was used with three replicates. The experimental unit area was 12 m² including three ridges, each four meters long and 70 cm apart, and the distance between the hills was 30 cm apart. Potatoes tubers; cv Spunta were obtained from Agric. Res. Center (ARC), Ministry of Agric.,

Egypt. Tubers were divided into pieces, averaging approximately 50 g weight. As recommended by the Pathology Dept. Ministry of Agric. Egypt, potato tuber pieces were sterilized with Vitavax Kapetan 1% at the rate of 1.25 kg/ton for 5 min.

Planting procedure:

The treated potato tuber pieces were sown in the ridges at 12-15 cm in depth on November 9th and 15th in both seasons respectively. As recommended by the Agric. Res. Center, Egypt, Nitrogen fertilizer was added in three equal portions, the 1st was applied after emergence, in the form of ammonium sulphate (20.5 %), then two and four weeks later in the form of ammonium nitrate (33.5 %) at the rate of 180 Kg N/ fed. Phosphorous and potassium was applied during the soil preparation in the form of calcium superphosphate (15.5% P₂O₅) and potassium sulphate (48 % K₂O) at a rate of 75 kg P₂O₅/fed and 20 kg K₂O fed⁻¹ respectively. Plants were foliar sprayed at early morning with a sprayer (20 l in volume) to run-off twice, at 50 and 60 days from planting in both experimental seasons. The experiment included the treatments as follows 1- Control (tap water). 2- Seaweed Extract (SWE) at 500 mg/l. 3- Potassium humate (KH) at 100 mg/l. 4- Horseradish (Moringa) Leaf Extract (MLE) was prepared and diluted according to **Fuglie (2000)**. 5- Lithovit (Lith) at 500 mg/l. 6- Boron (B) at 1000 mg/l chelated boron. 7- B + SE. 8- B + KH. 9- B + MLE. 10- B + Lith

Sampling dates and data recorded:

At the developmental stage of 15 leaves, dated at the active growth period (after 80 days from planting), a random sample of four plants was taken from each experimental unit to determine the growth parameters, i.e. (plant height "cm", branch number per plant, shoot fresh and dry weights "g" and leaf area per plant "cm²"). Leaf area per plant was calculated based on area unit using the disk method according to Koller (1972), briefly, samples of ten disks were taken from the 3rd foliage compound leaf from plant tip and leaf area per plant was calculated in square centimeters (cm²) using the following equation: leaf area (cm²) per plant = (10 disks area x fresh weight of the leaves)/ (fresh weight of 10 disks). In addition, some physiological parameters like photosynthetic pigment concentration in the 3rd upper leaf (mg/g FW), as well as total carbohydrates concentration and ion "N, P and K" percentages in the shoot were also determined.

At harvesting time (115 days from planting), the tuber number and yield per plant were determined. A representative sample of 10 healthy tubers from each experimental plot was selected from the larger sizes to obtain the quality of tuber as follows:

- a. The specific gravity was calculated according to Abdel-Aal (1971) where the specific gravity (g/cm³) = tuber mass (g)/tuber volume (cm³)
- b. Total soluble solids (%) were determined by using Karl Zeiss hand Refract meter according to AOAC (1990)
- c. Crude protein percentage "multiplying total nitrogen percentage by 6.25 will give the crude protein content" (Ranganna, 1977).
- d. Ascorbic acid concentration (mg/g FW).
- e. Total phenol concentration (mg/g FW)

f. Total soluble sugars and starch concentration (mg/g FW)

Leaflet and stem anatomy

Specimens (5 x 5 mm) from the terminal leaflet of the 3rd upper compound leaf and the middle part of the 2nd internode of the main stem from the plant tip were taken after 80 days from planting. The samples were killed and fixed in formalin aceto alcohol for at least 48 h, then washed and dehydrated in series of ethanol and embedded in paraffin wax (52-54 °C melting points). Cross sections were done at 12-15 µm thick using rotary microtome, stained in Toluidine blue O, cleared in xylene and mounted in canada balsam (Gerlach, 1977). Three sections taken randomly and examined microscopically for determining the anatomical changes under different treatments.

Biochemical determination

Total carbohydrate concentration in the dried shoots was determined by the phenol-sulfuric acid methods as described by Sadasivam and Manickam, (1996).

Photosynthetic pigment (chlorophylls a, b and their total as well as total carotenoids), were extracted from the blade of the 3rd terminal upper compound leaf of the main stem for 24 hr at laboratory temperature by methanol after adding a trace from sodium carbonate (Robinson and Britz, 2000), then photosynthetic pigments were determined spectrophotometrically (Lichtenthaler and Wellburn, 1985).

Ion percentage: ground dried shoot and tuber samples were wet digested with HClO₃/H₂SO₄ until the solution was clear, cooled, and brought to volume at 100 ml using deionized water and kept for ion determinations. Total nitrogen was determined by microkjeldahl method. Potassium was determined by flame photometer (Kalra, 1998), and phosphorous using ammonium molybdate and ascorbic acid (Cooper, 1977).

Ascorbic acid was extracted from potato tuber and titrated using 2,6-dichlorophenol indophenole as described by Sadasivam and Manickam (1996). Total phenol was determined using Folin-Ciocalteu reagent according to the method of Kähkönen et al. (1999).

Soluble sugars and starch concentration: Soluble sugars and starch were estimated following Malik and Srivastava (1979). For the estimation of soluble sugars and starch, 0.1 g of well ground dry materials was homogenized in 80% ethanol and centrifuged, the residue was retained which was repeatedly washed with 80% ethanol to remove all traces of soluble sugars. The filtrate thus obtained was used for the determination of soluble sugars. The residue was used for the determination of starch. Five ml of distilled water and 6.5 ml of 52% perchloric acid were added to the residue. Extraction of starch with perchloric acid was carried out at 0 °C for 20 min, and then centrifuged, and the extract was retained. With the residue, the above step was repeated using fresh perchloric acid and the extract of this step was combined with the extract of the first step and then the volumes of each of the sugar and starch extracts were made up to 100 ml by the addition of distilled water. The extracts for both soluble sugars and starch (0.1 ml) were determined by the phenol- sulphuric acid methods using spectrophotometer (Spekol 11, Uk) as described by Sadasivam and Manickam, (1996)

Statistical analysis: All data were analyzed statistically using one-way ANOVA and means were separated using Tukey's HSD (CoHortSoftware, 2008). Data were not transformed before analysis because normality and homogeneity of variance assumptions were not violated

RESULTS AND DISCUSSION

Vegetative growth characters

The results presented in the Table (2) clearly revealed that exogenous application of biostimulants and lithovit with or without boron significantly affected all growth characters (plant height, branch number/plant, shoot fresh and dry weights and leaves area per plant) in the two experimental seasons as compared with untreated control plants. The highest vegetative growth values were recorded by application of MLE plus B which recorded 67.6 and 61.6 cm of plant height, 9.6 and 8.6 branches per plant; 269.13 and 247.73 g of shoot fresh weight; 22.336 and 18.683 g for shoot dry weight and 619 and 582 cm² for leaves area per plant, compared with untreated control plants which recorded 49.0 and 48.0cm of plant height, 4.3 and 3.3 branches number per plant, 165.76 and 155.50 g of shoot fresh weight; 14.196 and 12.673 g for shoot dry weight and 390 and 359 cm² for leaves area per plant in the 1st and 2nd experimental seasons respectively.

Table 2:Vegetative growth characters of potato as affected by biostimulants and lithovit with or without boron at 80 days from planting in both seasons

Treatments	Plant height (cm)		Branches number/plant		Shoot fresh weight (gm)		Shoot dry weight (gm)		Leaves area per plant (Cm ²)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	49.0f ±3.71	48.0f ±3.29	4.3d ±1.154	3.3f ±2.309	165.76f ±17.21	155.50f ±21.01	14.196g ±1.556	12.673e ±3.818	390.00g ±34.871	359.33i ±1.154
Seaweed Extract (SWE)	60.6cd ±3.05	59.6ab ±3.61	7.6b ±1.154	6.6cd ±1.154	199.86de ±5.262	188.13d ±1.501	17.293de ±0.480	14.083cde ±0.618	558.33c ±26.102	527.00d ±21.166
Potassium Humate (KH)	56.3e ±1.15	54.3de ±1.15	6.0c ±0.000	5.0e ±0.000	190.33e ±8.220	180.8de ±6.264	16.653ef ±0.400	13.630de ±0.336	462.00f ±17.009	431.66h ±34.078
Moringa Leaf Extract (MLE)	62.6bc ±1.15	59.3ab ±3.05	7.0bc ±0.000	7.0bc ±0.000	212.16cd ±16.78	203.53c ±12.57	17.930d ±1.271	14.41cd ±0.697	504.3e ±8.082	481.33f ±14.742
Lithovit (Lith)	56.6e ±2.30	55.3cde ±3.05	7.0bc ±2.000	5.6de ±2.309	195.53e ±7.305	182.2de ±2.163	16.483ef ±0.456	13.266de ±0.486	497.33e ±16.165	457.00g ±14.000
Boron	55.3e ±3.05	52.3e ±2.30	6.3bc ±1.154	5.0e ±0.000	186.70e ±3.704	173.83e ±11.66	15.500f ±1.247	12.763e ±0.244	442.66f ±35.232	417.00h ±23.065
Boron + SWE	65.0ab ±4	60.6a ±4.03	9.0a ±2.000	8.0ab ±2.000	241.66b ±1.900	235.50b ±8.576	20.183b ±0.404	16.466b ±0.030	774.00a ±57.166	641.66a ±38.695
Boron + KH	58.3de ±1.15	56.3bcd ±1.15	7.3b ±1.154	6.3cd ±1.154	220.80c ±11.400	208.46c ±4.046	18.340cd ±0.603	15.476bc ±0.741	532.33d ±20.132	505.33e ±40.066
Boron + MLE	67.6a ±5.03	61.6a ±5.42	9.6a ±1.154	8.6a ±1.154	269.13a ±22.21	247.73a ±28.800	22.336a ±3.360	18.683a ±3.070	619.00b ±34.698	582.333b ±5.033
Boron + Lith	61.3cd ±3.05	58.6abc ±3.05	7.6b ±2.309	6.6cd ±1.154	234.66b ±7.521	214.20c ±15.108	19.256bc ±1.039	16.126b ±1.030	582.66c ±21.197	549.00c ±19.078

Means within columns followed by different letters are significantly different ($P < 0.05$); df for each analysis was 9, 29

The enhancement in plant growth obtained by foliar application of boron may be a result of its roles in stimulating plant biological activities such as photosynthesis, enzyme activities, nutrient uptake and rate of translocation of photoassimilates. Moreover, the stimulating effect of B on plant growth may be due to its role in cell development and the production of IAA which is

essential for the elongation of plants (Follet *et al.*, 1981). In addition, foliar application of B increase the photosynthetic compounds in plant tissue which ultimately reduced the leaf drop and give strength for their persistency (Puzina, 2004).

The promotive effects of biostimulants on plant growth are not fully clear, although there are some theories which probably work together, and can be summarized: 1) Biostimulant's effects on physiological processes in plants like macro- and micro- nutrient uptake, cell elongation, enzymatic activity and protein synthesis and finally activation of biomass production (Calvo *et al.*, 2014, Rady and Mohamed, 2015). In this concern, as indicated later application of biostimulants increased ion percentage, in special, phosphorous, which play an important role in the biosynthesis and translocation of carbohydrates and stimulation cell division as well as formation of DNA and RNA (Taiz and Zeiger, 1991). 2) Activate root cells and stimulate the biosynthesis of endogenous cytokinin (Schmidt, 2005). Cytokinin are known to promote cell division, inhibit leaf senescence by blocking export of photosynthate to new tissue and stimulating translocation of resources to treated leaves (Taiz and Zeiger, 1991), 3) Altering hormonal balance and favor cytokinin and auxin production (Stirk *et al.*, 2004, Schmidt, 2005 for SWE and Abdalla 2015, Rady and Mohamed, 2015 for MLE; Pizzeghello *et al.*, 2002 for HS), 4) Stimulation the biosynthesis of ascorbic acid, α -tocopherol and carotenoids in chloroplasts which protect photosynthetic apparatus of PSII and stimulation of chlorophyll biosynthesis (Zhang and Schmodt, 2000)., 5) Stimulation of chloroplast development and enhancing phloem loading and delay senescence (Demir *et al.*, 2004). 6) Enriched content of MLE and SWE in crude protein and growth promoting hormones, in special, auxin and cytokinin (Abdalla, 2015). Proteins are essential for the formation of protoplasm, while growth substances favored rapid cell division and cell multiplication as well as elongation.

Elevated CO₂ is likely to stimulate the growth of many plant species (Rawat and Melkania, 2015). The increase in plant growth in response to lithovit may be due to, its role as a long term reservoir supplying plants with CO₂ (Bilal, 2010; Kumar, 2011); thus, it can enhance plant growth and productivity, where elevated CO₂ concentrations generally increased carbon assimilation, biomass and leaf area of plants (Rebbeck and Scherzer 2002, Maswada and Abd El-Rahman 2014). It is well known that lithovit particles remain as a thin layer on the surface of leaves and penetrate frequently when they get wet with dew at night.

Photosynthetic pigments and carbohydrate concentrations

It is clear from the data presented in table (3) that the concentrations of photosynthetic pigments and total carbohydrate concentrations significantly increased with biostimulants alone or combined with boron fertilizer as compared with untreated control plants. The highest values of photosynthetic pigment concentrations in both seasons were obtained under the application of seaweed extract plus boron fertilizer.

As regards to total carbohydrate concentration in potato shoots, the tabulated data revealed that application of biostimulants and lithovit alone or in combination with boron fertilizer significantly increased total carbohydrate

concentrations. The most effective treatment was application of lithovit plus boron followed by MLE plus B which increased total carbohydrate concentrations up to 84.08 mg/g DW, 89.92 mg/g DW and 78.86 mg/g DW, 74.38 mg/g DW in both experimental seasons as compared with control plants (56.56 mg/g DW and 54.03 mg/g DW).

Table 3: Photosynthetic pigments (mg/g FW) in the 3rd upper leaflet and carbohydrates concentrations (mg/g DW) in the shoot of potato as affected by biostimulants and lithovit with or without boron at 80 days from planting in both seasons.

Treatments	Total Carbohydrates		Photosynthetic pigments (mg/g FW)							
			Chlor A		Chloro B		Total Chlorophyll		Total Carotenoids	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Control	56.56h ±11.192	54.03f ±9.838	1.073b ±0.036	0.967c ±0.230	0.632c ±0.093	0.568c ±0.120	1.645c ±0.124	1.535c ±0.347	0.140b ±0.095	0.127e ±0.049
Seaweed Extract (SWE)	69.13ef ±3.213	63.94cde ±2.563	1.094ab ±0.120	1.221a ±0.377	0.881a ±0.070	0.778b ±0.334	1.975ab ±0.083	1.9909a ±0.696	0.220ab ±0.083	0.199b ±0.141
Potassium Humate (KH)	65.59fg ±1.625	61.14de ±3.347	1.067b ±0.303	1.097b ±0.307	0.680b ±0.238	0.646bc ±0.216	1.747bc ±0.542	1.743bc ±0.523	0.143b ±0.019	0.160c ±0.023
Moringa Leaf Extract (MLE)	72.30de ±1.164	67.57bcd ±2.413	1.088ab ±0.111	1.132ab ±0.293	0.701ab ±0.095	0.636bc ±0.263	1.789bc ±0.180	1.768b ±0.529	0.151ab ±0.021	0.180bc ±0.147
Lithovit (Lith)	72.30de ±0.941	69.10bc ±1.132	1.112ab ±0.180	1.050bc ±0.438	0.660bc ±0.121	0.716b ±0.322	1.772bc ±0.285	1.766b ±0.749	0.160ab ±0.009	0.135de ±0.032
Boron (B)	62.86g ±1.593	58.96ef ±0.982	1.066b ±0.075	1.076b ±0.292	0.678b ±0.128	0.637bc ±0.118	1.744bc ±0.199	1.713bc ±0.410	0.159ab ±0.036	0.149d ±0.059
Boron + SWE	76.35bc ±1.131	68.06bc ±18.446	1.237s ±0.243	1.241a ±0.647	0.851a ±0.080	0.820a ±0.324	2.088a ±0.323	2.061a ±0.972	0.247a ±0.127	0.224a ±0.249
Boron + KH	74.05cd ±1.323	71.163b ±0.618	1.055b ±0.219	1.166ab ±0.223	0.940a ±0.434	0.769b ±0.114	1.995ab ±0.266	1.935a ±0.327	0.201ab ±0.022	0.175bc ±0.047
Boron + MLE	78.86b ±1.180	74.38b ±7.311	1.186ab ±0.132	1.193a ±0.277	0.807ab ±0.117	0.807a ±0.120	1.993ab ±0.096	2.000a ±0.388	0.235ab ±0.136	0.219a ±0.239
Boron + Lith	84.08a ±6.343	89.92a ±6.053	1.143ab ±0.068	1.212a ±0.089	0.739ab ±0.077	0.675bc ±0.432	1.882abc ±0.110	1.887b ±0.520	0.154ab ±0.044	0.156cd ±0.129

Means within columns followed by different letters are significantly different ($P < 0.05$); df for each analysis was 9, 29

The stimulative effects of biostimulants may be explained as; 1) All biostimulants containing a considerable amount of micro- and macro-nutrients, in special, potassium, magnesium, and iron (Murillo *et al.*, 2005 for KH; Abdalla, 2015 for MLE; and Sridhar and Rengasamy, 2010 for SWE), which are required for chlorophyll biosynthesis. Moreover, potassium increased the number of cells per leaves and number of chloroplasts per cell which reflect to increase the photosynthetic pigment concentrations, 2) Its content of plant growth substances or hormonal like activity (Pizzeghello *et al.*, 2002, 2013 for KH, Kurepin *et al.*, 2014 for SWE; Rady and Mohamed 2015 for MLE). Moreover, it is well known that SWE contains a number of betaines and betain analogues (Mackinnon *et al.*, 2010). One of the characteristic responses of SWE is an increase in chlorophyll content in the treated plants (Arafa *et al.*, 2011, Jannin *et al.*, 2013). The study of the molecular responses in plants in response to the treatment with SWE revealed that the increase in the chlorophyll content was largely due to an increase in the biogenesis of chloroplasts, a reduction in chlorophyll degradation and a delay in senescence (Jannin *et al.*, 2013). Senescence

associated cysteine proteases were down-regulated whereas expression of genes associated with photosynthesis, cell metabolism, and stress response were significantly also-up-regulated in plants treated with *A. nodosum* extract (Jannin *et al.*, 2013).

The promotive effect of boron on photosynthetic pigment concentration as compared to untreated plants may be attributed to its effects either directly by affecting the activity of enzymes responsible for the biosynthesis of photosynthetic pigments (Aizupiete, 1968) or indirectly through the influence of boron on nitrogen metabolism (Ashour and Thalooh, 1974).

Ion Percentage:

Data presented in Table (4) indicate that application of biostimulants and lithovit with or without boron significantly increased ion percentage in the shoot compared with untreated control plants. The most effective treatment in this concern was MLE plus boron, which increased N and P percentage from 1.495%, 1.188% and 0.463%, 0.339% to 3.041%, 2.361% and 0.859, 0.682 in the first and second seasons respectively. Moreover, the data in the same table proved that application of potassium humate alone or plus boron significantly increased potassium percentage to 2.847% and 2.128% in the first and second season compared with control plants (1.794% and 1.246%) or boron fertilizer only (1.900 and 1.473%) in both seasons.

Table 4: Ion percentage of potato shoot as affected by biostimulants and lithovit with or without boron at 80 days from planting in both seasons.

Treatments	Nitrogen		Phosphorous		Potassium	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Control	1.495f ±0.624	1.188e ±0.244	0.463g ±0.019	0.339f ±0.033	1.794h ±0.280	1.246f ±0.178
Seaweed Extract (SWE)	2.164bcd ±0.179	1.705cd ±0.216	0.617def ±0.017	0.477cd ±0.039	2.098ef ±0.155	1.661cde ±0.094
Potassium Humate (KH)	2.001de ±0.095	1.559d ±0.105	0.541fg ±0.066	0.419e ±0.038	2.522b ±0.144	2.078a ±0.374
Moringa Leaf Extract (MLE)	2.173bcd ±0.090	1.783bcd ±0.115	0.657cd ±0.085	0.492c ±0.035	2.243de ±0.111	1.852bc ±0.140
Lithovit (Lith)	2.042cde ±0.087	1.644cd ±0.071	0.605def ±0.149	0.435de ±0.067	2.040fg ±0.068	1.627de ±0.054
Boron	1.762ef ±0.101	1.318e ±0.213	0.568ef ±0.046	0.417e ±0.035	1.900gh ±0.157	1.473e ±0.306
Boron + SWE	2.478b ±0.377	1.988b ±0.236	0.789b ±0.119	0.606b ±0.035	2.286cd ±0.197	1.935ab ±0.326
Boron + KH	2.187bcd ±0.018	1.823bc ±0.144	0.632de ±0.040	0.499c ±0.079	2.847a ±0.350	2.128a ±0.047
Boron + MLE	3.041a ±0.628	2.361a ±0.552	0.895a ±0.168	0.682a ±0.068	2.454bc ±0.122	1.943ab ±0.107
Boron + Lith	2.347bc ±0.414	1.836bc ±0.204	0.726bc ±0.091	0.565b ±0.050	2.182def ±0.258	1.723cd ±0.263

Means within columns followed by different letters are significantly different ($P < 0.05$); df for each analysis was 9, 29

In plants with adequate B levels, increases in NR activity and in nitrate assimilation were demonstrated as B supply increased. It was hypothesized that the higher nitrate reductase activity and nitrate assimilation under normal B levels occurred as a result of either increased de novo

synthesis of the proteins involved in this metabolic process, or as a result of the facilitation of nitrate absorption (Ruiz *et al.*, 1998).

Several comparable studies confirmed the obtained results. For instance, Sivakumar and Ponnusami (2011) for MLE and Arafa *et al.* (2013) for SWE and HA who indicate that application of biostimulants inducing the uptake and accumulation of ion in the shoot and root of several plants. The promotive effect of biostimulants on ion accumulation in plant tissues may be due to stimulating root system growth, increasing proliferation of root hairs, production of smaller but more ramified secondary roots (Canellas *et al.*, 2002) and finally to improving membrane permeability as well as using efficiency of nitrogen fertilizers coupled with retarded nitrification processes and inhibition of urease activity (Adani *et al.*, 1998).

Recently, it is well stated that various seaweed extracts are known to affect the regulation of genes that played an important role in nutrient uptake, for example, *A. nodosum* extract unregulated the expression of a nitrate transporter gene NRT.1. that improved nitrogen sensing and auxin transport (Castaings *et al.*, 2011) resulting in enhanced growth of lateral roots and improved nitrogen assimilation.

Anatomical studies

Leaflet anatomy:

The data presented in table (5) and illustrated in figure (1) showed that exogenous application of biostimulants and lithovit with or without boron application markedly increased leaflet thickness in the midrib region, epidermis thickness, main vascular bundle dimension, thickness of xylem and phloem tissue thickness.

Table (5) Potato leaflet anatomical characteristics as affected by biostimulants and lithovit with or without boron at 80 days from planting during the second growing season

Treatment	Leaflet thickness in the midrib region (μ)	Thickness of leaf blade (μ)	Palisade parenchyma thickness (μ)	Spongy parenchyma thickness (μ)	Epidermis thickness (μ)	Main vascular bundle dimension (μ)		Thickness of xylem tissue (μ)	Thickness of phloem tissue (μ)
						length	width		
Control	720	260	150	90	20	170	180	90	80
Seaweed Extract (SWE)	1320	190	90	70	30	210	370	110	100
Potassium Humate (KH)	1250	200	110	70	20	180	230	100	80
Moringa Leaf Extract (MLE)	1300	180	80	70	30	200	390	100	100
Lithovit (Lith)	1180	310	220	60	30	200	210	100	100
Boron	1240	200	110	70	20	190	390	110	80
Boron + SWE	1400	190	80	80	30	220	430	120	100
Boron + KH	1250	190	80	90	20	190	400	100	90
Boron + MLE	1260	240	160	50	30	210	450	110	100
Boron + Lith	1250	220	130	60	30	190	390	100	90

The highest values were obtained in most cases due to an addition of boron plus seaweed extract as a foliar application. On the other hand, the data in the same table and figure clearly indicate that all treatments decreased thickness of leaf blade except lithovit treatment which increased

markedly the thickness of leaf blade. This increase is accompanied by increasing the thickness of the palisade parenchyma thickness. As regards to the width of vascular bundle, the data in the same table and figure showed that the highest value was obtained due to application of moringa leaf extract plus boron followed by application of seaweed extract plus boron.

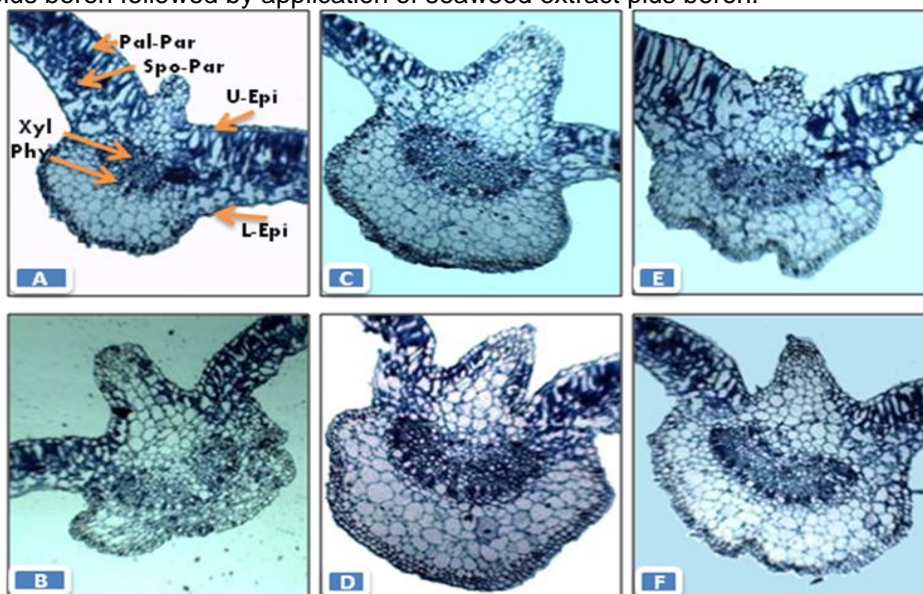


Figure (1) Cross section in the 3rd upper potato leaflet as affected by biostimulants and lithovit with or without boron at 80 days from planting in the second season (L-Epi, lower epidermis; Pal-Par, palaside parenchyma; Phy, phloem; Spo-Par, spongy parenchyma; Xyl, xylem; U-Epi, Upper epidermis; A, control; B, boron; C, seaweed extract; D, boron plus seaweed extract, E, lithovit; F, boron plus lithovit) (40x)

Stem anatomy:

The data presented in table (6) and illustrated in figure (2) showed that exogenous application of all biostimulants and lithovit with or without boron application markedly increased stem and pith diameter, thickness of epidermis, length and width of large vascular bundle. The results also indicated that the best treatment for increasing this parameter was an exogenous application of seaweed extract plus boron.

The data presented in the same table and illustrated figure clearly indicate that the cortex thickness in most cases decreased with the application of biostimulants and lithovit under boron application or without boron application, except the treatment of exogenous application of moringa leaf extract plus boron and seaweed extract plus boron application respectively. Also the data indicate that, application of potassium humate or boron application gave the same thickness of control plants.

Table (6) Potato stem anatomical characteristics as affected by biostimulants and lithovit with or without boron at 80 days from planting during the second growing season

Treatment	Stem diameter (μ)	Pith diameter (μ)	Epidermis thickness (μ)	Cortex thickness (μ)	Large vascular bundle dimension (μ)	
					Length	Width
Control	1700	704	8	350	140	200
Seaweed Extract (SWE)	2950	1446	12	340	400	290
Potassium Humate (KH)	1950	774	8	350	230	290
Moringa Leaf Extract (MLE)	2800	1376	12	310	390	300
Lithovit (Lith)	2700	1696	12	270	220	300
Boron	2270	1114	8	350	220	280
Boron + SWE	3370	1838	16	360	390	430
Boron + KH	3000	1812	14	340	240	420
Boron + MLE	3250	1618	16	410	390	310
Boron + Lith	3100	1732	14	270	400	310

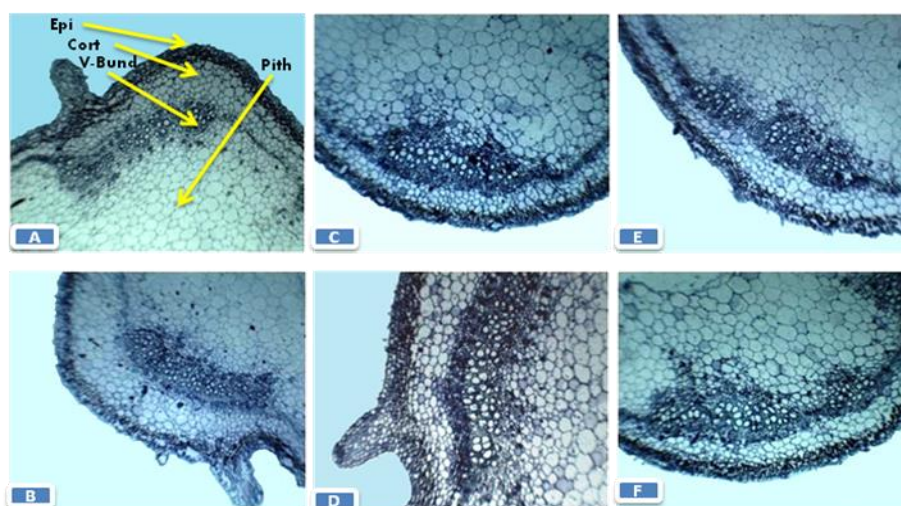


Figure (2) Cross section in the 2nd internode of potato main stem as affected by biostimulants and lithovit with or without boron at 80 days from planting in the second season (Epi, Epidermis; Cort, Cortex; V. Bund, Vascular Bundle, A, control; B, boron; C, seaweed extract; D, boron plus seaweed extract, E, lithovit; F, boron plus lithovit)(40x)

It could be concluded that application of seaweed extract plus boron proved to be more effective in inducing the anatomical characters either of potato leaf or stem.

Yield and its components

Data in Table (7) indicated that productivity of potato plants was significantly influenced by using of different biostimulants and lithovit with or without boron fertilizer as foliar spray. It is well shown from the table (7) that application of boron plus seaweed extract gave the highest potato yield per plant (1.483 and 1.217 kg/plant) and highest tuber number per plant (8.6 and 7.3) as well as ascorbic acid concentration (4.930 and 4.640 mg/g FW) as compared with untreated control plants.

Concerning total phenol concentration the data in the same table proved that all treatments, in special, potassium humate plus boron significantly increased total phenol, from 10.385 and 9.995 mg/g FW to 24.852 and 21.518 mg/g FW in both experimental seasons respectively.

Regarding the specific gravity and total soluble solids as well as starch and soluble sugar concentrations in potato tubers, the tabulated data proved that all biostimulants and lithovit with or without boron significantly increased all mentioned characters; the highest values were recorded with foliar application by lithovit plus boron as compared with control plants.

The promotive effect of B on increasing potato yield might be due to its role in cell differentiation and development, translocation of photoassimilates and growth regulators from sources to sink. These results are in accordance with that EL-Dissoky and Abdel-Kadar (2013) who documented that application of B significantly increased tuber yield and its quality. In addition, inducing sugar transport to the tuber and enhancing the synthesis of protein and regulation of carbohydrate metabolism (Mengle and Kirkby 2001). These results are in agreement with Bari *et al.*, (2001) and EL-Banna and Abd EL-Salam (2005).

The stimulation effect of biostimulants and lithovit with or without boron on tuber yield could be attributed to the presence of plant growth substances, in special, cytokinin in SWE (Kurepin *et al.*, 2014) and MLE (Rady and Mohamed, 2015) as well as hormones like substances in HA (Pizzeghello *et al.*, 2002, 2013), which increased overall plant growth, maintenance of green leaves, and number of branches per plant, increasing photosynthetic pigments as well (Table 3), followed by increasing sink capacity fulfilled supply of photoassimilates from green leaves (Saravanan *et al.*, 2003) and/or re-translocation of stem reserve. These finding was correlated with increasing leaf blade thickness as well as dimension of vascular bundles (Table 5, 6).

Concerning yield quality, It is well documented from the present investigation that foliar application of biostimulants improved yield quality. These results were confirmed with Farouk *et al.*, (2012) and Calvo *et al.*, (2014) for SWE, who found that fruit quality parameters such as TSS and total sugar contents showed a positive and gradual response relative to the applied seaweed extract compared to the control treatment. The favorable influences of biostimulants on the chemical characteristics of potato tuber may be ascribed to its stimulative effect on photosynthesis process and its concentration of some promoter hormones such as cytokinin which is closely involved in cell division, protein, carbohydrates and chlorophyll formation (Featonby-Smith and Van Standen 1984).

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Recently, Arafa et al. (2012) found that, foliar application of seaweed extract has resulted in increase in potato yield as well as fruit quality represented as minerals, total acidity, total soluble solids and ascorbic acid content, starch and total soluble sugars concentration and specific gravity. Application of seaweed extract significantly affected the biochemical constituents of potato tubers which might be due to efficient uptake of most of essential nutrients by the plants (Anantharaj and Venkatesalu, 2002). Although, it has been reported that application of HA increased vitamin C, carbohydrates and ions in potato plants was confirmed by Arafa *et al.*, (2012).

Lithovit ® natural CO₂ foliar fertilizer is a new nanotechnological fine powdered created by tribodynamic activation and micronization. Lithovit particles, sprayed finely onto the leaf surface, are taken up directly through the stomata and converted into carbon dioxide. In this way lithovit can considerably increase the photosynthesis rate, since the essential factor limiting photosynthesis, leading to yield increases

CONCLUSION

There is more promise for the use of non-chemical approaches in crop production in the light of the recent shift towards organic farming and growing public concern to minimize the use of chemicals. This particular potential widens the scope of biostimulants for use in other crops also. The results of this study showed that foliar spray of seaweed extract plus boron enhanced potato growth, yield and improved tuber quality of potato plants.

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تحسين نمو وإنتاجية البطاطس باستخدام بعض المنشطات الطبيعية والليثوفيت في وجود أو غياب البورون

سعد فاروق

قسم النبات الزراعي، كلية الزراعة، جامعة المنصورة، جمهورية مصر العربية

أجريت تجربتان حقليتان خلال موسمي الزراعة ٢٠١٤/٢٠١٥ و ٢٠١٥/٢٠١٦ بأحد المزارع الخاصة، بمركز شربين، محافظة الدقهلية، مصر، لتقييم دور بعض المنشطات الطبيعية والليثوفيت في وجود أو غياب الرش بالبورون علي نمو ومحصول نبات البطاطس بالإضافة لبعض الصفات الفسيولوجية والتشريحية للنبات.

أوضحت النتائج أن رش المنشطات الطبيعية والليثوفيت في وجود أو غياب البورون تزيد معنويًا صفات النمو الخضري لنبات البطاطس (ارتفاع النبات، عدد الأفرع للنبات، الوزن الطازج والجاف للمجموع الخضري و المساحة الورقية للنبات) بالإضافة إلي عدد ومحصول الدرناات للنبات. كذلك تحسنت صفات الجودة بالدرناات باستخدام المنشطات الطبيعية والليثوفيت مع إستعمال البورون. وكانت أفضل المعاملات في هذا الشأن هي الرش بمستخلص الطحالب البحرية مع التسميد بالبورون.

يُوصي برش نباتات الطماطم مرتين عند ٥٠ و ٦٠ يوماً من الزراعة ب ٥٠٠ ملليجرام/لتر مستخلص طحالب بحرية مع ١٠٠٠ ملليجرام/لتر بورون مخلبي للحصول علي أعلى محصول وأفضل جودة.

Table 7: Yield and its quality of potato plants as affected by biostimulafbnts and lithovit with or without boron at 80 days from planting in both experimental seasons.

Characters Treatments	Potato Yield/plant (kg)		Potato tuber number/ plant		Potato Quality															
	1 st	2 nd	1 st	2 nd	Specific Gravity (g/cm ³)		Total Soluble Solid%		Crude Protein %		Ascorbic Acid (mg/g FW)		Phenol (mg/g FW)		Starch (mg/g FW)		Soluble Sugars (mg/g FW)			
					1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	0.765h ±0.057	0.573h ±0.079	3.6f ±1.154	2.6f ±1.154	1.079e ±0.031	0.773d ±0.038	5.2g ±0.200	4.2h ±0.200	9.61g ±0.326	8.49e ±0.429	2.574d ±0.203	2.336e ±0.196	10.385g ±0.157	9.995g ±3.007	83.33f ±18.583	77.07f ±24.59	36.81e ±7.582	35.17d ±5.933		
Seaweed Extract (SWE)	1.066cd ±0.100	0.881c ±0.057	6.6bcd ±2.309	6.0ab ±2.000	1.130cde ±0.003	0.829bcd ±0.016	5.5ef ±0.230	4.5efg ±0.305	11.79de ±0.701	9.89d ±1.319	3.545bc ±0.326	3.185bcd ±0.026	13.657f ±0.137	12.965ef ±0.590	96.86de ±2.893	106.7de ±5.735	47.77cd ±1.833	45.15bc ±1.310		
Potassium Humate (KH)	0.868fg ±0.027	0.651fg ±0.029	4.3ef ±3.333	3.6def ±1.154	1.112cde ±0.018	0.823cd ±0.024	5.4f ±0.115	4.4fg ±0.115	10.81ef ±0.499	8.57e ±0.511	2.976cd ±0.392	2.661e ±0.227	16.497c ±1.793	15.103cd ±1.630	100.2d ±3.189	104.5de ±4.331	46.67d ±1.692	44.68bc ±2.566		
Moringa Leaf Extract (MLE)	0.913ef ±0.031	0.698ef ±0.022	5.6de ±2.309	4.3cde ±1.154	1.153cde ±0.010	0.846bc ±0.027	5.6de ±0.115	4.6de ±0.115	12.68cd ±0.850	10.36d ±0.611	3.843b ±0.263	3.480b ±0.193	14.361e ±1.181	12.444ef ±0.417	100.5d ±12.133	111.6cd ±8.693	52.52cd ±9.432	51.13b ±8.225		
Lithovit (Lith)	1.041d ±0.073	0.781d ±0.022	7.0abcd ±0.000	5.0bcd ±0.000	1.153cde ±0.024	0.843bc ±0.001	5.6de ±0.115	4.6def ±0.115	10.71efg ±1.543	8.67e ±0.608	3.318bc ±0.131	3.059d ±0.029	13.810ef ±0.530	12.877ef ±1.079	107.9cd ±4.460	114.6cd ±7.595	54.91bcd ±6.311	48.97bc ±3.555		
Boron	0.812gh ±0.021	0.619gh ±0.009	4.0f ±2.000	3.0ef ±2.000	1.094de ±0.044	0.867bc ±0.088	5.4f ±0.115	4.4g ±0.000	10.40fg ±1.307	8.452e ±1.540	2.871cd ±0.122	2.622e ±0.101	13.698ef ±1.373	11.679f ±1.353	87.21ef ±1.552	93.81e ±2.823	45.12de ±1.242	42.53c ±0.914		
Boron + SWE	1.483a ±0.176	1.217a ±0.123	8.6a ±1.154	7.3a ±1.154	1.192bc ±0.010	0.891b ±0.017	5.9bc ±0.305	4.9bc ±0.305	15.9s0b ±1.013	13.82b ±1.483	4.930a ±0.047	4.648a ±0.047	17.796b ±2.467	15.722c ±0.097	118.5c ±10.971	123.3c ±1.222	57.35bc ±14.084	52.45b ±13.586		
Boron + KH	0.951e ±0.041	0.746de ±0.038	6.3cd ±1.154	5.3bc ±2.309	1.177cd ±0.005	0.873bc ±0.015	5.7cd ±0.305	4.8cd ±0.200	13.77c ±1.374	12.51c ±0.736	3.431bc ±0.162	3.102cd ±0.133	24.852a ±7.633	21.518a ±2.911	113.2c ±13.192	123.8c ±5.922	53.59cd ±3.836	51.69b ±7.235		
Boron + MLE	1.303b ±0.149	0.944b ±0.038	8.3ab ±1.154	7.3a ±1.154	1.266ab ±0.063	0.962a ±0.094	6.0ab ±0.115	5.066ab ±0.115	17.54a ±2.354	15.67a ±1.000	4.884a ±2.206	4.648a ±1.143	20.035ab ±2.182	18.855b ±2.147	132.3b ±13.914	141.7b ±6.322	64.01b ±6.322	62.11a ±5.924		
Boron + Lith	1.138c ±0.043	0.951b ±0.086	7.6abc ±3.055	7.3a ±2.309	1.334a ±0.265	0.969a ±0.153	6.1a ±0.305	5.1a ±0.305	15.12b ±1.540	13.566b ±0.679	3.714b ±0.075	3.412bc ±0.072	15.228cd ±1.704	13.924de ±1.405	157.5a ±25.737	183.4a ±39.213	74.62a ±26.098	67.69a ±17.974		

Means within columns followed by different letters are significantly different ($P < 0.05$); df for each analysis was 9, 29