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EFFECTS OF BIOLOGICAL NANO-FERTILIZER ON THE MORPHOLOGICAL, PHYSIOLOGICAL AND PROLIFERATION TRAITS AND QUALITY OF *BUXUS HYRCANA* POJARK

BEHZAD KAVIANI* AND NASER NEGAHDAR[1]

Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran

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Abstract

The effect of various concentrations of biologic Nano-fertilizer special for ornamental plants (0.00, 0.60, 1.20, 1.80, 2.40 and 3.00 g per pot as drench and 0.00, 1.00, 2.00, 3.00, 4.00 and 5.00 g/l as leaf spray) on *Buxus hyrcana* Pojark were evaluated. Totally among 36 treatments, 3.00 g/pot drench + 2.00 g/l spray, also 1.80 g/pot drench + 2.00 g/l spray of Nano-fertilizer special for ornamental plants introduces as a good treatment for proliferation of *Buxus hyrcana* Pojark. The highest and the lowest concentrations of this Nano-fertilizer were no good.

Introduction

Buxus sempervirens L. or *Buxus hyrcana* Pojark. (Buxaceae), is a wild edible plant species (Orhan *et al*. 2012). Seeds are rarely used for proliferation and cuttings are commonly used. Biotechnology advances in protection and nutrition strategies for plants have attempted to provide some solutions for the problems caused by application of chemical fertilizers. Bio-fertilizers comprise environment friendly microorganisms that supply or improve availability of nutrients to promote soil fertility and crop productivity (Ghormade *et al*. 2011). Nanoparticles offered the advantage of effective loading due to the larger surface area, easy attachment and fast mass transfer (Ghormade *et al*. 2011). Organic fertilizers are now widely employed instead of chemical fertilizers (Inubushi *et al*. 2000). Encapsulation of fertilizers within a nanoparticle is one of these new facilities (Rai *et al*. 2012). Two methods of Nano-fertilizers application are practiced as foliar spraying and drenching. Foliar application of micronutrients, now a common horticultural practice, enhanced its uptake by the leaves (Martens and Westermann 1991). The mode of fertilizer application influences their efficiency and environmental impact (Ihsan *et al*. 2007, Matthews 2008). The aim of the present study was to evaluate the application methods and different concentrations of biological Nano-fertilizer, especially for the ornamental plants (containing microorganisms, especially phosphate solubilizing bacteria, plant growth promoter, nitrogen biological fixative, Fe and Zn) on some morphological, physiological and proliferation traits and enhancing the quality of *Buxus hyrcana* Pojark.

Materials and Methods

Buxus hyrcana Pojark., was used as mother plants. Cuttings with a height of 10 to 15 cm were prepared from maternal plants. Cuttings were planted in perlite for rooting. After rooting (60 days), cuttings were transferred to pots (4 kg) containing cocopeat, municipal compost and soil. Experiment had 36 plots and 108 blocks. Plots were pots containing Nano-fertilizers special for ornamental plants. Plants were grown in the greenhouse. Different ratios of Nano-fertilizer special for ornamental plants as the first experiment factor and different application methods as the second

*Author for correspondence: <b.kaviani@yahoo.com>. [1]Present address: Hyrcan Agricultural Sciences and Biotechnology Research Institute, Amol, Iran.

factor were prepared. The 0.00, 0.60, 1.20, 1.80, 2.40 and 3.00 g/pot (D_1, D_2, D_3, D_4, D_5 and D_6, respectively) of fertilizer were applied as drench at the beginning of the trial and 0.00, 1.00, 2.00, 3.00, 4.00 and 5.00 g/l as spray (S_1, S_2, S_3, S_4, S_5 and S_6, respectively) on leaves 30 days later. Spraying was repeated fortnightly. Used biological Nano-fertilizer containing bacteria enabling P uptake and biological fixation of N enriched with Fe and Zn Nano-chelates. Measurements were done on 20-month-old plants. In order to measure the dry weight of plant samples, they were dried in an oven with the temperature to 103°C for 24 hrs. The amount of productivity and proliferation rate was calculated by following formula: Productivity = Shoot numbers × mean of shoot length; Proliferation rate = Shoot numbers × node numbers. Total N concentration of leaf blade was determined by the Kjeldahl method. The content of P was measured by spectrophotometry. K content was calculated using flame photometry. Fe content of the samples was obtained using atomic absorption device. Chlorophyll contents of leaves were measured using spectro-photometry. The collected data were subjected to statistical analysis using a randomized factorial design. Each treatment was designed with three replicates. The mean values were compared using the least significant difference (LSD) test. Statistical tests were performed at $p \leq 0.05$ using MSTAT-C. EXCEL software was used to draw graphs.

Results and Discussion

Tables 1, 2 and 3 show that the biologic fertilizer special for ornamental plants can change the most characters in *Buxus hyrcana* Pojark. The minimum plant height (35.17 cm) was obtained in D_6S_5 of biologic Nano-fertilizer especial for ornamental plants. Plant height (38.10 cm) was less in D_6S_3. We focused on this treatment as a superior treatment to introduce. Maximum plant height (72.27 cm) was calculated in D_4S_3. There was no positive effect between increasing plant height and increasing Nano-fertilizer concentration (Table 1). Differences of plant height and most of traits in samples grown under different concentrations of Nano-fertilizer especial for ornamental plants were significant ($p \leq 0.01$) (Table 4). The results of this study indicate the role of biologic Nano-fertilizer especial for ornamental plants to change some quantity and quality characters in *Buxus hyrcana* Pojark. Given the new nanotechnology and the growing trend of studies in the technology, there are not reports about the effect of biologic Nano-fertilizer especial for ornamental plants on plant growth and development. Current study indicated that this Nano-fertilizer accelerated the growth of *Buxus hyrcana* Pojark. Similar results on other biological Nano-fertilizer were reported by other researchers (Bozorghi 2012, Tarafdar *et al*. 2014).

The largest number of shoots (9.30 and 8.83) was obtained in D_4S_5 and D_6S_3, respectively (Table 1). The smallest number of shoot (2.37) was calculated in D_4S_2. When the plants were inoculated with 3.00 g/pot drench + 2.00 g/l spray of Nano-fertilizer (D_6S_3), the best results were observed for node number (19.33) (Table 1). The least node number (5.67 and 6.00) was calculated in D_2S_1 and D_1S_3, respectively (Table 1). Maximum leaf number (133.30) was counted in D_6S_3. Minimum leaf number (34.00) was obtained in control plants. The highest leaf length (1.40 cm) was calculated in D_6S_5. The lowest leaf length (0.73 cm) was obtained in D_2S_5. Nano-fertilizer used in current study contains Fe and Zn. These two elements have key role in plant cell metabolism. Sabir *et al*. (2014) revealed that the treatment of Nano-fertilizer significantly affected the general leaf growth features of the grape. Our findings related to leaf number is severely similar to the Sabir *et al*. (2014) findings, so the least leaf was produced in control plants. The largest (7.93) and smallest (3.33) number of root were observed in D_3S_3 and D_1S_2, respectively (Table 1). The highest root length (6.83 cm) was measured in D_6S_3. Maximum root volume (178.70 ml) was measured with D_4S_6.

Table 1. Effects of the different treatments on morphological characters of box tree (*Buxus hyrcana*).

Treatments	Mean of Values							
	Root volume (ml)/plant	Root length (cm)/plant	Root No./ plant	Leaf length (cm)/plant	Leaf No./ plant	Node No./plant	Shoot No./ plant	Plant height (cm)/ plant
D_1S_1	155.00^{a}	5.50^{a}	4.20^{efgh}	1.10^{a}	34.00^{h}	16.33^{ab}	3.53^{bc}	44.30^{gh}
D_1S_2	122.00^{a}	6.37^{a}	3.33^{h}	1.30^{a}	80.33^{bcdefgh}	16.33^{ab}	6.77^{abc}	62.00^{abcde}
D_1S_3	119.33^{a}	4.70^{a}	4.90^{bcdefgh}	0.77^{a}	105.33^{abcdef}	6.00^{bc}	7.57^{abc}	51.63^{bcdefg}
D_1S_4	145.33^{a}	6.43^{a}	7.37^{abc}	1.20^{a}	116.00^{ab}	16.00^{abc}	5.50^{abc}	46.87^{defgh}
D_1S_5	134.67^{a}	5.87^{a}	7.60^{ab}	0.83^{a}	100.00^{abcdefg}	8.33^{bc}	2.50^{c}	50.20^{cdefgh}
D_1S_6	162.33^{a}	5.50^{a}	7.50^{abc}	1.10^{a}	77.67^{bcdefgh}	8.33^{bc}	5.37^{abc}	47.37^{defgh}
D_2S_1	154.33^{a}	5.57^{a}	4.63^{cdefgh}	1.23^{a}	98.67^{abcdefg}	5.67^{c}	7.57^{abc}	45.93^{fgh}
D_2S_2	157.33^{a}	5.57^{a}	6.53^{abcdefg}	1.30^{a}	105.33^{abcdef}	8.00^{bc}	7.67^{abc}	52.47^{bcdefg}
D_2S_3	166.67^{a}	5.70^{a}	5.13^{abcdefgh}	0.77^{a}	106.00^{abcde}	13.67^{abc}	7.17^{abc}	64.50^{abc}
D_2S_4	143.00^{a}	5.50^{a}	4.37^{defgh}	1.03^{a}	34.33^{h}	7.33^{bc}	3.53^{bc}	42.93^{gh}
D_2S_5	178.00^{a}	6.37^{a}	3.77^{gh}	0.73^{a}	73.67^{cdefgh}	8.00^{bc}	6.77^{abc}	45.57^{fgh}
D_2S_6	174.67^{a}	4.70^{a}	4.57^{cdefgh}	1.23^{a}	95.00^{abcdefg}	14.33^{abc}	7.57^{abc}	37.83^{gh}
D_3S_1	137.67^{a}	6.43^{a}	5.97^{abcdefgh}	1.03^{a}	122.67^{abc}	13.00^{bc}	5.50^{abc}	48.70^{cdefgh}
D_3S_2	166.67^{a}	6.20^{a}	6.83^{abcdef}	0.90^{a}	96.67^{abcdefg}	14.33^{bc}	2.50^{c}	51.80^{bcdefg}
D_3S_3	137.00^{a}	5.50^{a}	7.93^{a}	0.77^{a}	74.00^{cdefgh}	7.33^{bc}	8.70^{ab}	44.90^{gh}
D_3S_4	117.00^{a}	5.57^{a}	7.27^{abcd}	0.83^{a}	100.33^{abcdefg}	8.33^{bc}	7.57^{abc}	41.37^{gh}
D_3S_5	127.00^{a}	5.57^{a}	4.17^{efgh}	0.93^{a}	103.00^{abcdefg}	8.00^{bc}	7.67^{abc}	42.60^{gh}
D_3S_6	136.67^{a}	5.70^{a}	5.10^{abcdefgh}	1.37^{a}	107.67^{abcde}	7.33^{bc}	6.13^{abc}	41.07^{gh}
D_4S_1	170.00^{a}	5.70^{a}	5.37^{abcdefgh}	1.10^{a}	76.33^{bcdefgh}	6.67^{bc}	7.07^{abc}	62.00^{abcde}
D_4S_2	172.00^{a}	6.33^{a}	6.63^{abcdefg}	0.93^{a}	68.00^{defgh}	13.00^{abc}	2.37^{c}	61.43^{abcdef}
D_4S_3	162.00^{a}	5.70^{a}	6.13^{abcdefgh}	1.07^{a}	66.33^{defgh}	19.00^{a}	3.23^{bc}	72.27^{a}
D_4S_4	120.00^{a}	5.50^{a}	4.37^{defgh}	1.37^{a}	75.67^{bcdefgh}	12.67^{abc}	5.87^{abc}	47.13^{defgh}
D_4S_5	151.33^{a}	6.00^{a}	3.77^{gh}	0.93^{a}	68.67^{defgh}	10.00^{abc}	9.30^{a}	44.43^{gh}
D_4S_6	178.67^{a}	6.17^{a}	4.00^{fgh}	1.00^{a}	81.67^{bcdefgh}	7.33^{bc}	6.93^{abc}	53.07^{bcdefg}
D_5S_1	146.00^{a}	5.77^{a}	7.03^{abcde}	0.77^{a}	124.00^{abc}	13.67^{bc}	3.33^{bc}	67.47^{ab}
D_5S_2	153.00^{a}	5.70^{a}	4.30^{efdgh}	1.13^{a}	74.33^{cdefgh}	8.33^{bc}	7.00^{abc}	53.87^{bcdefg}
D_5S_3	170.33^{a}	5.73^{a}	3.57^{h}	1.33^{a}	124.00^{abc}	10.00^{abc}	6.30^{abc}	64.27^{abc}
D_5S_4	153.00^{a}	5.47^{a}	3.73^{gh}	1.30^{a}	69.33^{defgh}	10.00^{abc}	6.73^{abc}	43.87^{gh}
D_5S_5	141.00^{a}	5.40^{a}	4.63^{cdefgh}	0.90^{a}	75.33^{bcdefgh}	14.00^{abc}	6.30^{abc}	40.67^{gh}
D_5S_6	142.33^{a}	5.70^{a}	4.80^{bcdefgh}	0.87^{a}	66.00^{defgh}	10.00^{abc}	3.60^{bc}	49.87^{cdefgh}
D_6S_1	154.00^{a}	5.87^{a}	3.77^{gh}	0.97^{a}	114.00^{abcd}	13.67^{abc}	6.37^{abc}	46.20^{efgh}
D_6S_2	154.67^{a}	5.70^{a}	7.60^{ab}	0.93^{a}	75.67^{bcdefgh}	8.33^{bc}	7.40^{abc}	48.87^{cdefgh}
D_6S_3	163.00^{a}	6.83^{a}	7.63^{ab}	0.83^{a}	133.33^{a}	19.33^{a}	8.83^{ab}	38.10^{gh}
D_6S_4	162.00^{a}	4.93^{a}	5.47^{abcdefgh}	1.03^{a}	56.00^{fgh}	8.00^{bc}	5.17^{abc}	44.33^{gh}
D_6S_5	135.33^{a}	4.60^{a}	3.87^{gh}	1.40^{a}	53.67^{gh}	6.33^{bc}	5.33^{abc}	35.17^{h}
D_6S_6	119.33^{a}	4.60^{a}	4.60^{cdefgh}	0.87^{a}	57.00^{efgh}	13.00^{abc}	5.77^{abc}	45.57^{fgh}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test. D_1, D_2, D_3, D_4, D_5 and D_6, respectively the concentrations of 0.00, 0.60, 1.20, 1.80, 2.40 and 3.00 g/pot of Nano-fertilizer applied as drench and S_1, S_2, S_3, S_4, S_5 and S_6, the concentrations of 0.00, 1.00, 2.00, 3.00, 4.00 and 5.00 g/l Nano-fertilizer applied as spray.

Table 2. Effects of the different treatments on morphological and physiological characters of box tree (*Buxus hyrcana*).

Treatments	Mean of values					
	Dry weight (g)/plant	Fresh weight (g)/plant	Proliferation rate/ plant	Productivity/ plant	Root diameter (cm)/plant	Stem diameter (cm)/plant
D_1S_1	55.37^{cd}	91.00^{a}	57.93^{a}	8.41^{a}	0.29^{a}	0.36^{bc}
D_1S_2	145.33^{abcd}	218.17^{a}	92.40^{a}	20.97^{a}	0.33^{a}	0.42^{bc}
D_1S_3	38.10^{d}	62.30^{a}	60.70^{a}	38.31^{a}	0.37^{a}	0.52^{bc}
D_1S_4	117.53^{abcd}	143.80^{a}	58.13^{a}	19.49^{a}	0.28^{a}	0.92^{ab}
D_1S_5	46.33^{cd}	70.53^{a}	31.13^{a}	10.05^{a}	0.35^{a}	0.37^{bc}
D_1S_6	95.10^{abcd}	119.50^{a}	57.80^{a}	20.20^{a}	0.25^{a}	0.62^{bc}
D_2S_1	148.66^{abcd}	203.53^{a}	41.77^{a}	43.88^{a}	0.38^{a}	0.38^{bc}
D_2S_2	142.60^{abcd}	186.53^{a}	76.20^{a}	41.40^{a}	0.47^{a}	0.42^{bc}
D_2S_3	171.53^{abcd}	220.47^{a}	54.07^{a}	39.65^{a}	0.42^{a}	0.37^{c}
D_2S_4	172.67^{abcd}	202.43^{a}	35.67^{a}	9.02^{a}	0.31^{a}	0.58^{bc}
D_2S_5	49.60^{cd}	69.63^{a}	63.13^{a}	21.89^{a}	0.31^{a}	0.40^{c}
D_2S_6	85.53^{abcd}	119.17^{a}	57.80^{a}	35.18^{a}	0.33^{a}	0.36^{bc}
D_3S_1	89.30^{abcd}	143.57^{a}	55.10^{a}	20.25^{a}	0.30^{a}	0.36^{bc}
D_3S_2	54.27^{cd}	83.77^{a}	45.07^{a}	13.58^{a}	0.31^{a}	0.51^{bc}
D_3S_3	78.37^{abcd}	110.27^{a}	66.50^{a}	27.08^{a}	0.31^{a}	0.56^{bc}
D_3S_4	73.20^{bcd}	108.93^{a}	73.07^{a}	44.31^{a}	0.32^{a}	1.31^{a}
D_3S_5	116.67^{abcd}	161.77^{a}	62.40^{a}	30.40^{a}	0.35^{a}	0.34^{c}
D_3S_6	128.77^{abcd}	100.37^{a}	55.73^{a}	48.14^{a}	0.32^{a}	0.49^{bc}
D_4S_1	217.20^{ab}	272.73^{a}	47.20^{a}	30.08^{a}	0.43^{a}	0.35^{c}
D_4S_2	107.33^{abcd}	182.00^{a}	40.67^{a}	11.44^{a}	0.39^{a}	0.62^{bc}
D_4S_3	99.47^{abcd}	143.83^{a}	85.80^{a}	20.81^{a}	0.42^{a}	0.32^{c}
D_4S_4	44.03^{cd}	72.93^{a}	58.23^{a}	16.80^{a}	0.30^{a}	0.41^{bc}
D_4S_5	110.50^{abcd}	161.47^{a}	51.40^{a}	39.80^{a}	0.45^{a}	0.50^{bc}
D_4S_6	143.07^{abcd}	184.13^{a}	51.80^{a}	25.01^{a}	0.40^{a}	0.33^{c}
D_5S_1	169.70^{abcd}	255.00^{a}	71.73^{a}	21.03^{a}	0.39^{a}	0.33^{c}
D_5S_2	160.40^{abcd}	206.37^{a}	73.00^{a}	37.45^{a}	0.40^{a}	0.33^{c}
D_5S_3	133.53^{abcd}	185.27^{a}	49.60^{a}	28.25^{a}	0.37^{a}	0.40^{bc}
D_5S_4	93.77^{abcd}	136.23^{a}	62.73^{a}	31.39^{a}	0.35^{a}	0.40^{bc}
D_5S_5	211.50^{ab}	260.00^{a}	72.57^{a}	18.46^{a}	0.27^{a}	0.33^{c}
D_5S_6	132.33^{abcd}	190.73^{a}	71.20^{a}	33.15^{a}	0.41^{a}	0.33^{c}
D_6S_1	131.47^{abcd}	184.13^{a}	71.93^{a}	25.20^{a}	0.36^{a}	0.33^{c}
D_6S_2	181.73^{abc}	70.60^{a}	45.07^{a}	21.68^{a}	0.39^{a}	0.37^{c}
D_6S_3	147.97^{abcd}	199.93^{a}	133.53^{a}	24.84^{a}	0.36^{a}	0.48^{bc}
D_6S_4	90.03^{abcd}	128.60^{a}	41.30^{a}	21.71^{a}	0.42^{a}	0.53^{bc}
D_6S_5	213.70^{a}	259.73^{a}	41.47^{a}	16.15^{a}	0.42^{a}	0.37^{bc}
D_6S_6	106.37^{abcd}	150.30^{a}	64.73^{a}	18.00^{a}	0.44^{a}	0.35^{c}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test. D_1, D_2, D_3, D_4, D_5 and D_6, respectively the concentrations of 0.00, 0.60, 1.20, 1.80, 2.40 and 3.00 g/pot of Nano-fertilizer applied as drench and S_1, S_2, S_3, S_4, S_5 and S_6, the concentrations of 0.00, 1.00, 2.00, 3.00, 4.00 and 5.00 g/l Nano-fertilizer applied as spray.

Table 3. Effects of the different treatments on physiological characters of box tree (*Buxus hyrcana*).

Treatments	Mean of values						
	Content of Fe (ppm)/plant	Content of K (ppm)/plant	Content of P (ppm)/plant	Content of N (%)/plant	Total chlorophyll content (mg/m F.W.)/plant	Chlorophyll b content (mg m^{-1} F.W.)/plant	Chlorophyll a content (mg/m F.W.)/plant
D_1S_1	80.80^{cdef}	37.43^{ef}	112.50^{e}	0.11^{b}	1.71^{p}	0.56^{s}	1.17^{l}
D_1S_2	47.60^{ijklm}	29.22^{qr}	108.30^{n}	0.44^{ab}	6.41^{a}	2.98^{a}	3.28^{bcd}
D_1S_3	54.27^{hijklm}	41.26^{b}	122.30^{h}	0.24^{ab}	4.83^{de}	2.35^{c}	2.44^{ghij}
D_1S_4	48.43^{ijklm}	39.43^{d}	52.68^{z}	0.14^{b}	2.56^{o}	0.83^{r}	1.64^{lm}
D_1S_5	66.23^{efghij}	31.24^{p}	107.20^{o}	0.21^{ab}	2.72^{o}	1.12^{p}	1.58^{lm}
D_1S_6	48.87^{ijklm}	39.72^{cd}	135.00^{c}	0.17^{ab}	3.68^{lm}	1.31^{mno}	2.33^{hij}
D_2S_1	43.50^{ijklm}	38.36^{e}	90.57^{w}	0.11^{b}	3.99^{hi}	1.66^{fghij}	2.33^{hij}
D_2S_2	57.70^{ghijkl}	33.02^{mn}	115.30^{l}	0.25^{ab}	2.75^{o}	1.17^{op}	1.66^{l}
D_2S_3	49.57^{ijklm}	32.33^{no}	34.64^{z}	0.12^{b}	4.43^{g}	1.72^{efgh}	2.65^{fghi}
D_2S_4	151.00^{b}	32.77^{mn}	95.97^{t}	0.20^{ab}	4.05^{hi}	1.69^{fghij}	2.34^{hij}
D_2S_5	48.90^{ijklm}	27.43^{s}	92.23^{v}	0.39^{ab}	5.02^{cd}	1.84^{ef}	2.92^{def}
D_2S_6	93.90^{cd}	40.60^{bc}	112.50^{m}	0.13^{b}	3.28^{n}	1.52^{ijkl}	1.73^{kl}
D_3S_1	47.10^{ijklm}	36.73^{fg}	77.75^{z}	0.12^{b}	3.71^{klm}	1.58^{hijkl}	2.13^{jk}
D_3S_2	43.27^{ijklm}	29.35^{q}	131.80^{e}	0.26^{ab}	4.85^{de}	1.90^{de}	2.96^{cdef}
D_3S_3	47.87^{ijklm}	30.88^{p}	103.90^{q}	0.25^{ab}	6.49^{a}	3.04^{a}	3.38^{b}
D_3S_4	30.80^{m}	30.47^{p}	90.56^{w}	0.14^{b}	4.13^{hi}	1.41^{lmn}	2.66^{fghi}
D_3S_5	35.93^{lm}	28.35^{rs}	80.05^{y}	0.40^{ab}	3.76^{jkl}	1.61^{fghi}	2.11^{jk}
D_3S_6	292.50^{a}	34.58^{kl}	108.90^{n}	0.22^{ab}	1.87^{p}	1.10^{pq}	2.72^{efgh}
D_4S_1	51.80^{hijklm}	28.45^{qr}	106.10^{p}	0.05^{b}	2.58^{o}	1.10^{pq}	1.52^{lm}
D_4S_2	40.67^{jklm}	24.35^{t}	88.82^{x}	0.63^{a}	6.86^{a}	2.27^{c}	4.02^{a}
D_4S_3	85.53^{cdef}	36.01^{ghij}	200.20^{a}	0.36^{ab}	4.80^{ef}	2.04^{d}	3.21^{bcd}
D_4S_4	65.60^{efghijk}	36.59^{fgh}	149.20^{b}	0.11^{b}	2.75^{o}	1.23^{nop}	1.62^{lm}
D_4S_5	39.13^{lm}	35.48^{ijk}	94.45^{v}	0.43^{ab}	4.64^{efg}	1.52^{jkl}	3.09^{bcde}
D_4S_6	39.87^{klm}	35.20^{jk}	134.00^{d}	0.15^{b}	6.05^{b}	2.61^{b}	3.36^{bc}
D_5S_1	44.03^{ijklm}.	36.63^{fg}	99.57^{s}	0.07^{b}	1.93^{p}	0.76^{r}	1.25^{m}
D_5S_2	90.17^{cde}	39.83^{cd}	71.69^{z}	0.49^{ab}	4.59^{fg}	1.77^{efg}	2.75^{efg}
D_5S_3	46.33^{ijklm}	44.88^{a}	57.62^{z}	0.15^{b}	3.95^{ij}	1.63^{ghijk}	2.33^{hij}
D_5S_4	59.80^{fghijkl}	35.63^{hij}	199.70^{a}	0.21^{ab}	4.65^{ef}	1.80^{efg}	2.79^{efg}
D_5S_5	75.67^{defgh}	35.23^{jk}	100.80^{r}	0.25^{ab}	3.53^{m}	1.22^{op}	2.32^{ij}
D_5S_6	102.20^{c}	33.61^{lm}	121.30^{i}	0.13^{b}	3.50^{m}	1.20^{op}	2.32^{ij}
D_6S_1	106.20^{c}	38.33^{e}	118.60^{j}	0.12^{b}	4.11^{hi}	1.54^{hijkl}	2.51^{ghij}
D_6S_2	67.43^{efghi}	31.39^{op}	125.10^{g}	0.19^{ab}	4.21^{h}	1.47^{klm}	2.71^{efghi}
D_6S_3	82.60^{cdefg}	36.51^{fgh}	134.50^{cd}	0.12^{b}	3.92^{ijk}	1.53^{ijkl}	2.40^{ghij}
D_6S_4	87.53^{ghijkl}	33.39^{lm}	107.40^{o}	0.11^{b}	2.61^{o}	0.93^{qr}	1.67^{l}
D_6S_5	57.53^{ghijkl}	36.23^{ghi}	129.30^{f}	0.16^{ab}	3.27^{n}	1.15^{op}	2.16^{j}
D_6S_6	70.30^{f}	23.70^{t}	117.70^{k}	0.07^{b}	5.08^{c}	2.25^{c}	2.77^{efg}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test. D_1, D_2, D_3, D_4, D_5 and D_6, respectively the concentrations of 0.00, 0.60, 1.20, 1.80, 2.40 and 3.00 g/pot of Nano-fertilizer applied as drench and S_1, S_2, S_3, S_4, S_5 and S_6, the concentrations of 0.00, 1.00, 2.00, 3.00, 4.00 and 5.00 g/l Nano-fertilizer applied as spray.

Table 4. Analysis of variance for the effect of the different treatments on morphological and physiological characters of box tree (*Buxus hyrcana*).

Source of variations	df	Mean of squares											
		Proliferation rate	Productivity	Root diameter	Stem diameter	Root volume	Leaf length	Leaf No.	Root No.	Root length	Node No.	Plant height	Shoot No.
Replication	2	708.31	204.95	0.02	0.01	471.23	0.05	361.00	1.58	0.17	3.73	91.64	4.14
Treatment	35	1041.32^{ns}	343.92^{ns}	0.01^{ns}	0.12**	943.65*	0.12^{ns}	1914.97**	5.91**	0.75**	39.62**	248.73**	9.88**
Error	70	1098.51	387.61	0.01	0.02	570.25	0.13	185.35	0.61	0.38	7.59	17.99	2.10
CV		54.77	75.93	28.52	34.39	16.00	35.04	15.81	14.68	10.95	25.58	8.49	24.33

* And **: Significant at $\alpha = 5\%$ and 1%, respectively, ^{ns}: not significant.

Table 4. Continued.

Source of variations	df	Mean of squares								
		Fresh weight	Dry weight	Chlorophyll a content	Chlorophyll b content	Total chlorophyll content	Content of N	Content of P	Content of K	Content of Fe
Replication	2	261.28	1900.67	0.47	0.004	0.018	0.048	1.00	0.138	1.81
Treatment	35	10599.57^{ns}	7222.46**	1.20**	0.98**	4.33**	0.054**	3326.60**	66.29**	638.75**
Error	70	1246.30	1382.25	0.014	0.003	0.004	0.019	0.054	0.085	5.57
CV		21.83	31.10	4.89	3.41	1.65	62.75	0.21	0.85	5.10

* And **: Significant at a = 5 and 1%, respectively, ^{NS}: Not significant.

Maximum fresh (272.73 g) and dry weight (217.20 g) were obtained in D_4S_1 (Table 2). Most of Nano-fertilizer treatments led to increments of more leaf fresh and dry weights of box tree in comparison to the control. Similar results were reported by Sabir *et al*. (2014) on grapevines and Tarafdar *et al*. (2014) on *Pennisetum americanum*. The most productivity (48.14) was obtained in D_3S_6 (Table 2). The least productivity (8.41) was calculated in control plants. The highest proliferation rate (133.53) was obtained in D_6S_3, the treatment that we focused on it (Table 2). The lowest proliferation rate (31.13) was seen in D_1S_5.

Nano-fertilizer notably enhanced the concentrations of N, P, K and Fe in leaves (Table 3). The highest amount of N (0.63%) was obtained from D_4S_2 (Table 3). This amount is six times higher than the amount of N in control plants (0.11%). Conversely, the least values on N (0.04%) were determined in plants treated with highest concentrations of Nano-fertilizer (3.00 g/pot drench + 5.00 g/l spray). The highest rate of P (200.20 ppm) in leaves was obtained from D_4S_3 (Table 3). This amount is two times higher than the amount of P in control plants (112.50 ppm). Maximum K (44.88 ppm) in leaves was calculated from D_5S_3 (Table 3). Minimum K (23.70 ppm) was obtained in plants treated with the highest concentrations of Nano-fertilizer (3.00 g/pot drench + 5.00 g/l spray) (Table 3). The most rate of Fe (292.50 ppm) in leaves was obtained from D_3S_6 (Table 3). The least rate of Fe (30.80 ppm) was determined in D_3S_4 (Table 3). Leaf treatments with Nano-fertilizer had significant positive effects on the leaf N, P, K and Fe contents compared with control. Similar results reported by Sabir *et al*. (2014). Contrary to our findings, some researchers demonstrated that leaf blade N, P and K concentrations did not significantly differ among the treatments (Poni *et al*. 2003, Arrobas *et al*. 2014). Such different results among the studies might be due to the differences between fertilizer, ecologies and plant genotype. In many studies, the effectiveness of Fe treatment was greatly variable due to the several plant-related, environmental and physicochemical factors that may affect the physiology of plant in different ways (Abadía *et al*. 2011, Sabir *et al*. 2014). Fernández *et al*. (2009) showed that foliar application of different Fe-containing compounds increased Fe concentration in peach leaves.

The highest chlorophyll a and total chlorophyll concentration (4.02 and 6.86 mg/g F.W., respectively) was determined in D_4S_2 (Table 3). The highest chlorophyll b (3.04 mg/g F.W.) was determined in D_3S_3 (Table 3). The least concentration of all chlorophylls was seen in control plants. Our results revealed that all treatments significantly increased the leaf chlorophyll contents of box tree. The least chlorophyll content was determined in leaves of untreated box tree. This result is consistent with Sabir *et al*. (2014). The results of Tarafdar *et al*. (2014) on *Pennisetum americanum* demonstrated that photosynthetic pigment chlorophyll was increased by 24.4%, by application of Zn Nano-fertilizer at 10 mg/l concentration with respect to control. Increasing the chlorophyll content in leaves treated with Nano-fertilizer in current study is because of the presence of Fe and Zn in this fertilizer.

When the application forms of Nano-fertilizer (drench and foliar spray) were compared with each other, significant differences between traits were observed. Totally, spray application was better than drench. Similar results on some plants were reported (Karp *et al*. 2002, Mohammadipoor *et al*. 2013). It seems that in the indexes of corresponding to the root such as fresh and dry weight of roots, soil method is better because of more activity of root for nutrient uptake from medium and increases this indexes (Mohammadipoor *et al*. 2013). This matter can be useful for plants that their ground part is important and economic. Soil application was not a suitable method. Similar results were reported by some other researchers (Erdal *et al*. 2002). This decrease in the concentration of the elements on soil application treatment can be due to low speed of iron intake and transmission from this fertilizer that causes disturb the balance of nutrients absorption such as P and K in soil.

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