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ORIGINAL ARTICLE

The Effects of Nano-bio Fertilizer on Vegetative Growth and Nutrient uptake in Seedlings of three apple cultivars

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ABSTRACT
For investigation the effect of nano-bio fertilizer on growth characteristics and nutrient uptake in three cultivars of apple, performed an experiment in a randomized complete block design with eight replications in Eqlid of Fars (Iran) providence in 2015. Treatments that applied at the end of May, were contained of four levels of nano-bio fertilizer (0, 1, 2 and 3 g/ pot) and three cultivars of apples ("Red Delicious", "Golden Delicious" and "Starking Delicious"). At the end of period, vegetative characteristics and concentration of elements were analyzed. The results showed that the application of nano-bio fertilizers in apple plants growing medium increased significantly plant height, stem diameter, leaf number and area and amount of chlorophyll a and b compared to the control .The concentration of copper, iron and phosphorus of leaves decreased, but their absorption increased in all three cultivars. Based on the results, using of 1 g/pot nano-bio fertilizer had a greater impact on the growth of apple plants. However, according to the results, the most characteristics did not show significant difference between 2 and 3 gram nano-bio fertilizer per pot.
***Keywords**: Chlorophyll, Micro Elements, Nanotechnology, Nutrition, Phosphorus*

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INTRODUCTION

The use of excess chemical fertilizers can result in environmental contamination while applying biological and nano-fertilizers can increase soil fertility, crop production and at the same time prevent environmental pollutions [6]. Actually, nanotechnology has provided new opportunities in order to provide higher nutrient use efficiently and minimize the cost of protecting the environment [3].
Nano fertilizers have been designed to release their nutrient contents gradually and synchronize release time with the pattern of a plant mineral uptake as needed over an extended period of time, therefore the most of the nutrients is taken up by the crop and the lowest nutrient losses due to leaching will occur [3]. However, over application of macronutrients followed by the low use efficiency (about 30-50%) have led to large inputs of these nutrients (P, N) to ground water and surface water which can affect human health and aquatic life that rely on water for habitat [14]. Therefore, more studies would be strongly needed around macro nano nutrients which are the best environmentally friendly replacements for common N and P fertilizers [14]. Humic compounds of nano-fertilizers improve the efficiency of water and nutrients absorption in the soils under adverse conditions such as high pH, salt stress and etc [14]. Foliar application of nano-chelate super plus zinc, iron and manganese significantly increased the micronutrients concentration in leaf of apricot [13]. The results have shown that by spraying four different Fe-fertilizers on apricots, replacement of iron fertilizer produced with nanotechnology even in lower concentration in comparison with conventional one could effectively increase chlorophyll content and leaf sugar [16].

However, studies have clearly indicated that applying more Fe nano-chelate fertilizer on olive increases the chlorophyll content a, b (a+ b) and carotenoids [2]. Using biological fertilizers have significantly improved yield of cucumber [20]. Spraying Fe nano-chelate fertilizer (Khazra) on pistachio has led to 50% Fe and 55% Ca content increase. Foliar application of two forms of ferrous sulfate consisted of nanoparticles and conventional fertilizers also have shown similar effects on photochemical efficacy, concentration of chlorophyll b, sodium, potassium and iron content in aerial parts of the trees [10]. Application the nanoparticles form of the nutrients has a profound impact on modifying salinity effects for zinc concentration compared with conventional fertilizers in aerial parts. In addition, regarding the key structural role of micronutrients such as Fe, Mn and Zn on some enzymes as well as important role in protein synthesis, applying micronutrients can significantly enhance crop productivity and plant resistance under environmental stress [12]. Absorption and storage of many nutrients such as Fe, P and etc in plant tissues is limited, considering characteristics of limestone soils in Iran. The present study was conducted to evaluate the effects of biological nano-fertilizer on growth characteristics and nutrients absorption rate in limestone soils of apple seedlings and finally recommended the best nano-fertilizer application rate on apple seedlings.

MATERIAL AND METHODS

Experimental site and treatments

The experiment was conducted in two growing seasons 2016 in the suburbs of Eghlid, Fars province (Iran), in the region with latitude 32˚56′ N, longitude 59°13′ E and elevation above sea level, 2158 m. This experiment was actually performed as a randomized complete block design (RCBD) with two factors including: three types of apple ' Golden Delicious', 'Red Delicious', and 'Starking Delicious' and four levels of nano biological fertilizer (0, 1, 2, 3 gram per pot), in eight replication. Each pot was as a replication.

Apple seedlings (three cultivars: 'Golden Delicious', 'Red Delicious', and 'Starking Delicious' that were grafted on 'Malling' three year-old rootstock) were prepared from Abedini nursery (Eghlid) that has received certification from Seed and Plant Improvement Institute, Karaj, Iran.

Each apple seedling was planted in a plastic pot (with 45cm length and 25 cm diameter (top)) filled with sandy-loam soil. Distances between the pots and replications were 30 and 50 cm, respectively. In order to avoid transplant shock, seedlings were kept in a greenhouse (with temperature around 23±2°C, photoperiod 16/8 cycle) then pots were moved outside with outdoor condition (zero for rainfall, maximum and minimum temperature, 35°C and 4°C).

Soil physicochemical properties

According to soil analysis results, soil texture was classified as sandy loam (68.8% sand, 28.4% silt, and 2.8% clay); soil pH and EC was determined 7.3 and 3.6ds.m^{-1}, respectively. Soil composite sample contained %0.04 N, 158 ppm K, 9.5 ppm K, 0.6 ppm Fe, 0.34 ppm Zn, 0.، ppm Cu and 2.4 ppm Mn.

Treatment of seedlings with Bio-nano fertilizer

Biological nano fertilizer of Biozar® (containing 2 ppm fulic acid, 32 ppm humic acid, 14 ppm P, 5.96 ppm Fe, 4.3 ppm Mn, 0.33 ppm Mg, 10 ppm Zn, 0.36 ppm Ca, 1.44 ppm B and containing *Rhizobium*, *Azospirillium*, *Pseudomonas* and *Bacillus* microorganisms and nanoparticles 1000 ppm) was inoculated in the rhizosphere zone of seedlings pots. Control was without fertilizer with eight replicates. Data collected at the end of growth season in September. Immediately after transplanting to pots, irrigation was done at the rate of 0.5 liter per pot, and irrigation schedule was carried out in periods of 3, 5, and 7 days, depending on environmental condition during the test period. As the soil dried, soil water tension was measured by using tensiometer. In order to control two-spotted spider mite, Envidor pesticide was applied in two stages at rate 50 ml/100 l, and weeds were removed by hand without applying herbicide.

For leaf sampling, midway and fully expanded leaves were collected at the end of season's growth from September till October. The following traits were evaluated: leaf area, leaf number, dry and fresh weight, chlorophyll a, b content, seedling height and stem diameter, macronutrients and micronutrients measurement (N, P, Cu and Fe) measurement.

Vegetative growth measurements

Plant height and stem diameter of the main shoot were measured using measuring tape and Vernier callipers respectively, at 5 cm above the soil surface. Leaf area was assessed with the help of the leaf-area meter model Win Area-UT-11 and it was expressed in square centimetres (cm^2). For calculating dry weight, the leaves were rinsed with distilled water and oven-dried at 65 ˚C for 72 hours [16]. Chlorophyll content of the leaf was estimated according to the process given by Halfacre *et al*. [9].

Total N content of leaves was determined using Nitrogen Kjeldahl's method. P content of leaves determined by the phosphovanadomolybdate method [11]. K content was determined by using a flame photometer. Micronutrients viz. Fe, Cu, Zn, and Mn were determined at wavelength of 214–589 nm using

atomic absorption spectrophotometer (GBC UV-Visible Spectrometer Cintra 5T model) [22]. Concentrations of the macronutrients were expressed as percentages of dry weight, while those of micronutrients were expressed as ppm in dry weight. Nutrient uptake measured according to the relation 1 and expressed as mg per pot.

Relation (1): $U(\frac{mg}{pot}) = \frac{C \times W}{100} \times 1000$

U: Amount of element that absorbed by the plant

C: Concentration of element in plant according to the percent

W: Dry weight of plant according to the g pot^{-1}

Data analysis

The experiment was laid out in a random block design. Each treatment was replicated four times. The data thus obtained were subjected to analysis of variance (ANOVA). The least significant differences (LSD) were used to compare the means at 5% level of significance.

RESULTS

Vegetative traits

According to the results, nano-fertilizers improved growth factors (height and diameter). Table 1 shows that nano fertilizers increased plant height. In current study, different reaction of cultivars to nano-fertilizer was indicated (table1).

Maximum plant height was obtained in 'Golden Delicious' cultivar compared to 'Red Delicious', and 'Starking Delicious' (table1). Maximum plant height was obtained at 1 and 3 g nano-fertilizer per pot (table1). Application of nano-fertilizer increased stem diameter compared to controls (table1). As depicted in table1, significant increase of stem diameter was shown in response to each three nano-fertilizer levels on all three cultivars of seedlings. Maximum stem diameter was obtained in 'Golden Delicious' cultivars that were treated with 1g nano-fertilizer and the minimum one was calculated in control samples in all three cultivars.

Table1. The interaction effect of cultivar and nano-fertilizer on apple seedling vegetative traits (the columns with common letters, show non-significant difference at the 1% level by Duncan test)

Nano-fertilizer (g/pot)	cultivar	Height (cm)	Stem diameter (cm)	Leaf number	Leaf area (cm^2)	chlorophyll a (mg/g fresh weight)	chlorophyll b (mg/g fresh weight)	Fresh weight (g)	Dry weight (g)
0	Starking Delicious	2^{f}	0.047^{f}	48^{e}	5.37^{c}	2.21^{cd}	1.80^{c}	3.89^{d}	2.94^{e}
	Golden Delicious	7^{bc}	0.05^{f}	70de	7.23^{bc}	2.01^{d}	1.92^{cd}	4.62^{bcd}	3.45^{bcde}
	Red Delicious	4.83^{e}	0.04^{f}	59^{e}	7.63^{bc}	2.55^{abc}	2.04^{bcd}	3.89^{d}	2.86^{e}
1	Starking Delicious	9.83^{bc}	0.11^{bc}	159^{a}	13.63^{a}	1.92^{d}	2.13^{abcd}	4.09^{cd}	3.08^{de}
	Golden Delicious	12.83^{a}	0.09^{de}	84^{cde}	10.67^{ab}	2.88^{a}	2.57^{ab}	5.19^{bc}	3.58^{bcde}
	Red Delicious	9.67^{bc}	0.16^{a}	162^{a}	7.31^{bc}	2.64^{abc}	2.43^{ab}	5.08^{bcd}	3.47^{bcde}
2	Starking Delicious	8.67^{c}	0.11^{bc}	77de	14.26^{a}	2.33^{abcd}	2.20^{abcd}	5.34^{bc}	3.92^{abc}
	Golden Delicious	9.83^{bc}	0.08^{e}	132^{abc}	10.4^{ab}	2.80^{ab}	2.14^{abcd}	5.07^{bcd}	3.76^{abcd}
	Red Delicious	6.67^{d}	0.12^{b}	137^{abc}	11.09^{ab}	2.80^{ab}	2.74^{a}	7.56^{a}	4.38^{a}
3	Starking Delicious	11.17^{ab}	0.10^{cde}	120^{abc}	13.73^{a}	2.42^{abcd}	2.34^{abcd}	4.90^{bcd}	3.21^{cde}
	Golden Delicious	11.67^{a}	0.09^{e}	115^{abcd}	9.55^{abc}	2.26^{bcd}	1.90^{cd}	5.21^{bc}	3.86^{abc}
	Red Delicious	5.33^{de}	0.12^{b}	147^{ab}	9.46^{abc}	2.67^{abc}	2.50^{abc}	5.54^{b}	3.58^{bcd}

As shown in table 1, the nano-fertilizer treatment has significant effect on leaf number and leaf area. 'Starking Delicious' and 'Red Delicious' cultivars that were treated by 1 g nano-fertilizer showed maximum leaf number (table 1). All three levels (1, 2 and 3g per pot) of nano-fertilizer showed the

maximum leaf area in 'Starking Delicious' cultivar and the minimum one observed in control samples of 'Starking Delicious' (table1). By increasing the nano-fertilizer levels in soil, chlorophyll content of leaves improved in all apple cultivars (table 1). The highest amount of chlorophyll a content was obtained in 'Golden Delicious' cultivar that was treated with 1 g nano-fertilizer per pot and the maximum chlorophyll b content was recorded in 'Red Delicious' that was treated with 2 g nano-fertilizer per pot. In this study, fresh and dry weight of leaves increased by the application of nano-fertilizer, as maximum fresh and dry weight was obtained in 'Red Delicious' cultivar with 2g nano-fertilizer per pot (table1). By contrast, minimum fresh and dry weight was obtained in control samples of 'Starking Delicious' and 'Red Delicious' cultivars (table1).

Leaf macronutrients concentration and absorption

According to table 2, by increasing the nano-fertilizer levels in soil, P concentration of leaves significantly reduced in all apple cultivars while phosphorus absorption in leaves significantly increased. Maximum phosphorus concentration (0.45%) was recorded in 'Starking Delicious' cultivar without fertilizer treatment, while the minimum one obtained in 'Golden Delicious' treated with 2 and 3 g nano-fertilizer per pot (table 2). However, maximum amount of phosphorus absorption (17.59 g) was obtained in 'Starking Delicious' cultivar treated by 3 g nano-fertilizer per pot and the minimum one (9.26 g) recorded in 'Red Delicious' without fertilizer treatment.

According to the results leaf nitrogen content significantly increased in 'Red Delicious' and 'Golden Delicious' by increasing nano-fertilizer level while in 'Starking Delicious', leaf nitrogen content decreased significantly compared with control (table 2). In present study, maximum leaf nitrogen content (3.70%) was obtained in 'Red Delicious' treated by 1 g nano-fertilizer per pot.

Our results showed a marked increase in leaf nitrogen absorption with incorporation of nano-fertilizer. Of course this increase wasn't accompanied with nano-fertilizer levels, as maximum leaf nitrogen absorption observed in 'Red Delicious' with 1g per pot treatment (17.59 g) (table 2). The highest leaf nitrogen absorption was also obtained in 'Golden Delicious' treated by 2 g nano-fertilizer (16.7 g) and in 'Starking Delicious' treated by 3 g nano-fertilizer per pot (14.67 g). Therefore, variable responses of each cultivar to different levels of nano-fertilizer were identified.

Table 2. The effect of various levels of nano-fertilizer macronutrients concentration and absorption on apple seedling (the columns with common letters show non-significant difference at the 1% level by Duncan test)

cultivar	Nano-fertilizer (g/pot)	macronutrients concentration and absorption			
		N (%)	N(gr)	P (%)	P(gr)
Red Delicious	0	3.16^{bc}	9.26^{efg}	0.42^{ab}	9.26^{e}
Red Delicious	1	3.70^{a}	17.59^{a}	0.28^{d}	12.70^{cd}
Red Delicious	2	3.20^{abc}	14.90^{b}	0.35^{c}	14.90^{b}
Red Delicious	3	3.10^{bcd}	10.25^{cde}	0.28^{d}	10.25^{de}
Golden Delicious	0	2.87^{cd}	10.41^{fg}	0.39^{bc}	10.41^{de}
Golden Delicious	1	3.40^{ab}	12.35^{bc}	0.26^{d}	12.35^{cd}
Golden Delicious	2	3.40^{ab}	16.70^{a}	0.27^{d}	14.70^{b}
Golden Delicious	3	3.30^{abc}	12.14^{bc}	0.41^{ab}	12.14^{cd}
Starking Delicious	0	2.70^{d}	11.42^{fg}	0.45^{a}	11.42^{de}
Starking Delicious	1	3.30^{abc}	12.70^{cde}	0.28^{d}	14.67^{b}
Starking Delicious	2	3.10^{bc}	12.48^{bc}	0.35^{c}	10.48^{cd}
Starking Delicious	3	3.50^{ab}	14.67^{bc}	0.37^{bc}	17.59^{a}

Leaf micronutrients concentration and absorption

The maximum concentration and absorption of Cu was obtained in 'Starking Delicious' cultivar without any fertilizer treatment (control) (table 3). However, various leaf copper absorption were characterized, as Cu absorption decreased with enhancing nano-fertilizer level. in 'Red Delicious' also, the absorption amount did not show significant increase at 2 and 3 g treatments compared to 1g treatment. In addition in 'Golden Delicious', treatments and control sample did not show difference in Cu absorption. However, Cu concentration in 'Starking Delicious' leaves was low significantly and the maximum rate of it (20 g /kg) obtained in control pots (without treatment). According to table 3 the most Cu absorption (54 g) was obtained in 'Starking Delicious' without any nano-fertilizer (control). In 'Red Delicious', by increasing in nano-fertilizer level, Cu absorption significantly increased until 2 g per pot, but in 3 g treatment, Cu absorption decreased.

Maximum Fe concentration (341 g/kg) was identified in 'Red Delicious' without any treatment (control), as the highest Fe absorption (941 g) was obtained in 'Red Delicious' treated by 1 g nano-fertilizer. According to table 3, by enhancing nano-fertilizer level Fe concentration significantly decreased.

Table 3. The effect of cultivar and various levels of nano-fertilizer on apple seedling micronutrients concentration and absorption (letters in common, in vertical columns show non-significant difference at the 1% level by Duncan test)

cultivar	Nano-fertilizer (g/pot)	micronutrients concentration and absorption			
		Fe (mg/kg)	Fe (gr)	Cu (mg/kg)	Cu (gr)
Red Delicious	0	341^{a}	578^{cde}	14^{b}	39^{bc}
Red Delicious	1	193^{de}	941^{a}	10^{cd}	39^{bc}
Red Delicious	2	192^{de}	845^{ab}	12^{bcd}	39^{bc}
Red Delicious	3	140^{efgh}	686^{abc}	12^{bcd}	45^{ab}
Golden Delicious	0	262^{bc}	653^{ef}	10^{cd}	34^{bc}
Golden Delicious	1	172^{cde}	884^{ab}	10^{cd}	38^{bc}
Golden Delicious	2	145^{efg}	649^{bcd}	12^{bcd}	40^{bc}
Golden Delicious	3	95^{gh}	518^{cde}	10^{cd}	35^{bc}
Starking Delicious	0	300^{ab}	244^{f}	20^{a}	54^{a}
Starking Delicious	1	215^{cd}	859^{b}	8^{d}	31^{c}
Starking Delicious	2	78^{h}	852^{ab}	14^{b}	40^{bc}
Starking Delicious	3	119^{fgh}	393^{def}	10^{cd}	33^{bc}

DISCUSSION

According to the results, it was revealed that while there are significant differences between cultivars of apple in terms of genetic, application of nano-fertilizers in all experimented cultivars considerably stimulated their growth and development.

Data analysis of vegetative traits showed that incorporation of nano-fertilizer in high pH soil led to growth stimulation and vegetative traits improvement such as plant height, stem diameter, shoot numbers, fresh weight and dry weight. Findings in this study were according to many previous researchers [20]. Nano-fertilizer contains metal and non-metal oxide nano-particles and a structure similar to the soil that is rich of the available micronutrients for plant to be absorbed. However, each needed nutrient will be selected and absorbed by the plant. Also, organic acids of this nano-fertilizer improve micronutrients chelation rate that lead to maintaining soil fertility and as a result nutrition balance in plant that finally plant growth balance in all the aspects such as foliage growth, leaf production occurs [21]. Therefore, nano-fertilizer treatment at optimum level increases plant growth.

Nano-fertilizer at different levels significantly affected on seedling stem diameter but, cultivar type did not show significant effect on stem diameter. Our results revealed that treatment of 'Red Delicious' cultivar by 2 gram nano-fertilizer per pot led to 0.123 cm stem diameter increase compared to 0.043 cm in control. In 'Golden Delicious', nano-fertilizer treatment at different levels showed the least effect on stem diameter as at 1g nano-fertilizer 0.087 cm increase was measured in comparison to 0.05 cm in control samples. However, different levels of nano-fertilizer did not show significant difference in stem diameter of seedlings. Our results were according to Clark *et al*., [5], Caoa *et al*., [4]. By increasing nano-fertilizer levels, fresh and dry weight of leaves in all treatments significantly increased. It proved the role of nano-fertilizers in stimulation of plant growth. It has been reported by James *et al*., [12], Clark *et al*., [5], Caoa *et al.,* [4]. In present study, using nano-fertilizer enhanced chlorophyll content of leaves which leads to higher photosynthesis and improvement of root and stem growth.

Maximum fresh weight of leaves was obtained in 'Red Delicious' cultivar compared to the others. Also, maximum phosphorus absorption was recorded in 'Red Delicious' cultivar with 1 g nano-fertilizer treatment. However, leaf nitrogen absorption increased with enhancing nano-fertilizer levels in all three apple seedlings and maximum nitrogen absorption was determined in 'Red Delicious' cultivar with 1 g nano-fertilizer treatment. Biological nano-fertilizer has an important effect that supplies nitrogen for plant by absorbing air nitrogen which leads to enhancing plant nitrogen.

Phosphorus contain nano-fertilizers reacts less weak with soil particles than ions like PO^{-3}_4, HPO_4^{2-}, $H_2PO_4^-$ or Ca_2^+. Therefore, considerable amount of phosphorous remains in the soil solution for roots

absorption while most of the phosphorous ions in common fertilizers are absorbed by soil particles and are not available for plant [14].

With enhancing nano-fertilizer levels, Fe and Cu concentration of the leaves decreased as in 'Starking Delicious' cultivar, it was about quarter compared to control sample. However, minimum Cu concentration was observed in 'Starking Delicious' cultivar that was treated by 1g nano-fertilizer and maximum Cu concentration obtained in 'Starking Delicious' cultivar without any treatment. Maximum Fe absorption was observed in 'Red Delicious' cultivar with 1g nano-fertilizer treatment. Totally, micronutrients are added to N, P and K (that is called complete fertilizer). Generally, complete fertilizers provide micronutrients for plant growth efficiently and reduce environmental problems. However, micronutrients are less available for plant and their deficiency will occurs in alkaline, sandy soil or soil with little organic matter [8]. Nano-fertilizer contains micronutrients increase plant availability even under such undesirable conditions. Since, the development and use of nano-fertilizers are still in the early stages, there have been relatively few studies of advantages and disadvantages of micro nano-fertilizers application in field condition.

Low concentration of Fe nano-fertilizers in comparison to other forms of Fe fertilizers increased chlorophyll content in apricot leaves. In addition, low concentration of Cu nano-fertilizer enhanced rate of photosynthesis by 35% compared to control [17]. By applying nano-fertilizer, plant chooses and absorbs needed nutrients in significant amount. Nano-fertilizers maintain soil fertility and as a result nutrition balance in plant occurs that finally leads to branch growth, leaf production balance. However, applying nano-fertilizer as well as maintaining soil structure, improve soil texture and nutrition absorption that results in plant growth improvement and reduces fertilizers consumption and costs. According to the most studying traits, with enhancing nano-fertilizer level, growth characteristics of apple seedlings increased and 1g nano-fertilizer considered the best treatment.

CONCLUSION

In conclusion our results proved that plant growth characteristics (such as plant height, diameter, leaf number and leaf area) increased with nano-fertilizer treatment. Furthermore, different nano-fertilizer levels lead to increasing chlorophyll content, fresh and dry weight. Although applying nano-fertilizer reduced Cu, Fe, N and P concentration of the leaf, it increased nutrients absorption in all three cultivars. In conclusion, with applying nano-fertilizer in most of the estimated traits, an increase in the apple seedlings growth characteristics was observed. Results of this study suggest that 1g nano-fertilizer is the best treatment for apple seedlings in alkaline soil.

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