HUMIC SUBSTANCES APPLICATIONS IMPACT QUALITY AND YIELD OF COMMERCIALLY-PRODUCED POMEGRANATE SAPLINGS IN NANGARHAR, AFGHANISTAN

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Abstract: The efficacy and reliability of humic substances for increasing crop yields have not been widely established in the scientific literature. The aim of this research was to measure the effect of humic and fulvic acid application on the number of harvested pomegranate saplings, which meet the required standards and to compare it with saplings produced without humic substances. The application of humic substances increased by 34% the number of harvested pomegranate saplings meeting the requirements of the established standard. It improved quality characteristics of the saplings as well, such as increased weight and volume of the root system and increased diameter and height of the plant.

Keywords: Humic acid, nutrient uptake, yield components, saplings quality standards

Introduction

Pomegranate (*Punica granatum*) production is a significant contributor to the Afghan agricultural economy and a significant source of income for farmers and rural communities of many provinces. However, varietal and nursery technology degradation have been caused by years of war. For example, in Nangarhar Province, commercial fruit nurseries have been in operation just since 2006. That is why a survey study for that year indicated that only 45% of the saplings were acceptable for transplant (Sandor 2007). The large majority of unacceptable saplings were too small to transplant. Climatic and soil conditions in this Eastern region are challenging, characterized by low average annual precipitation, high temperature during the growing season and limited soil development. The use of biostimulants such as humic substances may significantly improve plant growth and yields.

Diercks (1983) concluded that the way to increase soil nutrient capacity is to introduce organic matter bound nutrients into the soil or to mix free nutrient based fertilizers with organic carbon compounds. He emphasized that in order to maintain the long-term balance of the soil and surrounding ecosystems, agriculturalists should pay greater attention to the practical application of organic manure and humus substances; a practice that has been historically neglected. Because of its molecular structure, it provides numerous benefits to crop production. Humic acid is the end product of organic matter decay and is primarily found in manure, peat, lignite coal, and leonardite. Humic acid is characterized by forming chelates with metallic micronutrients, iron, copper, zinc and manganese (Kussow 2002). The cation exchange capacity (CEC) of humic acid is in the range of 500 to 600 milliequivalents per 100 grams. This is about five times greater than the CEC of good quality peat moss and twice higher as the CEC of soil humus (Bigman 1996, Stevenson et al. 1982). Albayrak and Camas (2005) indicated that humic acid significantly affects most yield components. Root and leaf yields and their yield components increase along with increase rates of humic acid. Further studies by Kotob (2009) show that humic acid applications

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mitigate the harmful effects of salinity and enhance seedling emergence and plant growth.

Katkat et al. (2009) published their results of a humic acid experiment with wheat indicating that humic acid applications increase dry matter and N, P, K, Ca, Mg, Na, Fe, Cu, Zn and Mn uptake of plants in non-limed pots at 0.1% rate of humic acid. Higher rates, such as 0.2% was found much more effective on increasing dry matter and nitrogen uptake at high lime conditions. The foliar application of humic acid significantly affected Mg, Fe and Mn uptake with the highest dry matter accumulation and nutrient uptake obtained at the rate of 1g kg-1 of humic acid treatment. Khattab et al. (2012) indicated that the use of humic acid and amino 3 acids enhanced vegetative growth and fruiting in pomegranate production. In contrast, Mackowiak et. al. (2001) were unable to show any increase in wheat yield caused by humic acid applications, but successfully cured nutrient deficiency symptoms in the crop. Timothy and Bottoms (2010) reported similar results regarding phosphorus deficiency in lettuce and tomato. Cimrin and Yilmaz (2005) established that additional phosphorus application into the soil increased the uptake of nitrogen in the plant but they could not observe this in the humic acid treatment.

The main purpose of this study was to measure yield and quality changes in commercial pomegranate sapling production as affected by applications of humic substances. Also studied

Table 1. Comparison between reference values and average soil test results

Characteristic	Measured Value	Reference Range	Description	
$CaCO_3$ (%)	10.56	5,0-19,9	Moderately Alkaline	
K_A	35	30-38	Sandy-Loam	
pH_{H2O}	8.14	7.2-7.9	Moderately Alkaline	
Salt (%)	0.02	<0.05	Low	
Characteristic	**Measured Value**	**Reference Range**	**Description**	**Conditions**
P_2O_5 (mg/l)	47.03	26.0-50.0	Moderate	If $CaCO_3$ >1% and K_A value is >30
K_2O (mg/l)	129.5	101-160	Moderate	If K_A value is >30
NO_3-N (ppm)	3.5	<10	Low	-

Table 2. Methods for the pomegranate saplings production in the experiments

Characteristics	Description
Land Area	2.0 ha
Crop	Pomegranate saplings
Date of planting	March 2007 (Year 1) and 2008 (Year 2)
Date of harvesting	December 2007 (Year 1) and December 2008 (Year 2)
Planting distance	70 x 10 cm
Physical layout	Ridges
Starter fertilizer	150 kg DAP/ha and 75 kg urea/ha
Top dressing	May-June 2007 – 50 kg DAP/ha and 25 kg urea/ha by side dressing method
Water source	Canal (River)
Irrigation method	Furrow
Irrigation efficiency	40.08%
Irrigation interval	From 4 to 7 days
Weeds control	During growing season weeds were controlled by mechanical methods (hand labor)

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were changes of the soil physical and chemical properties and soil nutrient availability when compared to the baseline soil test.

Materials and Methods

The humic and fulvic acid used in this experiment was produced by Farmfert Formulators INC from South Africa and registered under the code number PCT WO 2006/092720AI. The four farms of the experiments were located in Behsod District, Nangarhar Province in the Eastern Region of Afghanistan. The water table can be found between 4 to 6 m. The initial soil characteristics of the four farms showed little differences. The soil pH is slightly-moderately alkaline with low salt accumulation. The soil was developed under forest and has a tendency to become sodic. The cation exchange capacity value indicates the dominance of 2:1 type secondary clay minerals, mainly Montmorrilonit and Smectit. The value of exchangeable sodium is very low. The phosphorus and potassium content in the soil is moderately good (Table 1).

The experiments were conducted in commercial pomegranate nurseries managed according to traditional production techniques described in Table 2.

For propagation we used simple non-rooted cuttings containing 5-6 buds each. The selected branches for cuttings were purchased from a commercial nursery located in Kandahar Province, Afghanistan. The cuttings were a year old, approximately 25-30 cm in length, populated with buds approximately 5 cm apart, and the resulting cuttings had a green cambium ring inside indicating that they were alive and healthy.

The four experiments consisted of two randomized treatments with four repetitions each within the four pomegranate nurseries (0.4 ha each) managed under traditional production methods. Additionally to the 4 treated plots per experiment, each one of them included one control (untreated) plot. In 2007, the four future experimental areas were standardized to a uniform production method (Table 2) and baseline data was collected. We used these data as a background information for the four experiments conducted in 2008. Standardization of the experimental areas was important to reduce the impact of soil neglect caused by more than 20 years of internal war. In 2008, the experiments followed the standard production method established in 2007 and treated plots received two applications of humic acid applied in shallow furrows with 15 cm distance from the plants. The application depth was 10 cm. The humic acid solution contained humic acid powder (50% concentration) mixed with water using 1:8 ratio during the preparation. The total application rate of humic acid was 100 kg/ha. The first soil application (50 kg/ha) occurred 60 d after planting and the second at d 90 at a rate of 50 kg/ha. After 120 d of planting we applied fulvic acid (10 l/ha) on the forming canopy of the saplings using a backpack sprayer. The saplings were harvested after 210 day.

During the field trial we conducted weekly measurements of sapling stem diameter and height of 20 saplings from treated and untreated plots. The sampling selection method is known as a point-intercept transect (laying down the center of a quadrat over each point of a line transect and then counting every plant inside the square) using transect tools, which are straight lines typically established through the use of a cord, wire or measuring tape, in this case a metal “Z” frame with a one meter length. The data was measured from the plants between the two ends of the frame. Harvested saplings that meet acceptable quality standards for commercial use were defined as those meeting a minimum height of 0.8 m and minimum diameter of 8-9 mm (Cselötei at al. 1985). The diameter of the 10 saplings was measured at three levels: root-stem transition region, stem midsection, and upper-third section. In addition, 10 plants per plot were measured for root weight and quantity after sapling harvest. Roots were separated from soil using a 6 mm sieve.

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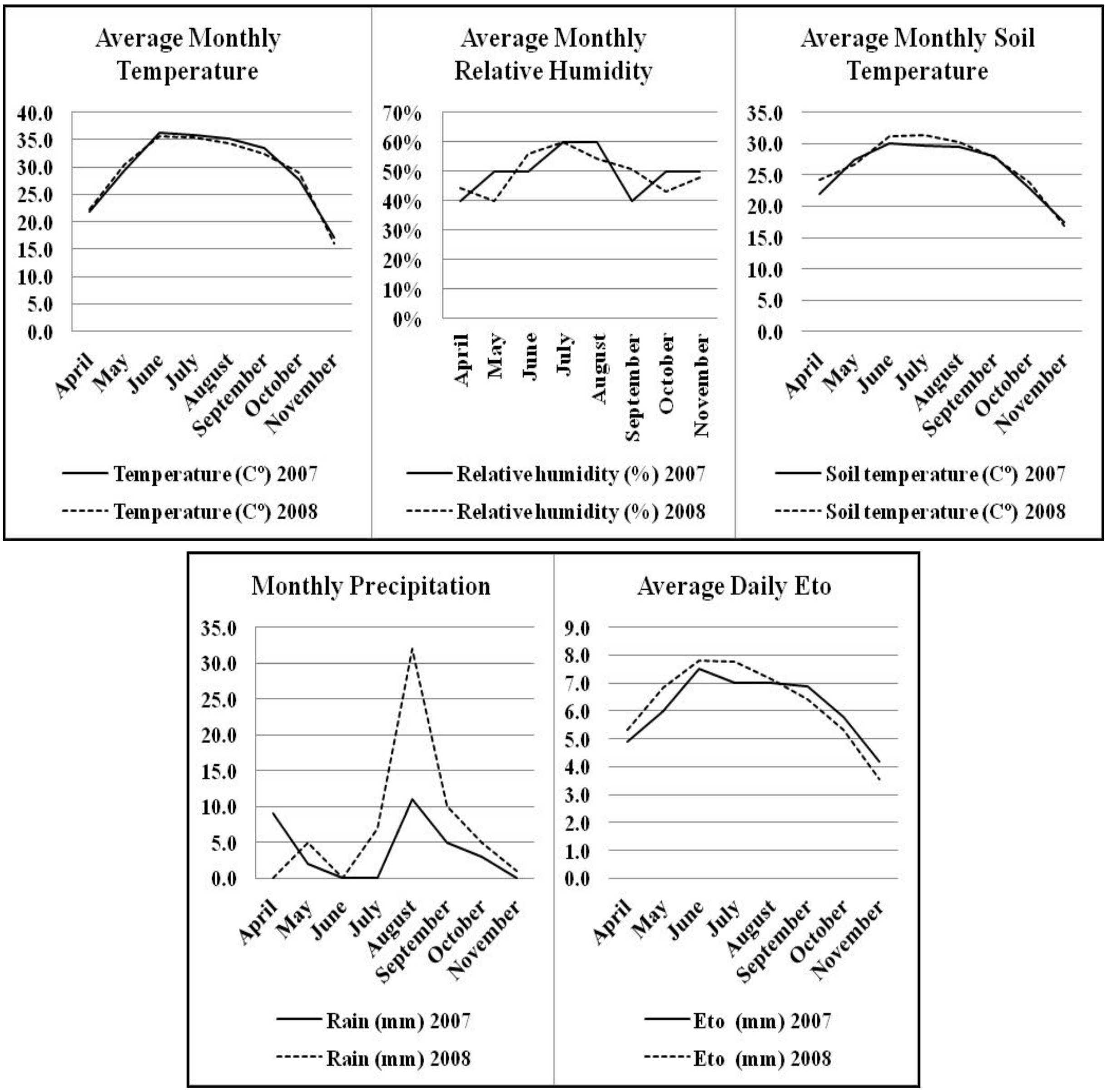
Table 3. Methods used for testing the soil samples from the area of the experiment

HACH Soil Testing Methods
Calcium sulphate extraction and cadmium reduction method for $N\text{-}NO_3$
Mehlich 2 extraction and ascorbic acid method for PO_4^-
Mehlich 2 extraction and tetraphenylborate method for potassium
Aqueous extract and electrode method for pH
Aqueous extract and electrode method for EC and salinity (Twin Cond B-173)

Soils were sampled twice: before and two months after the humic substances applications. Tests were performed to determine the main changes in the soil as a result of the treatment. The tests were conducted using HACH kits (Table 3). The soil samples were taken from 0-15 cm, 15-30 cm, and 30-45 cm depth. We registered local climatic data from the experimental site (Figure 1) using a Min Max Thermometer of 25 cm, an Outdoor Metal Barometer of 16 cm, a Class A Evaporation Pan, Rain Gauge Standard, and Soil Probe Thermometer.

Figure 1. Climatic conditions in the area of the experiment (Samarkhel, Afghanistan)



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The climatic conditions in 2007 and 2008 were similar which allowed for comparison of experimental data from both years. The analysis included the comparison between the baseline data from 2007 and untreated saplings data from 2008. Resulting data were processed using the Student t-test to examine characteristics of the pomegranate saplings. Additionally, the results of the four experiments were tested with one and two-factor analysis of variance (ANOVA) and Fisher test. The differences between the means of the samples were analyzed with the standard error of the mean which is a good estimate for standard deviation of a large number of samples drawn from the population. In order to compare the effects of the humic substances treatment, the differences between the samples means were compared with the value of the $SD_{5\%}$ and a 95% confidence interval.

Figure 2. Average monthly values between the saplings height of treated (Test) and untreated (Control) plants

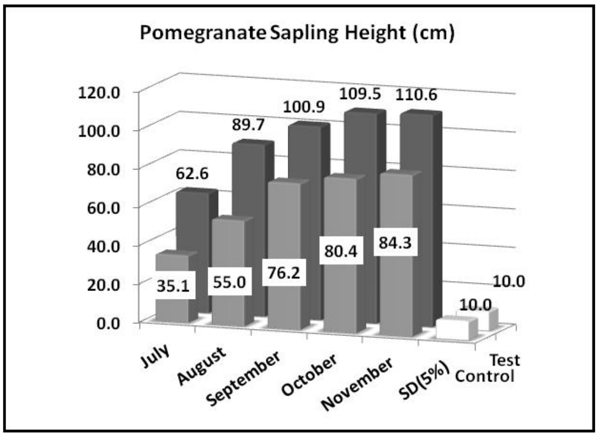
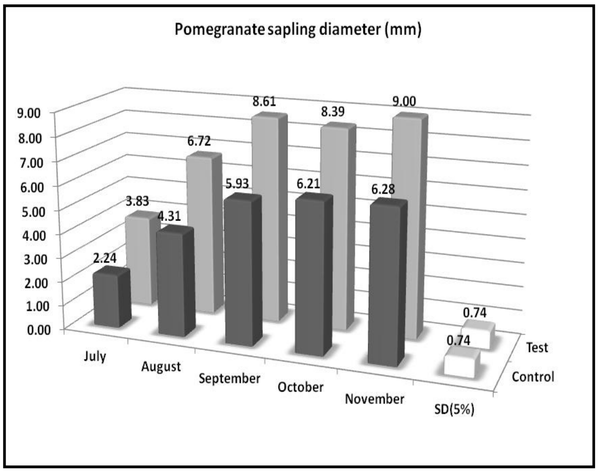


Figure 3. Average monthly diameter values of treated and untreated pomegranate saplings



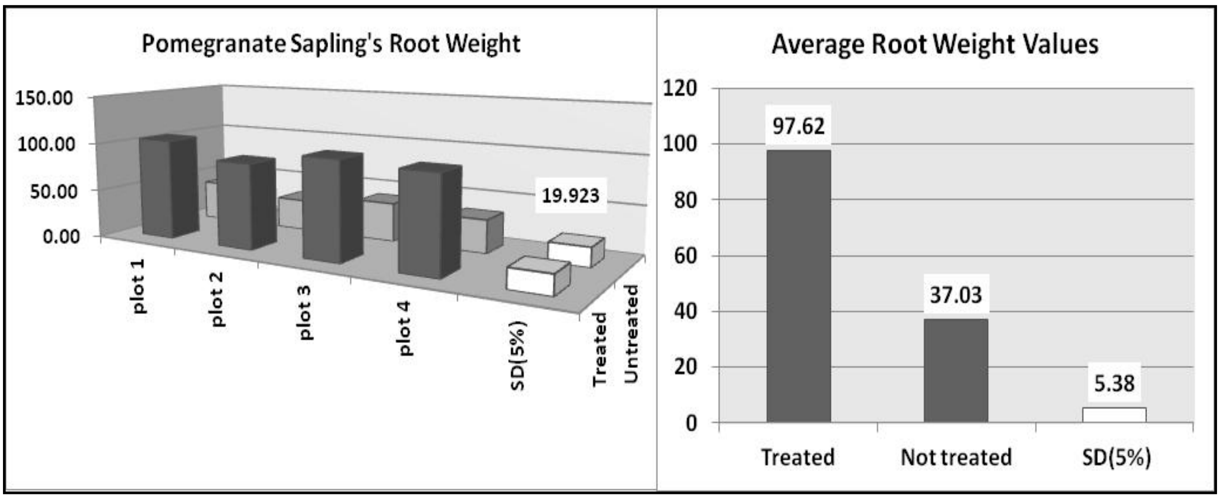
Results and Discussion

At the end of the production cycle, plant height and stem's diameter from the treated plants were 30 - 35% greater than those from the untreated plants. The analysis and comparison of the average monthly values between the saplings from treated and untreated plants showed similar tendency for growth and significant differences between the two groups (Figure 2).

The treated samples always resulted in significantly higher values for both diameter and height when compared to the untreated samples (Figure 3).

Besides the analysis of sapling's height and diameter, several other tests were used for the qualification of the harvested pomegranate saplings. For example, the analysis of sapling's

Figure 4. Weight comparison between treated and untreated pomegranate saplings



average root weight values showed similar differences between the treated and untreated plants. While the pomegranate saplings from the treated soil developed a roots system with an average weight of 98 g, the root system of the control plants averaged of 37 g. The difference was considerably larger than SD(5%)=5.38 value of the one-way ANOVA analysis (Figure 4).

The data from the untreated plants supports the positive effects of humic substances application. However, what would be the effect on the variance generated by variability of the root weight? Would the measured values show a more scattered data base or the variability of the data would be negligible? During the Fisher test, the comparison of the highest variance value of the treated samples (34.0 for the samples of the 4th plot) with the lowest variance value of the untreated samples (9.0 for the samples of the 2nd plot) resulted in significant difference (p=0.026). Considering the fact that the average root weight from the treated plants are considerably higher than those from the untreated plants, in order to determine the effect of humic acid on the variance of the root weight, it seemed to be more trustable result the comparison between the calculated coefficients of variance of the untreated and treated samples. The Fisher test for the comparison between the CV values (respectively $CV_{(TEST)}$=35.0% and $CV_{(CONTROL)}$=26.0%) did not show significant difference (p=0.194), which means that humic substances does not negatively affect the variance in the values of root weight. The comparative analysis of the differences in the root weight between treated and untreated plants showed higher value than the calculated value of the $SD_{(5\%)}$ ($SD_{5\%}$=19,923 for plots and $SD_{5\%}$=5.38 for average values). Based on the received "F" value from the analysis of variance it can be proved statistically that the observed differences in the root weight between treated and untreated samples are significant and the weight of the treated samples are always higher than the root weight of the control plants (Figure 4).

Root volume plays an important role in the sapling survival. The analysis of volume of the root system indicated that the average number of roots over 2 mm diameter was 63 for the treated saplings in comparison to 18 for the untreated saplings. The largest difference was found in the number of roots with a diameter range of 4-7 mm. The number of roots of the pomegranate saplings from the humic substances treated soil was 4 times higher than those found in the untreated sapling population. Number for the roots over 8 mm and in the range of 2-4 mm was 4 times and 3 times higher, respectively, than the number of roots in the untreated population. The occurrence of the different root diameters was tested with two-factor analysis of variance. In both samples (test and control) the occurrence of thin roots (2-4mm diameter) was the most frequent. In all three diameter's categories (root-stem transition region, stem mid-section, upper-third section) the difference between treated and untreated samples was highly significant. The difference increased drastically with the decrease of root's diameter (Figure 5).

The collected data were compared using two-factor analysis of variance. The difference between treated and untreated samples was highly significant at all three points. The largest difference was measured in the diameters of the root-stem transition region. In the cases of stem mid-section and upper-third section the observed differences were also significant (Figure 6).

The research team expected similar results from the baseline (2007) and the untreated samples (2008) because the climatic conditions were similar and the applied technology was the same in both years. Usually, in the area where the experiment was conducted climatic conditions vary very little from one year to other (Figure 1). The collected data during

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Figure 5. The occurrence of different root diameters in control and test samples (>8, 4-7, 2-4mm) and the calculated difference between the categories

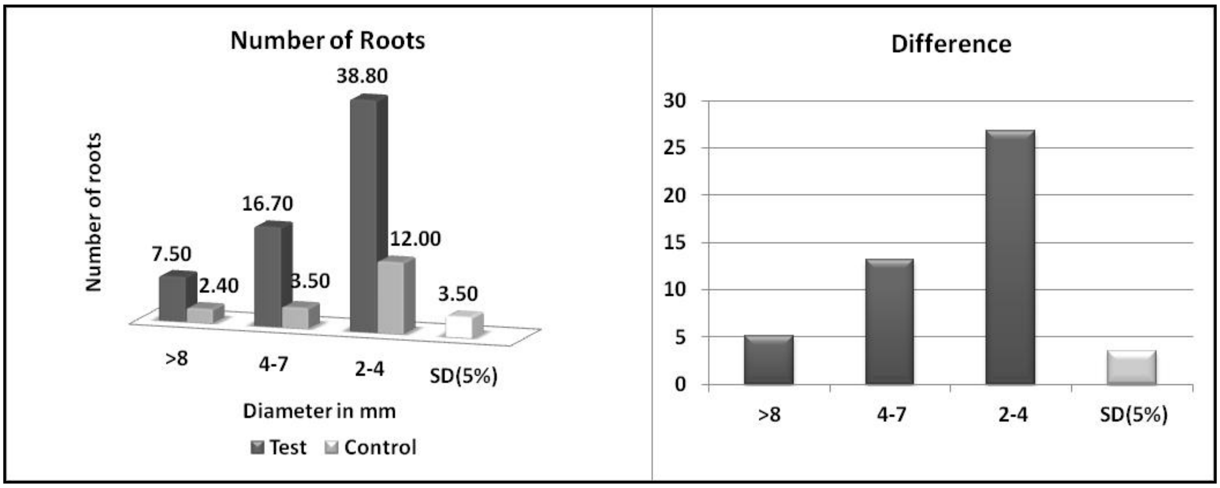
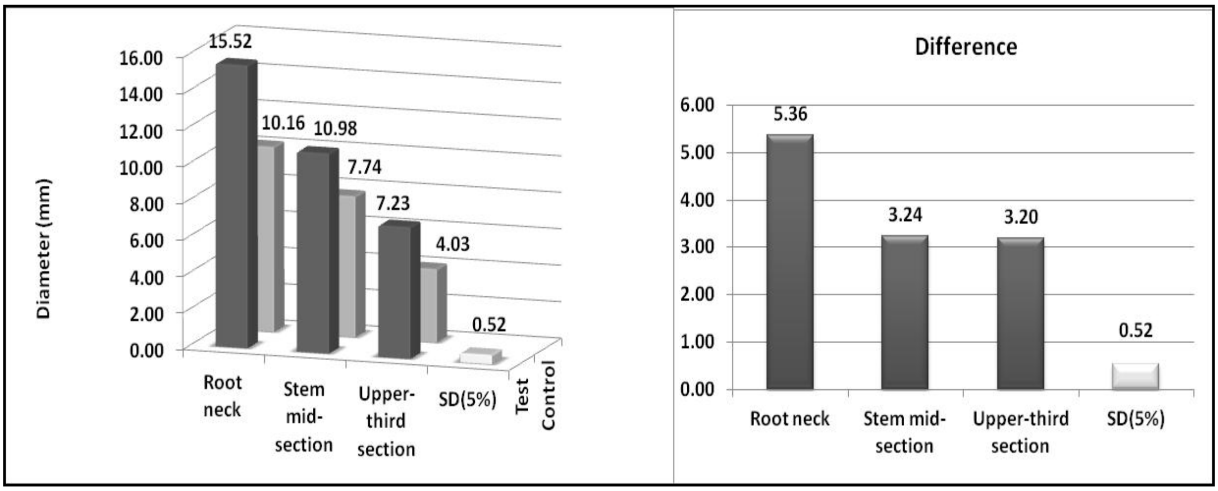


Figure 6. Sapling diameters at different points (Root-stem transition region, Stem mid-section, Upper-third section) and the calculated difference between the categories



the experiment indicated that the differences between treated plants and untreated plants (based on the quality standards established for commercial sapling production) were considerably high. The treated soil produced significantly higher number of saplings that met the standards when compared to those untreated. In addition to meeting a minimum height of 0.8 m and minimum diameter of 8-9 mm, harvested saplings meeting the quality standard were free of pests, diseases, and physical damage. No differences were observed between the 2007 and 2008 untreated samples. The use of humic substances reduced the number of substandard saplings from 57% to 22% (Figure 7).

The soil test results revealed significant differences in all three sampling depths. However, the major changes were observed in the 0-15 cm sampling depth. These major changes after 2 months were the increase in nitrogen, phosphorus and exchangeable

Figure 7. Comparison of pomegranate sapling's samples according to the quality standards

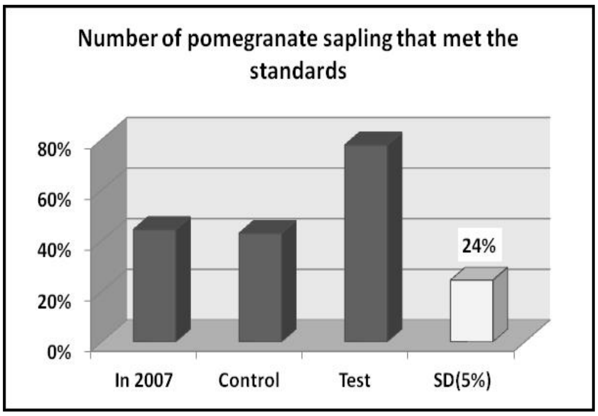


Table 4. Soil properties before and two months after humic substances treatment

Soil depth (cm)	Before humic substances application			After 2 months of humic substances application		
	Nitrate-nitrogen (mg/L)	Phosphate-phosphorus (mg/L)	Exchangeable potassium (mg/L)	Nitrate-nitrogen (mg/L)	Phosphate-phosphorus (mg/L)	Exchangeable potassium (mg/L)
0-15	3.50	49.97	181.50	11.30	85.53	211.17
15-30	3.00	47.03	129.50	7.12	69.44	227.67
30-45	2.08	20.02	118.50	5.12	59.81	156.25

Soil depth (cm)	Before humic substances application			After 2 months of humic substances application		
	$pH_{(H2O)}$	EC (mS/cm)	Salinity (%)	$pH_{(H2O)}$	EC (mS/cm)	Salinity (%)
0-15	8.14	0.41	0.01	7.75	0.78	0.01
15-30	8.07	0.45	0.02	7.83	0.36	0.02
30-45	7.90	0.44	0.02	7.93	0.29	0.01

potassium content in the soil, the increase of electrical conductivity and the decreased pH value. The tests did not reveal significant difference in soil salinity (Table 4).

This nursery experiment contrast the work of Feibert et al. (2000) which questions the effect of humic substances for yield and quality characteristics of crops. However, observations by Albayrak and Camas (2005) support the results of this experiment indicating strong positive effect of the humic substances on plant growth in various aspects (diameter, height, and root growth). The significant increase in volume and number of the root system, especially in the number of roots with a diameter less than 2.0 mm indicates higher capacity for nutrient and water uptake. This is essential for the sapling to survive hard climatic conditions and for healthy development. Also, the large differences measured in the sapling's diameter and height between treated and untreated plants, the positive effect of humic substances was confirmed by the experiment.

Conclusions

The humic substances application significantly increased the number of pomegranate saplings, which met the standard requirements for nursery production. This fact showed the efficacy and reliability of the application of humic substances in order to increase crop yields and quality. The trial in Afghanistan was conducted under harsh environmental conditions (very arid area with extremely high temperatures and poorly developed alkaline soil showing nutrient deficiencies and lack of organic matter). Under these conditions, which are also typical for other areas in the region, the application of humic substances in nursery production can have a significant impact on the feasibility and sustainability of the nursery and horticultural business in Afghanistan.

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