School of Environmental Sciences

The effect of humic substances in nursery production

Thesis of the doctoral dissertation

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OBJECTIVES

During the last decades in the countries of the 3rd world gradually increased the importance of the fruit production sector in order to satisfy the export and the domestic market's demand for this type of products. At the same time, insufficient production technologies and the lack of good quality reproductive material does not serve the purpose. Even the central nurseries, with highly advanced technologies, have to face up to problems like increasing the cutting's secondary root system development and achieving the established quality standards for saplings.

Organic carbon compounds occur naturally in the environment in the form of humic substances. Their non-soluble, solid phase gives color to the soil and builds up soil structure. Therefore, they affect the soil's physical properties. The soluble phase forms colloid solutions in the soil and determines fundamentally soil fertility, as well as the utilization level of organic and inorganic fertilizers. Several experiments observed the effect of humic substances on the yield of different crops and vegetables. Though it may appear, there are only a few experiments with fruits and nothing at all for nursery production.

The Eastern Region of Afghanistan is well known for its large scale of horticulture production. But it is also knowledgeable that climatic and soil conditions are very difficult. In this region, where the annual precipitation is very low and the heat is very high, the soil shows a very weak development. The tested soil is young soil in the early stages of weathering. It is originated from sedimentary material, which was deposited in the area. Consequently there is little difference between the horizons. The soil is compact and poorly structured as well. The water penetration is also limited into the soil. The chemical property tests show that nitrogen and generally the organic matter content in the soil are low. The soil pH value is above 8.0; therefore the plant's nutrient uptake is limited. In this kind of soil the development of the plant's secondary root system is limited, which decreases the sapling's survival rate and the percentage of quality saplings according to the established standard.

The nursery has been producing pomegranate saplings of the indigenous "Kandahar" variety since February 2006. The survey study in the end of 2006 showed that only 44-45% of the saplings achieved the standard quality for transplantation.

The main objective of the research was to increase the pomegranate cutting survival rate and the number of quality saplings in the nursery production through the application of humic and fulvic acid during the growing period. The main condition to satisfy the quality standard for sapling had been the increase of the sapling's secondary root system in mass and volume.

The research objectives of the experiment:

- Increase the number of saplings, which fulfill the established specifications for the standard sapling quality
- Study the effects of humic acid on seed germination in different application doses
- Analyze the changes of the NPK uptake by plants
- Study the major changes of the soil's physical and chemical properties

MATERIALS AND METHODS

There were two groups of experiments conducted. The first group involved the experiments conducted on the open field of the nursery and the second group contained the experiment in a closed environment (laboratory tests). The humic acid that was used during the research originates from South Africa. It was produced by Farmfert Formulators INC. and registered under the code number of PCT WO 2006/092720AI.

Nursery experiment

The location of the nursery field for the experiment is in the Nangarhar Province of the Eastern Region of Afghanistan and belongs to the Eastern Region Water Basin. The selected area is plain valley with a sloping angle of less than1%. The underground water can be found between 4m and 6m. The field is divided into plots by the irrigation channels. The surface type irrigation system is gravity-based furrow irrigation.

The soil's pH is slightly-moderately alkaline. Based on the soil' salt content the it is not belong into the salty type of soils, tough it has the tendency to become a sodic soil. The alkaline saturation and cation exchange capacity value indicates, that the dominant secondary clay minerals is, Montmorrilonit. It is moderately calciferous soil. The percentage value of exchangeable sodium is very low. The phosphorus and potassium content in the soil is moderately good.

The experiment was conducted with pomegranate saplings (Punica granatum, Kandahar variety). For the purpose of propagation, we used simple and not rooted cuttings with 5-6 buds on it.

The experiment was conducted in four plots of the pomegranate cuttings nursery. We used 330 test and 80 control rows equal to a total of 2.0 hectare area. Each plot covered approximately half a hectare area. In every 5th row, there was a control row without any treatment application. In the treated rows, we applied the humic acid in shallow furrows with a 15cm distance from the plants. The application depth was 10cm. The humic acid solution contained humic acid powder (50% concentration) mixed with water using 1:8 ratio during the preparation. The total quantity of the applied humic acid was of 200 kg/ha. The first application occurred after two months of the plantation and it was repeated again five weeks later. After four months of the planting time we applied fulvic acid (10 l/ha dose) on the forming canopy of the saplings with a backpack sprayer. The total time period used for the experiment covered was of seven months.

During the field trial, I conducted measurements on a weekly basis. This involved the measurement of the sapling stem's midsection (diameter) and the height from 400 test and 100 control plants. We used line transect method, with the help of a measuring "U: frame, to recollect the samples. Additionally, we registered data about the on-going climatic conditions.

Plant experiment with ryegrass

Fast growing ryegrass (Lolium perenne) was tested during the experiment. The selected seed type was perfect for the one month trial because of its size and its fast growing characteristics. The seeds were planted in small 200 ml plastic containers filled with soil. In each one, we sowed 200 seeds. The dry soil was previously sieved using a 2 mm diameter sieve and treated with humic acid in different concentrations. Equal number of tests and control samples were used for each treatment (10 tests and 10 control containers).

After five weeks of a growing period, the leaves were removed from the root system and dried. Parallel with the ryegrass experiment treated and untreated soil was placed into similar plastic containers and kept wet during the time period of the experiment for further soil analysis.

Seed germination experiment had been conducted to establish the effect of humic acid on seed germination. For that experiment 25 seeds were placed on a cotton layer in small Petri cups, which had been soaked with the humic acid solution and water. In this case we also prepared 10 test and 10 control experiments for each treatment's doses. The humic acid solution was prepared in different concentrations. During the experiment, we observed and registered data on daily bases.

Sample analysis

These samples were tested in the soil laboratory of SZIE University, Godollo:

- Phosphorus and Potassium content (given in P_2O_5 and K_2O) from soil sample extracted with Ammonium-Lactate
- Soil sample's Nitrate-Nitrogen content soluble in nKCl
- NH_4-N content from soil sample extracted with KCl
- Cu, Fe, Mn, Zn content from soil sample extracted with HNO_3
- K, Na, Ca, Mg content from soil sample extracted with NH_4 (Ammonium-Acetate)
- Measurement of pH_{H2O} and pH_{KCl}
- Measurement of exchangeable and hydrolytic acidity
- CaCO3 content
- CEC and S% measurement
- Weight loss of heated soil sample
- Measurement of humus content (0.25mm and 2.00mm)
- Total nitrogen content
- Measured and dried leaf sample's Nitrogen, Phosphorus and Potassium content

RESULTS

The humus content analysis of the soil samples indicated that the value of the coarse size humus content (0.25-2.00mm) in the top 0-20 cm soil layer was half in the treated soil than the measured value of the untreated soil. While in the treated soil the accumulation of the fine humus content (less than 0.25mm) occurs in the top 0-20 cm surface layer, in case of the untreated soil the measured values are close to equal in both surface layers, in the 0-20cm and 20-40cm layers. The value of the coarse humus content (0.25-2.00mm) resulted equally similar in both, the treated and untreated soil, independently from the sampling soil depth. In case of the treated samples and comparison with the untreated ones, the measured low level of coarse humus content smaller than 0.25mm in the root system's zone (20-40cm) indicates an increasing tendency for organic matter decomposition, mineralization and plant's nutrient uptake (Table 1).

Table 1: Average values of soil's organic matter content of the humic acid treated and untreated nursery soil after harvest in the pomegranate nursery

	Organiz matter					
Soil depth (cm)	Stability index % K		Hu% <0.25 mm		C% <0.25 mm	
	Treated	Not treated	Treated	Not treated	Treated	Not treated
0-20	0.65	0.77	1.17	0.90	0.68	0.52
20-40	1.59	1.16	0.33	0.93	0.19	0.54
Soil depth (cm)	Heating loss i.v.% 2mm		Hu% 0.25-2 mm		C% 0.25-2 mm	
	Treated	Not treated	Treated	Not treated	Treated	Not treated
0-20	4.20	4.90	0.70	1.40	0.41	0.81
20-40	4.00	5.00	0.70	1.40	0.41	0.81

While the parameters of the saplings improved, parallel to that, the nutrient uptake by the plants also increased. This fact may explain why the values of the NO_3-N and K2O were smaller in the treated soil than in the untreated soil after the harvest. At the end of the production period the humus content in the treated soil was lower than in the untreated one, which also indicates the increasing tendency of organic matter mineralization. Consecutively, the ammonium loss decreased in the treated soil as a result of the effect from the humic acid. Therefore the NH4-N value in the treated soil was significantly higher than in the untreated soil. The value of the phosphorus content had been found higher in the treated soil.

The Arany fixation number in the top 0-20 cm layer was higher in the case of the treated soil than in the untreated soil resulting from the improvement of the soil structure, as a result of a positive effect of the humic acid treatment (Table 2).

Table 2: Average values of soil's NPK content of the humic acid treated and untreated nursery soil after harvest in the pomegranate nursery

Soil NPK status						
Soil depth (cm)	Arany-index K_A		Free lime content $CaCO_3$%		AL-P_2O_5 P_2O_5	
	Treated	Not treated	Treated	Not treated	Treated	Not treated
0-20	41	35	9.40	10.56	439	260
20-40	36	38	10.05	11.35	237	144
Soil depth (cm)	AL-K_2O K_2O		NH_4-N N		NO_3-N N	
	Treated	Not treated	Treated	Not treated	Treated	Not treated
0-20	183	263	4.6	3.7	10.0	12.0
20-40	168	185	4.5	4.5	14.0	14.2
Soil depth (cm)	Total N ΣN		Humus Hu%		pH H_2O	
	Treated	Not treated	Treated	Not treated	Treated	Not treated
0-20	0.08	0.09	1.87	2.30	8.16	8.24
20-40	0.08	0.09	1.03	2.33	8.32	8.36

The lower pH also resulted in a lower free calcium-carbonate content. However the exchangeable sodium content slightly increased in the treated soil. Based on the measured CEC value we were able to determine that the dominant soil clay mineral is 2:1 type Montmorrilonit (Internationally knowen as Smektit). The presence of the Allofan type clay minerals was excluded, because the AL^{3+} and $H3O^{+}$ content were low (Table 3).

Table 3: Colloid status of the humic acid treated and untreated nursery soil after harvest in the pomegranate nursery

Soil Colloid Status						
Soil depth (cm)	Salt %		Exchangeable cations T		Exchangeable S	
	Treated	Not treated	Treated	Not treated	Treated	Not treated
0-20	0.08	0.06	68.00	91.80	54.43	76.48
20-40	0.06	0.05	87.30	86.80	72.04	71.37
Soil depth (cm)	T-S		V		U	
	Treated	Not treated	Treated	Not treated	Treated	Not treated
0-20	13.57	15.32	80.04	83.31	19.96	16.69
20-40	15.26	15.43	82.52	82.22	17.48	17.78
Soil depth (cm)	pH KCl		pH H_2O		Exchangeable acidity y2	
	Treated	Not treated	Treated	Not treated	Treated	Not treated
0-20	7.22	7.39	8.16	8.24	0.30	0.30
20-40	7.31	7.45	8.32	8.36	0.30	0.30
Soil depth (cm)	Hydrolytic acidity y1		Free lime $CaCO_3$%		Exchangeable Sodium Na%	
	Treated	Not treated	Treated	Not treated	Treated	Not treated
0-20	0.75	0.75	9.40	10.56	0.37	0.04
20-40	0.75	0.75	10.05	11.35	0.08	0.17

Results of soil sample analysis from the ryegrass experiment

The result of the soil analysis for the soil treatment with lower concentration of humic acid solution (0.075%) indicated that the available N-NO_3 content is higher in these samples than in the soil samples, which had been treated with a solution of higher concentration (0.5%) (Table 4).

Table 4: NPK and organic carbon content of soil samples, which were treated with humic acid solutions of different concentration comparison to the untreated samples

Treatment	Sample	mg/kg				mg/100g
		NH_4-N	NO_3-N	P	K	C
0.075% Humic acid	Treated	13.90	406.90	0.347	45.47	19.67
	Not treated	3.40	126.40	2.751	50.18	48.18
0.5% Humic acid (1)	Treated	2.80	27.10	0.08	265.59	55.66
	Not treated	3.00	19.60	0.04	32.70	31.02
0.5% Humic acid (2)	Treated	1.40	18.30	0.12	198.05	46.61
	Not treated	1.50	17.10	0.05	71.76	35.60

However, in both cases the available N-NO_3 content was higher than in the untreated soil samples. The comparative analysis of available potassium and phosphorus content between the treated and untreated soil samples indicated a different tendency. The treatment with 0.075% humic acid solution resulted in a lower potassium content in the treated soil than in the control samples, while the treatment with 0.5% solution resulted in higher potassium, as well phosphorus content in the treated soil.

The single factor analysis of variance (ANOVA) of the soil test results of the samples taken during the beginning and the last third part of the production cycle showed a significant difference for the available N-NO3 and the P-PO4content in the 0-20cm and 20-40cm soil layers. However, the analysis for potassium content could only show a similar difference in the 20-40cm layer. This indicates that potassium leaches intensively from the surface layer into the deeper soil layers with the irrigation water (Table 5).

Table 5: Single factor analysis of variance to compare the NPK content of humic acid treated and control soil samples from different soil depths

Analysis of variance - $F_{0.05;\,1,\,22} = 4.4$						
Nutrient	Soil depth: 0-20 cm			Soil depth: 20-40 cm		
	F	Df	Prob.	F	Df	Prob.
N-NO_3	42.790	23	0.000	73.372	23	0.000
P-PO_4	60.511	23	0.000	28.076	23	0.000
K	1.909	23	0.181	55.798	23	0.000

The analysis of the PH value changed in the soil during the 8-month period, which determined a significant decrease in the top 0-20cm layer, while in 20-40 cm soil depth, the measured difference is statistically not significant (Table 6).

Table 6: Single factor analysis of variance to compare the pH values of humic acid treated and control soil samples from different soil depths

Analysis of variance - $F_{0.05;\,1,\,22} = 4.4$						
pH	Soil depth: 0-20 cm			Soil depth: 20-40 cm		
	F	Df	Prob.	F	Df	Prob.
Jan-09 Sep-09	86.186	23	0.000	0.318	23	0.579

The test for salt content measured an increased value in the top 0-20cm surface layer and the same value decreased in the 20-40cm soil depth. This effect was a result of two factors. First, the soil hydrologic conditions are characterized by high evaporation and second, the hardness value of the water is high. However, the calculated "F" value only in the 20-40cm soil depth determined a significant difference. The humic acid able to regulate salt content in the soil and probably this effect kept the salt content at a relatively low level in the top surface layer (Table 7).

Table 7: Single factor analysis of variance to compare the salt content of humic acid treated and control soil samples from different soil depths

Salt	Analysis of variance - $F_{0.05; 1, 22} = 4.4$					
	Soil depth: 0-20 cm			Soil depth: 20-40 cm		
	F	Df	Prob.	F	Df	Prob.
Jan-09 Sep-09	2.366	23	0.138	48.279	23	0.000

Results of the pomegranate nursery experiment

The field trial indicated that at the end of the production period the height and the diameter of the test plants increased with an average of 30-35% more than the diameter and height of the control plants (Table 8). During the harvest time, the diameter of the control plants was only 69.7% of the diameter of the test plants. This is a 95.4% difference in development and a 30.3% in proportion measurement. In harvest time, the height of the control plants was 76.2% of the height of the test plants. This is a difference of 23.8% in proportion measurement.

Table 8: Average diameter and height of pomegranate saplings during the production period

2008	Average diameter (mm)											
	Test plant					Control plant					Kontroll / Teszt	
	Jul	Aug	Sep	Oct	Nov	Jul	Aug	Sep	Oct	Nov	Initial	Final
Plot 1	5.00	7.92	10.54	9.70	9.71	3.62	5.70	7.08	6.60	6.59	114.0%	67.9%
Plot 2	1.54	5.29	7.89	6.88	7.58	0.74	2.96	5.00	5.13	5.14	192.4%	67.8%
Plot 3	5.03	7.39	8.10	8.51	9.48	2.81	4.70	6.49	6.54	7.10	93.6%	74.9%
Plot 4	3.76	6.29	7.93	8.48	9.21	1.78	3.87	5.14	6.57	6.28	102.8%	68.2%
Ave.	**3.83**	**6.72**	**8.61**	**8.39**	**9.00**	**2.24**	**4.31**	**5.93**	**6.21**	**6.28**	**125.7%**	**69.7%**
2008	Average height (cm)											
	Test plant					Control plant					Kontroll / Teszt	
	Jul	Aug	Sep	Oct	Nov	Jul	Aug	Sep	Oct	Nov	Initial	Final
Plot 1	82.02	100.36	123.09	138.16	127.41	55.45	75.68	86.16	93.35	95.98	92.3%	75.3%
Plot 2	28.72	70.70	91.08	93.03	93.31	13.94	39.80	65.90	66.94	70.90	138.6%	76.0%
Plot 3	82.06	99.08	95.20	107.85	113.79	44.13	56.50	85.33	84.43	88.95	68.9%	78.2%
Plot 4	57.80	88.58	94.16	99.11	107.75	26.88	48.05	67.55	76.96	81.24	83.1%	75.4%
Ave.	**62.65**	**89.68**	**100.88**	**109.54**	**110.56**	**35.10**	**55.01**	**76.23**	**80.42**	**84.27**	**95.7%**	**76.2%**

The analysis of the root system also showed large differences. While the average weight of the test plant's roots had been 97.62g after harvest, the roots of the control plants weighted only 37.23g. The number of roots higher than 2mm resulted in an average number of 63 in the case of the test plants, against the counted average number of 18 in the case of the control plants. These measurements showed that the measured value of the test plants for the root system average weight 2.62 times and for the average number of roots over 2mm diameter was 3.5 times higher than the measured values of the control plants. Similar comparison for number of roots between 2mm and 4 mm and over 8mm diameter established 4 and 3.5 times higher proportion values in favor of the test plants. The average diameter of the root neck was 15.52mm in the case of the test plants and 10.16mm for the control plants (Table 9).

Table 9: Plant's average characteristics of the pomegranate saplings after harvest

Description	Unit	Test plant 1	2	3	4	5	6	7	8	9	10	Ave.
Height	cm	190.00	183.00	182.00	179.00	180.00	183.00	178.00	181.00	181.00	180.00	**181.70**
Root neck diameter	mm	16.13	15.17	15.32	15.11	16.10	16.00	15.07	15.71	15.23	15.35	**15.52**
Stem mid-section diameter	mm	11.12	10.92	10.83	11.12	10.95	11.00	10.89	11.15	10.87	10.91	**10.98**
Stem top section diameter	mm	6.41	8.85	7.21	6.05	6.37	6.97	7.01	8.24	7.92	7.25	**7.23**
Root weight	g	105.40	170.50	75.90	67.60	112.50	79.70	69.00	107.90	106.50	81.20	**97.62**
No. of roots (>8 mm)	db	9	11	4	7	8	7	5	9	8	7	**8**
No. of roots (4-7 mm)	db	19	28	12	15	19	13	11	17	21	12	**17**
No. of roots (2-4 mm)	db	56	49	39	31	35	37	34	41	31	35	**39**
Total no. of roots	db	84	88	55	53	62	57	50	67	60	54	**63**
Description	**Unit**	**Control plant 1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Ave.**
Height	cm	109.00	104.00	112.00	107.00	107.00	103.00	109.00	111.00	105.00	102.00	**106.90**
Root neck diameter	mm	10.23	10.50	9.48	10.72	10.21	10.14	10.55	9.71	10.12	9.89	**10.16**
Stem mid-section diameter	mm	7.27	8.34	6.61	8.91	8.45	7.39	8.21	7.46	7.85	6.87	**7.74**
Stem top section diameter	mm	3.32	4.52	3.29	5.11	4.92	3.86	4.17	3.75	4.11	3.28	**4.03**
Root weight	g	46.20	43.70	19.70	44.50	37.80	41.70	28.10	43.20	21.90	45.50	**37.23**
No. of roots (>8 mm)	db	3	2	1	4	3	2	2	4	1	2	**2**
No. of roots (4-7 mm)	db	5	4	1	3	3	6	3	2	3	5	**4**
No. of roots (2-4 mm)	db	7	14	11	15	12	12	9	14	12	14	**12**
Total no. of roots	db	15	20	13	22	18	20	14	20	16	21	**18**

The single factor analysis of variance established a significant difference for both, diameter (F=23.268, P=0.003) and height (F=9.507, P=0.022) between test and control plants. The calculations for the side shootings did not offer a similar result, but this does not mean that the difference did not exist. The excessively high probability value (P=0.968) seems to support the idea of the existing differences between the two groups (Table 10).

Table 10: Analysis of variance for the characteristics of pomegranate saplings

Analysis of variance - $F_{0.05;\,1,6} = 6.0$			
Description	**F**	**Df**	**Prob.**
Diameter	23.268	7	0.003
Height	9.507	7	0.022
No. of sprout	0.002	7	0.968
Vigor	9.475	7	0.022

The value of the Person correlation index stated a strong correlation for the three sapling's characteristics (diameter R=0.766; height R=0.727; Side shootings R=0.917) and a moderately strong correlation for development vigor (R=0.478) (Table 11).

Table 11: Pearson correlation analysis for the characteristics of pomegranate saplings

Person Correlation Index Diameter			**Person Correlation Index Height**		
Correlation Matrix (R)			**Correlation Matrix (R)**		
	T- Diameter	K- Diameter		T- Height	K- Height
Test- Diameter	1	0.766	Test- Height	1	0.727
Control- Diameter	0.766	1	Control- Height	0.727	1
Person Correlation Index No. of sprout			**Person Correlation Index Vigor**		
Correlation Matrix (R)			**Correlation Matrix (R)**		
	T- Sprout	K- Sprout		T-Vigor	K-Vigor
Test-Sprout	1	0.917	Test-Vigor	1	0.478
Control- Sprout	0.917	1	Control-Vigor	0.478	1

The separate statistical analysis of the sapling's diameter in each production plots also indicated significant differences between the test plants and the control plants. The received "F" values varied between 65.6 and 938.4 with the probability between P=0.000 and P=0.06 (Table 12). The same analysis for the plant height resulted in an "F" value between 233 and 539 with the probability of P=0.000 (Table 13).

Table 12: Analysis of variance for the diameter of pomegranate saplings in each experiment plot

Analysis of variance				
Diameter	F	Df	Prob.	"F" table
Plot 1.	165.025	79	0.062	$F_{0.05;\,78,2}$ = 20
Plot 2.	938.470	79	0.000	$F_{0.05;\,75,4}$ = 5.6
Plot 3.	423.194	79	0.000	$F_{0.05;\,73,6}$ = 3.7
Plot 4.	65.562	79	0.003	$F_{0.05;\,76,3}$ = 8.5

Table 13: Analysis of variance for the height of pomegranate saplings in each experiment plot

Analysis of variance				
Height	F	Df	Prob.	"F" table
Plot 1.	513.883	79	0.000	$F_{0.05;\,60,19}$ = 1.9
Plot 2.	518.544	79	0.000	$F_{0.05;\,62,17}$ = 2.0
Plot 3.	233.837	79	0.000	$F_{0.05;\,47,32}$ = 1.8
Plot 4.	539.169	79	0.000	$F_{0.05;\,48,31}$ = 1.8

The Pearson correlation index showed a strong positive correlation between the test plants and the control plants supporting the suggested positive effect of the humic acid treatment for plant growth (Table 14).

Table 14: Pearson correlation analysis for the diameter and height of pomegranate saplings in each experiment plot

Correlation Matrix R			Correlation Matrix R		
Diameter	Correlation	Significance	Height	Correlation	Significance
Plot 1.	0.993	0.000	Plot 1.	0.984	0.000
Plot 2.	0.913	0.000	Plot 2.	0.951	0.000
Plot 3.	0.970	0.000	Plot 3.	0.984	0.000
Plot 4.	0.968	0.000	Plot 4.	0.982	0.000

Results of the ryegrass laboratory experiment

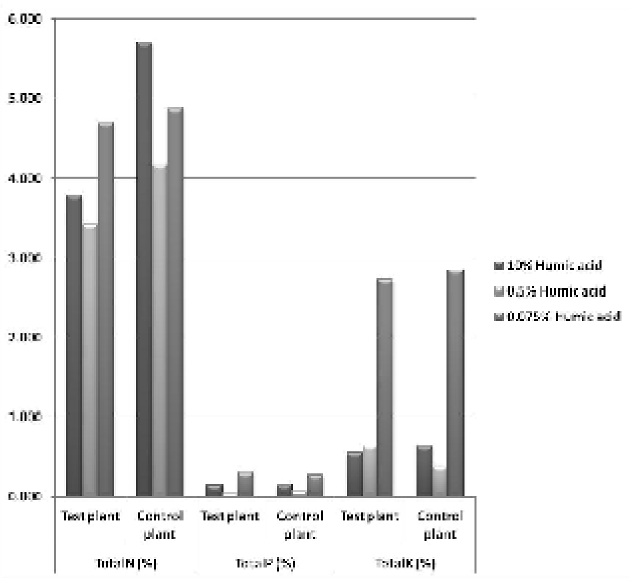
The data of the nitrogen uptake by the indicator plant indicated that the percentage value of total nitrogen content, in comparison to the control plant is lower in those plants, which grew on soil treated with 0.5% humic acid solution and almost the same in the plants, which grew on soil treated with 0.075% humic acid solution. This result is interesting if we consider that the soil $N\text{-}NO_3$ content was higher in the treated soil than in the untreated when the humic acid solution had been established at the 0.5% concentration level. Meanwhile, the soil $NH_4\text{-}N$ content gave a similar value. The treatment with a 0.075% humic acid solution increased almost three times the $N\text{-}NO_3$ and $NH_4\text{-}N$ content in the soil samples comparison to the control soil. The rest of the analysis indicated that the percentage value of total nitrogen and phosphorus uptake depends on the concentration of the applied humic acid solution. In the case of both nutrients, the best result was given by the treatment with 0.075% humic acid solution (Table 15).

Table 15: Percentage values of NPK content in ryegrass leaves that grown in soil, which was treated with humic acid solution of different concentration

Humic acid solution	Total N (%)		Total P (%)		Total K (%)	
	Test plant	Control plant	Test plant	Control plant	Test plant	Control plant
10%-os	3.780	5.700	0.152	0.158	0.554	0.640
0.5%-os	3.410	4.170	0.048	0.070	0.650	0.380
0.075%-os	4.700	4.880	0.310	0.280	2.730	2.850

The result of the plant potassium content analysis indicated that the percentage value of the total potassium content, in comparison to the control plant, is higher in those plants, which grew on soil treated with 0.5% humic acid solution. The results of the soil sample analysis showed that the humic acid treatment in higher concentration increases the percentage value of the available nutrients in the soil (Figure 1).

Figure 1: Comparison between the percentage values of NPK content in ryegrass leaves that grown in soil, which was treated with humic acid solution of different concentration



The NPK and dry matter analysis indicates that the soil treatment with 0.5% humic acid solution increased primarily the plant's dry matter and potassium content, meanwhile the treatment with 0.075% solution mainly increased the phosphorus and nitrogen content in comparison to the control plants (Table 16).

Table 16: Ryegrass's leaves NPK and dry matter content in mg, that grown in soil, which was treated with humic acid solution of different concentration

Humic acid solution	Total N (mg)		Total P (mg)		Total K (mg)		Dry matter (mg)	
	Test plant	Control plant	Test plant	Control plant	Test plant	Control plant	Test plant	Control plant
10%-os	5.8968	9.234	0.23712	0.25596	0.86424	1.0368	0.156	0.162
0.5%-os	6.7518	6.5052	0.09504	0.1092	1.287	0.5928	0.198	0.156
0.075%-os	7.943	7.3688	0.5239	0.4228	4.6137	4.3035	0.169	0.151

The statistical analysis of soil sample's test results indicated a huge difference between the treated and untreated soil samples regarding the NH_4-N and NO_3-N content. In the case of the NH4-N content, the "F" value was 89.987 with a 0.000 probability level ($F_{0.0;\ 3,4}$=6.6). The Pearson correlation index value could not explain with certainty whether the difference was caused by the concentration value of the treatment or only by the fact that we treated the soil with humic acid. This uncertainty can be seen in the 0.319 and 0.252 correlation index values established between treated and untreated soil samples. However, the analysis within the treated soil samples indicated undoubtedly that the differences between the different treatments are certainly the results of the different concentrations of humic acid solution. This fact is reflected in the strongly negative correlation index (R= -0.994; P=0.006). The NO_3-N content analysis of the samples indicated a more reassuring result. Both, the treatments itself and its concentration caused significant differences between the treated and untreated samples (F=732.6 P=0.000 and F=687.7 P=0.000) (Table 17).

Table 17: Analysis of variance and analysis of Pearson correlation's result for NH_4-N and NO_3-N content of soil samples, which was treated with humic acid solution of different concentration

Analysis of variance: NH4-N			
Origin	F	Df	Prob.
Model	89.987	7	0.000
$F_{0.05;3,4}$	6.6		
Test effect			
Test-Control	75.480	1	0.001
Concentration	114.401	1	0.000
T.-C.*Cont.	80.079	1	0.001
Pearson correlation: NH4-N			
Origin	Correlation	Significance	
Concentration*Control	-0.681	0.319	
Concentration*Test	-0.994	0.006	
Control*Test	0.748	0.252	

Analysis of variance: NO3-N			
Origin	F	Df	Prob.
Model	1,193.255	7	0.000
$F_{0.05;3,4}$	6.6		
Test effect			
Test-Control	732.613	1	0.000
Concentration	2,159.405	1	0.000
T.-C.*Cont.	687.746	1	0.000
Pearson correlation: NO3-N			
Origin	Correlation	Significance	
Concentration*Control	-0.999	0.001	
Concentration*Test	-0.999	0.001	
Control*Test	1.000	0.000	

The phosphorus content analysis of the soil samples gave a similar result. The observed significant differences, measured with the "F" value, are consequences (with a high probability value) of the treatment and the applied concentration of the solution. In this case, the correlation index was strongly negative between the solution's concentration and the measured available phosphorus content in the soil (Table 18).

Table 18: Analysis of variance and analysis of Pearson correlation's result for P-PO_4 content of soil samples, which was treated with humic acid solution of different concentration

Analysis of variance: P-PO4			
Origin	F	Df	Prob.
Model	33.351	7	0.003
$F_{0.05;\,3,4}$	6.6		
Test effect			
Test-Control	26.014	1	0.007
Concentration	45.243	1	0.003
T.-C.*Cont.	28.796	1	0.006
Pearson correlation: P-PO4			
Origin	Correlation	Significance	
Concentration*Control	-0.974	0.026	
Concentration*Test	-0.992	0.008	
Control*Test	0.951	0.049	

The statistical analysis between the samples for potassium content established some differences but it did not give a reassuring answer in regards to the question on whether these differences are caused by the treatment or whether they are spontaneous (Table 19).

Table 19: Analysis of variance and analysis of Pearson correlation's result for Potassium content of soil samples, which was treated with humic acid solution of different concentration

Analysis of variance: K			
Origin	F	Df	Prob.
Model	22.014	3	0.006
$F_{0.05;\,3,4}$	6.6		
Test effect			
Test-Control	20.046	1	0.011
Concentration	23.650	1	0.008
T.-C.*Cont.	22.345	1	0.009
Pearson correlation: K			
Origin	Correlation	Significance	
Concentration*Control	0.096	0.904	
Concentration*Test	0.969	0.031	
Control*Test	-0.153	0.847	

The results of the single factor analysis of variance for the ryegrass NPK content supports the hypothesis that the quantity of nitrogen, phosphorus and potassium uptake by the plant depend on the concentration of the applied humic acid solution. This fact is especially true for the volume of phosphorus and potassium because the calculations established significant differences for each treatment according to their concentration value (Table 20).

Table 20: Single factor analysis of variance's result for ryegrass's leaves NPK content that grown in soil, which was treated with humic acid solution of different concentration

Analysis of variance for Nitrogen content (F-8.6 P=95%)					
Origin	Type III SS	Df	Ave. Sq.	F	Prob.
Model	15.843	3	5.281	3.852	0.015
Error	63.057	46	1.371		
Total	78.900	49			
Analysis of variance for Phosphorus content (F-8.6 P=95%)					
Origin	Type III SS	Df	Ave. Sq.	F	Prob.
Model	0.346	3	0.115	15.999	0.000
Error	0.332	46	0.007		
Total	0.678	49			
Analysis of variance for Potassium content (F-8.6 P=95%)					
Origin	Type III SS	Df	Ave. Sq.	F	Prob.
Model	26.817	3	8.939	9.696	0.000
Error	42.408	46	0.922		
Total	69.225	49			

Results of the ryegrass germination test

The experiment reinforced with certainty the view that humic acid stimulates the seed germination processes. Each experiment with treated seeds resulted in a higher germination percentage than in the case of the untreated seeds. This positive effect was also observed in the development vigor. However, the shocking result was that all the treatments with a solution concentration higher than 0.1%, resulted in a fewer percentage value of germination, gradually decreasing the germination percentage, while the treatment's concentration increased. The best result of 95.6% germinated seed, showed the 0.1% humic acid treatment against the germination percentage of 84.8% of the untreated seeds. The 1.0% treatment resulted in a 92.0% of germination rate (Table 21).

Table 21: Germination rate and growing vigor of ryegrass seeds, which were treated with humic acid solution of different concentration

Plant:	Meadow grass	
Treatment	**Average**	
	Germination (%)	**Growing vigor**
Humic acid 1.0%	92.0%	3
Humic acid 0.5%	94.4%	3
Humic acid 0.1%	95.6%	4
Not treated	84.8%	2

The received "F" values of the analysis of variance (F=13.865, 13.550, 13.483; P=0.000) were higher than the "F" table's value at the significance level of 95%, which indicates a significant difference between the different test populations (Table 22).

Table 22: Analysis of variance's result and Pearson correlation analysis for the germination test's result of ryegrass seeds, which were treated with humic acid solution of different concentration

MANOVA - $F_{0.05;\ 69,282} = 1.3$			
Germination test	Df	F	Prob.
Test-Control	69; 282	5.696	0.000
ANOVA - $F_{0.05;\ 23,96} = 1.57$			
Concentration	Df	F	Prob.
Hu-1.0%	23; 96	13.865	0.000
Hu-0.5%	23; 96	13.550	0.000
Hu-0.1%	23; 96	13.483	0.000

Pearson correlation: Test-Control plant		
Concentration	R	Prob.
Hu-1.0%	0.745	0.000
Hu-0.5%	0.790	0.000
Hu-0.1%	0.764	0.000

Discussion of the results

The results of the experiment with the pomegranate saplings showed, that all of the vegetative organs developed a higher weight, diameter, height and volume of the root system than that of the control plants. The observations of the sapling developments and changes in the soil during the experiments also indicated some positive tendencies. The improvement of the yield characteristics when a limited quantity of fertilizer is applied indicates that the humic acid treatment increases the nutrient uptake from the soil. This observation is in line with the statement expressed separately by Chen and Pena-Mendez about the cause-effect relationship between the increasing nutrient uptake by the plant and the humic acid application.

The results from Katkat's work and his research group indicated that 0.1% of the application of the humic acid solution mainly increases the nutrient uptake meanwhile the humic acid application in 0.2% doses primarily increases the dry matter weight and nitrogen uptake in the plant. They drew the line between the two effects in the same level of doses where the results of the ryegrass experiment also indicated, which would be the doses around 0.075%.

It was observed that parallel to the increasing level of the concentration dose in the solution, that the nitrogen uptake by the plant decreased. The weight of the dry matter and the weight of each absorbed nutrient expressed in milligrams indicated, that the applications in low concentrations affects the nutrient uptake, in the first place nitrogen and phosphorus uptake, while applications in higher doses affects the dry matter content in the plant.

Yusuf, Ahmad and Majib (2009) observed that the humic acid application decreases the loss of soil ammonium content. This could also be observed during the testing procedure of the soil samples from the pomegranate nursery experiment. Whereas the untreated samples from the top 0-20cm soil surface layers contained 3.7 mg/kg ammonium content, in the case of the treated soil samples this value was 4.6 mg/kg. In the lower layers of 20-40cm, the ammonium content in both types of soil samples remained at a similar level (4.5 mg/kg).

Similarly, the observations confirmed that the available soil nitrogen content increased during the midterm period of the production cycle, but this value decreased gradually by the end of cycle at a lower level than the measured value of the control soil samples. From this, two conclusions can be drawn. First, MIKKELSEN view (2005) that the humic acid application increases nutrient uptake by the plant and KUSSOW statement (2005) that the same application increases the exchangeable cation content in the soil, are both correct. Second, the risk that the humic acid freed nutrients leach off from the surface soil layer will increase. The exchangeable cation content decreased in the top 0-20cm soil layer, whereas it increased in the lower 20-40cm layer in comparison to the measured valued in the untreated soil samples.

The results of the experiment do not support CIMRIN and YILMAZ (2005) point of view, which states that humic acid does not affect the nutrient uptake from the soil. The attained results from the experiment indicate that the macronutrient uptake depends on the application dose of the humic acid treatment even in the case of the phosphorus uptake.

The germination tests with ryegrass seeds unambiguously supported PENA-MENDEZ (2004) and KOTOB statements (2009) that humic acid stimulates seed germination, but there is no reference in the available literature, that parallel to the increasing value of the treatment doses, the percentage value of the germinated seeds are gradually decreasing. The highest percentage value of the germinated seeds had been the result of the humic acid treatment in the 0.1% concentration dose.

NEW SCIENTIFIC RESULTS

1. The production figures of pomegranate saplings (diameter, height, root weight and number) show a higher value for the saplings, which grow on humic acid treated soil than for the ones that grow on the untreated soil.

2. The humic acid application increased the sapling's secondary root system development in mass and volume, which also increased the sapling's survival rate. With that, the strong cutting practice is avoidable in the nurseries, which unfavorably affects the plant's survival later on.

3. Parallel to the increasing value of the treatment doses, the percentage value of the germinated seeds is gradually decreasing. The highest percentage value of the germinated seeds can be achieved with a treatment of 0.1% concentration dose.

4. The humic acid treatment decreased the coarse size humus content rate (>0.25mm) in the soil, whereas the small size humus content increased.

5. The quantity of potassium uptake by the plant is in direct proportion, whereas the nitrogen and phosphorus uptake is in inverse proportion to the concentration value of the humic acid solution.

CONCLUSIONS AND RECOMMENDATIONS

The results of the experiments indicate that the treatment with humic acid improves the soil's structure. Two aspects can support this fact. Firstly, that the value of the Arany's Fixation index increases in the soils that are treated with humic acid and secondly, that the coarse size humus content in the top 0-20 cm soil layer decreased, whereas the small size humus content nearly doubled in the treated soils. This fact indicates that the decomposition of organic matter in the soil increases when humic acid is applied.

Although all the treated soil samples in comparison to the control samples contained a higher volume of available NO_3-N, the treatment with the lower concentration of humic acid solution resulted in the highest value of available NO_3-N content. In the case of the available potassium content, the higher doses produced the same effect. It follows from the foregoing that when changing the concentration of the humic acid solution, we are able to change the volume of available nutrients for the plant in the soil.

Despite all the different doses of the humic acid application, the NO_3-N content was higher than the experiments with indicator plants (ryegrass) showed the opposite effect. The nutrient uptake by the plant is in inverse proportion with the concentration value of the humic acid solution. Concurrently, the plants that were growing in soils treated with low doses of humic acid solution increased their NO_3-N uptake. The plant analysis indicated that the range for the best result is around 0.075-0.1% of the treatment concentration.

From a practical point of view, the most important results have been delivered with the observed changes in the yield characteristics. In this case, because we are dealing with the sapling production, the sapling's height, diameter and the development stage of the root system should be considered under the concept of yield characteristics.

In case of the fruit trees developed from cuttings, the inclination to develop secondary root system, which subsequently determines its survival and development, is always a challenge. The humic acid treatment stimulates the secondary root system development and help to grow high quality saplings.

Recommendations

The obtained results from the field and the laboratory experiments provided valuable information for the use of humic acid in agriculture. Based on these experiences, the recommendations are as follows:

- From the humic acid products, the humate powder can be handled easily and it is the most cost effective.

- It is recommendable to enrich humate with macro and micro nutrients in order to use it for the plant nutrition. In this way, we can increase the degree of effectiveness of nutrient availability in the soil and the plant's nutrient uptake. The use of enriched humate will improve the nutrient transport in the soil without risking a possible loss of a considerable quantity of nutrients from the root zone through leaching. This will allow a considerable reduction of the chemical fertilizer's dose per hectare and it will also allow the targeting of specific plant nutrition management.

- During the compost production, the raw materials can be treated with humic acid in order to reduce the composting time period, to increase compost quality and to reduce nutrient loss.

- In the case of soils, which tend to be sodic, the humic acid fixes the sodium ions and leach out together with them from the soil surface layer and consequently, it loosens up the soil structure.

- The dilution and preparation of humic acid solution requires attention. The best humic acid-water ration is 1:10.

PUBLICATIONS

IF - Articles in foreign language

MARTON L.-SANDOR F.- KOSTYUCHENKO Y.V. [2010]: Organic Carbon changes over 40-years in a Haplic Luvisol type farmland in Hungary, Journal of Agricultural Science and Technology, p.913-920, (IF: 0,68)

MARTON L.-SANDOR F. [2015]: Essential plant environmental impacts on corn yield, Academia Journal of Agricultural Research (ISSN: 2315-7739), 3(9), September 2015, p.189-194, (IF: 0,342)

Non IF - Articles in foreign language

SANDOR F. [2009]: The effect of humic substances on pomegranate nursery production in Nangarhar, Afghanistan, Bulletin of the Szent István University, Gödöllő, 2009, p.5-24.

SANDOR F.[2011]: The effect of humic substances on pomegranate nursery production in Nangarhar, Afghanistan, (JESE-E20101025-01), Journal of Environmental Science and Engineering, ISSN1934-8932, USA, p.214-226.

SÁNDOR F.-TOLNER L.-SIMON B.-FÜLEKY GY. [2014]: The effects of humic acid product on soil fertility measured by ryegrass test plant, XIII. Alps-Adria Scientific Workshop Villach, Ossiacher See, Austria, Növénytermelés 63 Suppl. p.245-248.

SÁNDOR F., TOLNER L., FÜLEKY GY., ABDIANP S. A., SANCHE J. E. (2015): Humic substances applications on quality and yield of commercially-produced pomegranate saplings in Nangarhar, Afganistan. Columella – Journal of Agricultural and Environmental Sciences, Vol 2. No. 2. 59-67. pp.

Other articles in foreign language

SANDOR F. [2002]: Good practice in gardening, Horticulture Magazine, Lilongwe-Malawi

SANDOR F. [2010]: The Gardener, Monthly newsletter for the agricultural community, 1/1 szam, June 2010, RoP-USAID Kabul, Afghanistan, p.1-4.

SANDOR F. [2010]: The Gardener, Monthly newsletter for the agricultural community, 1/2 szam, July 2010, RoP-USAID Kabul, Afghanistan, p.1-6.

SANDOR F. [2010]: The Gardener, Monthly newsletter for the agricultural community, 1/3 szam, August 2010, RoP-USAID Kabul, Afghanistan, p.1-10.

SANDOR F. [2010]: The Gardener, Monthly newsletter for the agricultural community, 1/4 szam, September 2010, RoP-USAID Kabul, Afghanistan, p.1-8.

SANDOR F. [2010]: The Gardener, Monthly newsletter for the agricultural community, 1/5 szam, October 2010, RoP-USAID Kabul, Afghanistan, p.1-6.

SANDOR F. [2010]: The Gardener, Monthly newsletter for the agricultural community, 1/6 szam, November 2010, RoP-USAID Kabul, Afghanistan, p.1-10.

SANDOR F. [2010]: The Gardener, Monthly newsletter for the agricultural community, 1/7 szam, December 2010, RoP-USAID Kabul, Afghanistan, p.1-10.

Conference’s abstract in foreign language

SANDOR F. [2009]: Humic substances effect on pomegranate nursery production, International Scientific Conference-Humic Substances In Ecosystems 8, ŠOPORŇA, Slovakia, 13 – 16. september 2009

Books in foreign language

SANDOR F. [2008]: Soil testing, No. 2008-001-AFG, USAID-DAI-ROP megrendelesere, Jalalabad, Afghanistan (English and Pashtun language)

SANDOR F. [2007]: Vegetative propagation techniques, No. 2007-003-AFG, USAID-DAI-ROP, Jalalabad, Afghanistan (English and Pashtun language)

SANDOR F. [2008]: Fruit orchards, No. 2008-005-AFG, USAID-DAI-ROP, Jalalabad, Afghanistan (English and Pashtun language)

SANDOR F. [2008]: Irrigation, No. 2008-002-AFG, USAID-DAI-ROP, Jalalabad, Afghanistan

SANDOR F. [2008]: Apple production, No. 2008-004-AFG, USAID-DAI-ROP, Jalalabad, Afghanistan (English and Pashtun language)

Scientific films in foreign language

SANDOR F. (2005). Seed of Freedom (Good farming practices in prisons,)Penal reform International – Malawi, Lilongwe-Malawi, documental

SANDOR F. (2003). Compost production, Penal reform International – Malawi, Lilongwe-Malawi, documental