EFFECT OF HUMIC ACID ON GROWTH AND PRODUCTIVITY OF EGYPTIAN LIME TREES (*Citrus aurantifolia* Swingle) UNDER SALT STRESS CONDITIONS.

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ABSTRACT

A field experiment was carried out during 2014/2015 and 2015/2016 seasons on Egyptian lime trees (*Citrus aurantifolia* Swingle) at Baltim region, Kafr El-Sheikh governorate, Egypt, to study the effect of humic acid applications on soil properties, growth, yield, fruit quality and leaf nutrient content of Egyptian lime trees. Four treatments 0, 10, 20 and 30 ml/tree soil application were arranged in a randomized complete block design with three replicates.

The obtained results showed that application of Humic acid profoundly alleviated salinity effect by enhancing available nutrients and microbial activity. In addition, to it is induce a significant improvement of tree size and growth in terms of shoot length, leaves number/shoot, leaf area and canopy volume. Moreover, yield as kg/tree or number of fruits per tree was significantly increased with increasing humic acid level in both seasons. Fruit quality as weight (g), volume (cm^3), juice% and SSC% were significantly increased by increasing humic acid levels, whereas acidity was slightly decreased. Soil application of humic acid increased leaf N, P, K, Fe, Mn and Zn contents. The results suggested that humic acid treatment at 20 ml/tree have great potential in alleviating salinity stress on growth and productivity of Egyptian lime trees under saline soil conditions.

Key words: humic acid, salt stress, Citrus aurantifolia, fruit quality.

INTRODUCTION

Egyptian lime (*Citrus aurantifolia* Swingle) is one of the most popular fruit among citrus in Egypt, due to its high yield, fresh consumption, consumes throughout the year, aromatic flavor and high fruit quality. Egyptian lime reached 55797.47 feddan, represented about 10.3% of total area of citrus (541723 feddan) and produced, 424204.1 ton represented about 10.35% of the total citrus production (4098590 tons) according to Ministry of Agriculture and Land Reclamation statistics, (2015). Salinity is one of the major factors that affect growth, yield, fruit quality and nutritional status of citrus trees. Soil salinity resulting from natural processes, excessive application of mineral fertilizers or crop irrigation with saline water (West, 1986). In Egypt, lack of water source especially at the end of canal, pays farmers to use agriculture drainage water for irrigation in Baltim region, with poor drainage system, water table increase; this led to an increase in salinity in soil. Salinity decreases citrus tree growth and yield, due to various degrees depending on citrus species, salinity level, water deficit, effects on plant metabolism and ion toxicity as well as nutritional imbalance as accumulation of Na^+ or Cl^- or both (Chatzissavvidis *et al.,* 2008). So, salts in soil and irrigation water are being serious problems for commercial agriculture in this area. The production potential of Egyptian lime can be maximized encouraging the use of bio-stimulants like humic acid, due to enormous beneficial effects on soil and plant attributes. Humic acid may improve the physical, chemical and biological properties of soil. Ali *et al.,* (2013) study the effect of humic acid, Uni-sal, magnetic iron and arbuscular mycorrhizal fungi to avoid or minimize salt hazard on grapevine, they reported that application of humic acid and Uni-sal are more affecting in reducing salinity hazard of soil, it gave the lowest EC being horizontally and vertically at direction of vines. Also, Tenshia and Singaram (2005) revealed that addition of humic acid 20kg/ha improved the availability and uptake of macro and micronutrients. Cavalcante *et al.,* (2013) reported that, humic acid improve soil structure and change physical properties, promote the chelation of many elements and make these available to plants. Enhancement of total chlorophyll contents, stomatal conductance, net photosynthesis rate and transpiration rate has resulted in greater plant growth with humate application (Abbas *et al.,* 2013). Increase the permeability of plant membranes due to humate application resulted in improving growth of various groups of beneficial microorganisms, accelerate cell division, increased root growth and all plant organs for different fruit trees (Nardi *et al.,* 2002 and Ferrara and Brunetti, 2010).

So, the aim of this study was to investigate mitigation effect of Humic acid on growth and productivity of Egyptian lime trees under saline soil conditions.

MATERIALS AND METHODS

The present study was carried out during 2014/2015 and 2015/2016 seasons on fifteen years old Egyptian lime trees (*Citrus aurantifolia* Swingle) budded on sour orange (*Citrus aurantium* L.) rootstock, planted at 5x5 meter in a private orchard situated at Baltim, Kafr El-Sheikh governorate. The trees received the same cultural practices and the fertilization program as usually done in this area (0.5kg/tree superphosphate (37%) was applied at one dosage in December, 4.5kg/tree ammonium sulphate (20.5%) was given at three equal doses on February, May and late of June, 1.25kg/tree potassium sulphate was applied at two equal doses on March and late June). The soil texture was clayey (57.9% clay, 18.0% silt and 24.1% sand), 1.10% organic matter, 3.85 dSm^{-1} an electrical conductivity and a pH of 8.15. Thirty six trees were arranged in a randomized complete block design with four treatments, replicated three times with three trees. Treatments were consist of four levels of humic acid i.e. 0 (control), 10, 20 and 30 ml/tree applied via soil at Marsh, May and July in both seasons, and the source of Humic acid was Actosol, contains 2.9 % humic acid + 10, 10, 10 % NPK.

The following data was recorded:

1. Soil nutrient contents:

Before applying the treatments and at the end of experiment, soil samples were taken from each treatment at major root zone (0 – 60 cm depth). Soil samples were prepared for analysis according (Jackson, 1967). These soil samples were dried, sieved through a 2 mm and analyzed for texture and available N, P, K, Fe, Mn and Zn. Nitrogen was extracted by 1N KCl, P was extracted by 0.5N $NaHCO_3$, K was extracted by 1N NH_4AC and Fe, Mn, Zn were extracted by DTPA according to Page *et al.,* (1982).

2. Microbial activity:

Microbial activity was measured as rate of CO_2 (mg kg^{-1} day^{-1}) evolution from soil. Fresh soil sample were collected from each treatment before beginning treatments and after harvesting fruits. The CO_2 evolution was measured according to the procedure adapted by Gaur *et al.,* (1971). A 50 g fresh soil sample was taken into 500 ml conical flask. A glass vial containing 10 ml of 0.3 M NaOH solution was suspended carefully in each flask. Flasks were sealed using rubber bung and then incubated at 30°C for 20 day, while the CO_2 evolved and subsequently absorbed in NaOH was determined by titrating the NaOH solution against 0.1 M HCl.

3. Vegetative growth:

Shoot length (cm), leaves number per shoot, leaf area (cm^2) and canopy volume (m^3) was calculated according to the formula: 0.5238 x tree height x diameter square (Turrell, 1946).

4. Leaf nutrient contents:

Fully mature leaves were separated from nonbearing shoots, washed, cleaned and oven dried to constant weight at 60-65C° and reweighted. The dried leaves samples of each replicate were finally grounded and digested with H_2SO_4 and H_2O_2 according Evenhuis and DeWaard (1980). In digested solution samples N, P, K, Mn, Fe and Zn were determined as follows: nitrogen was determined by micro-Kjeldahl method (A.O.A.C. 1985), K by flame photometer, P by spectrophotometer, Mg, Fe and Zn were assayed with Atomic Absorption spectrophotometer (Unican SP 1900) according to Chapman and Pratt (1961).

5. Yield:

At harvest time (September); the yield of each tree was determined as number and weight (kg) of fruits per tree.

6. Fruit quality:

15 fruits were taken at random from the yield of each tree for determination the physical and chemical characteristics such: fruit weight (g), fruit volume (cm^3), juice (%), soluble solids content by hand refractometer and total acidity as citric acid according to (A.O.A.C., 1985).

Statistical analysis was done as analysis of variance according to Snedecor and Cochran (1967), and the least significant differences (L.S.D. at 5%) were used to compare the means values.

RESULTS AND DISCUSSIONS

1. Soil nutrient contents:

Data presented in Fig (1) clearly indicated that, there was a positive effect of humic acid at the three levels (10, 20 and 30 ml/tree) on soil available nutrients. The highest available N, P, K, Fe, Mn and Zn contents in soil was observed in treatment of humic acid at 20 ml/tree followed by 30 ml/tree. While, the lowest availability of N, P, K, Fe, Mn and Zn were noticed with the control. Similar results were reported by El-Galad *et al.,* (2013) resulted that application of humic acid or compost in saline soil gave the highest soil available N, P , K, Fe, Mn and Zn values after harvesting.

2. Microbial activity:

The effect of humic acid on CO_2 mg kg^{-1} day^{-1} as indicator to soil microbial activity is presented in Fig (2). CO_2 rate is indicator to microbial activities which enhanced with increasing doses of humic acid from 10 to 30 ml/tree as compared to the control treatment. The obtained results are in harmony with those obtained by Abd El-Ghany *et al.,* (2010) and Mohamed *et al.,* (2013). In this respect, Khattak *et al.,* (2013) concluded that humic acid treatments significantly increased microbial activities, measured in terms of CO_2 evolution in salt affected soil.

Generally, it is clear from Figures (1and 2) that, soil application of humic acid was enhanced all the fertility properties of salt-affected soil. Thus, application of humic acid at 20 or 30 ml/tree levels in Egyptian lime orchard appeared to be superior in improving saline soil by increasing CEC, available nutrients and microbial activity as compared with the other levels (0 and 10 ml/tree). These beneficial effects of humic acid on positively changes in the studied of soil properties could be attributed to humic acid forming cheleated compounds, induce more activity of microorganisms and it makes as like hormones. These results were in accordance with the findings of Fahramand *et al.,* (2014) who concluded that humic acid have an important role in improving soil aggregation and water movement leaching the excessive soluble salts. In this respect, Mohamed (2012) found that the electric conductivity (EC) of the soil treated with humic acid application was lower compared to the non-treatment of humic acid. Moreover, in salt affected soil, the sodium percentage in water generally increases, in this situation humus complex is considered to be the effective amelioration methods to removal exchange and soluble sodium and changing the ionic composition of soils with, at the same time, leaching the sodium salts out of the soil profile (Ouni *et al.,* 2014).

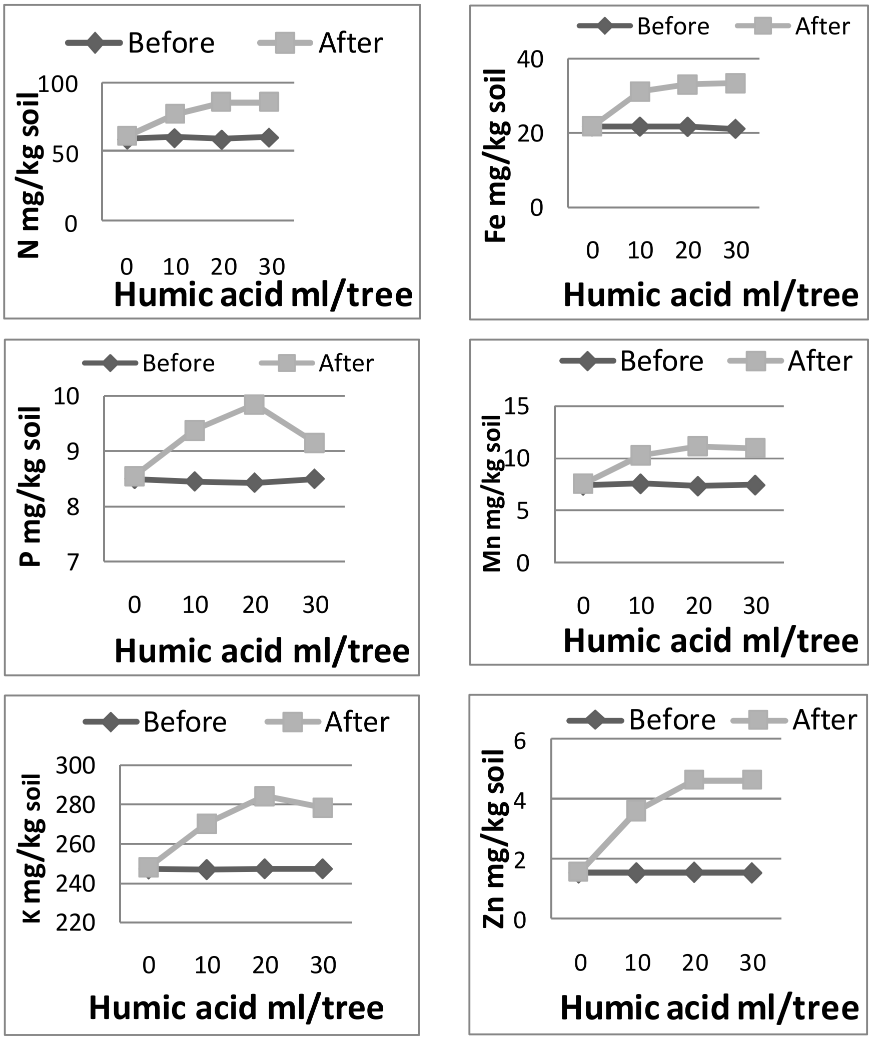

Fig 1: Effect of humic acid on soil N, P, K, Fe, Mn and Zn (mg/kg soil) before beginning and after the end of experiment

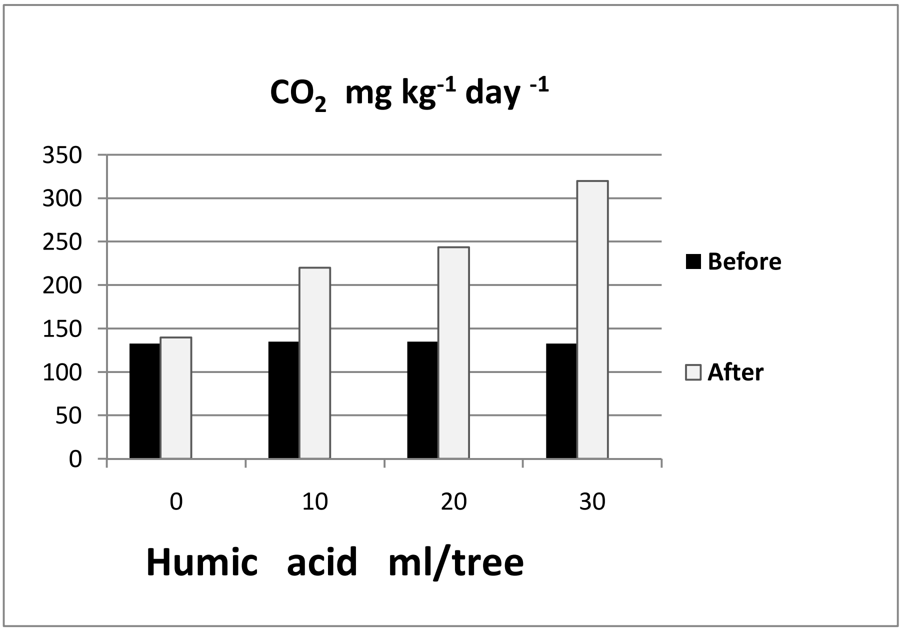

Fig 2: Effect of humic acid on soil microbial activity as rate of CO_2 (mg kg^{-1} day^{-1}) before beginning and after the end of experiment

3. Vegetative growth:

Data in Table 1 showed that, soil application of humic acid had a significant effect on all vegetative growth parameters in both seasons, it is clear that shoot length, leaves number per shoot, leaf area and canopy volume were significantly increased by increasing soil application levels of humic acid from 0 to 30 ml/tree in both seasons. The highest values of vegetative growth parameters were obtained with 20 and 30 ml/tree levels without significant differences between them in both seasons. Similar results were obtained by Webb *et al.,* (1988) on citrus. In this respect, Hagagg *et al.,* (2011) observed that, Egazy olive seedlings treated with humic substance gave the best results concerning percentage of plant height, shoot number per plant, leaves number per plant and stem diameter. Also, Fathy *et al.,* (2010) concluded that soil application of humic acid levels from 0.0 to 75 cm^3/tree had a positive effect on shoot length, leaves number per shoot and leaf area of Canino apricot trees. Generally, it is obvious from Tables (1) that, tree size and growth vigour of Egyptian lime trees were significantly enhanced by humic acid treatments under salt affected soil. Similar results were obtained by Ali *et al., (*2013) who reported that, soil application of humic acid (9 liter/feddan) was more effective in reducing EC from 4.2 dS/m in soil to 1.8 dS/m and avoiding the adverse effects of salinity on growth and fruiting of vines. In this line, El-Khawaga (2013) reported that application of humic acid and anti-salinity were very effective in alleviating the adverse effect of salinity on the leaf area, yield and fruit quality of Sewy, Zaghloul and Hayany date palm cultivars. Such results was recorded by Eissa *et al.,* (2007) on peach and apricot, they concluded that under salt stress, doses of both soil and foliar application of humic acid increased growth, uptake of nutrients and alleviating adverse effect of salinity. Humic acid influence plant growth directly and indirectly. Directly, humic acid compounds may have a variety of biochemical effects either at cell wall, membrane level or in the cytoplasm, including improved photosynthesis and respiration rates in plants, better protein synthesis and plant hormone like activity (Abbas *et al.,* 2013). The indirect effects of humic compounds on soil fertility include, (i) raise the soil microbial population including beneficial microorganisms, (ii) Better soil structure, (iii) Enhance the cation exchange capacity and the pH buffering capacity of the soil (Morard *et al.,* 2011).

Table (1): Effect of humic acid application on vegetative growth of Egyptian lime trees in 2015 and 2016 seasons

Humic acid ml/tree	Shoot length cm		Leaves number per shoot		Leaf area cm^2		Canopy volume m^3	
	2015	2016	2015	2016	2015	2016	2015	2016
0	32.4	35.2	19.2	20.8	14.5	15.6	23.7	25.8
10	41.5	43.0	27.4	26.6	15.6	16.5	25.2	27.2
20	45.4	45.3	26.4	27.1	16.0	16.8	26.8	29.4
30	47.8	49.8	29.8	30.7	16.7	17.4	28.1	31.6
L.S.D.at 5%	2.25	1.23	1.34	1.30	1.56	1.14	1.70	2.29

2. Leaf nutrient contents:

Data in Table 2 show the effect of soil application of humic acid on leaf N, P, K, Fe, Mn and Zn content of Egyptian lime trees. Soil application of humic acid had a significant effect on leaf nutrient contents as compared with control treatment. The highest values of leaf nutrient content recorded with 30 ml/tree followed by 20 ml/tree and 10 ml/tree in both seasons. These results were similar to those obtained by Rengrudkij and Partida (2003) who noted that leaf analysis of avocado trees showed a high level of N, K, Mn and Fe when treated with humic acid compared with untreated trees. Tenshia and Singaram (2005) concluded that, soil application of humic 20 kg/ha improved uptake of N, P, K, Fe and Zn than control. Generally, our results in Table (2) indicated that, soil application of humic acid increased leaf N, P, K, Fe, Mn and Zn concentrations of Egyptian lime trees. Improving mineral nutrient absorption by humic acid in plants grown under saline conditions came true with those reported by Ali *et al.,* (2013). In this respect, Mahmoudi *et al.,* (2013) revealed that, under salt stress humic acid enhanced nutrient uptake in terms of N, P, K, Cu and Zn in leaves of kiwifruit. In this line Khattak and Muhammad (2010) reported that, humic substance can ameliorate negative soil properties and improve nutrients uptake under salinity conditions. Humic acid can be improved efficiency of program fertilization, due to microbiological activity can be stimulated by humic substances, by which it is possible to enhance the uptake of minerals. If the adequate amount of humic substances is present within the soil, then it is a fertile soil. So, it can be concluded that humic acid may enhance growth, the uptake of some nutrients, reduce the uptake of toxic elements and could improve plant response to salinity.

Therefore, it can be concluded from Tables (1 and 2) that, soil application of humic acid at 30 and 20 ml/tree gave the best results concerning shoot length, leaves number per shoot, leaf area, canopy volume and nutrient elements concentrations in leaves of Egyptian lime trees without significant differences between them in both seasons.

Table (2): Effect of Humic acid application on leaf nutrient concentration of Egyptian lime trees

Humic acid ml/tree	N %	P%	K%	Fe ppm	Mn ppm	Zn ppm
	2015 season					
0	1.38	0.113	1.04	83	27.2	33
10	1.53	0.126	1.07	88	28.6	38
20	1.99	0.305	1.12	91	28.9	39
30	2.12	0.139	1.21	95	29.7	46
L.S.D.at 5%	0.08	0.005	0.04	2.85	0.91	2.08
	2016 season					
0	1.50	0.123	1.06	87	26.8	32
10	1.76	0.132	1.14	91	27.3	33
20	2.06	0.137	1.20	96	27.6	38
30	2.15	0.146	1.22	100	28.5	40
L.S.D.at 5%	0.07	0.003	0.03	2.67	1.05	1.93

3. Yield:

Data in Table (3) clear that, yield as weight (kg/tree) and number of fruits per tree was significantly increased by increasing soil application of humic acid as compared with untreated trees. Meanwhile, the high level at 30 ml/tree had the highest yield followed by 20 ml/tree without significant differences between them in both seasons. These results agree with those obtained by El-Mohamedy and Ahmed (2009) concluded that humic acid caused the highest yield as number of fruits/tree or weight (kg/tree) compared with untreated trees of mandarin. In this respect, Abbas *et al.,* (2013) showed that kinnow mandarin tree received humic acid at 30 ml exhibited highest number of fruits per tree.

Table (3): Effect of humic acid application on yield, fruit weight and fruit volume of Egyptian lime trees in 2015 and 2016 seasons

Humic acid ml/tree	Yield				Fruit weight g		Fruit volume cm^3	
	Kg/tree		Number/tree					
	2015	2016	2015	2016	2015	2016	2015	2016
0	25.7	28.4	1148.3	1224.5	21.9	23.2	20.8	22.8
10	27.3	29.8	1155.5	1294.4	23.2	24.1	22.8	23.4
20	30.0	32.7	1174.4	1345.3	24.7	25.6	23.6	24.5
30	31.4	35.3	1223.2	1352.2	25.1	27.0	24.6	24.7
L.S.D.at 5%	1.17	2.17	4.64	6.44	1.27	1.48	1.59	1.73

4. Fruit quality:

Data in Tables (3 and 4) show the effect of soil application of humic acid on fruit quality in terms of fruit weight (g), fruit volume (cm^3), juice %, SSC % and acidity % of Egyptian lime trees. As for the effect of soil application of humic acid, it is clear that heaviest and largest fruits were harvest from trees treated with humic acid at 30 ml/tree followed by 20 ml/tree and 10 ml/tree. The lowest values of such physical characters were recorded by control trees (Table 3). Also, the highest values of juice% and SSC% were found in fruits harvested from trees treated with humic acid at 30 ml/tree followed by 20 ml/tree and 10 ml/tree as compared with untreated one (Table 4). Acidity was slightly decreased with increasing levels of humic acid in both seasons. These results are in accordance with Ferrara and Brunetti (2010) and Abbas *et al.,* (2013) on different fruit crops.

Table (4): Effect of humic acid application on fruit juice weight %, fruit SSC % and fruit acidity of Egyptian lime trees in 2015 and 2016 seasons

Humic acid ml/tree	Juice %		SSC %		Acidity %	
	2015	2016	2015	2016	2015	2016
0	40.1	42.6	9.69	10.05	7.94	8.03
10	44.2	46.1	10.21	10.45	7.82	7.90
20	46.5	47.9	10.45	10.59	7.76	7.80
30	49.4	49.7	10.72	10.76	7.62	7.72
L.S.D.at 5%	1.35	1.62	0.03	0.33	0.04	0.17

Conclusion

Consequently from the previously mentioned results, it was clear the great role of humic acid as soil application for Egyptian lime trees grown in a slightly saline soil. It is indispensable for improvement of soil properties, growth and the nutritional status of the Egyptian lime trees and production of maximum yield and quality. So, it should be recommended the superiority of application of humic acid, especially

20 ml/tree soil application, it is lower cost than 30 ml/tree and gave the best growth, yield, fruit quality and nutritional status of Egyptian lime trees.

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تأثير حمض الهيوميك على نمو و إنتاجية أشجار الليمون البلدي تحت ظروف الإجهاد الملحي

حسن أبو الفتوح عناب

قسم الموالح - معهد بحوث البساتين – مركز البحوث الزراعية الجيزة مصر

أجريت تجربة حقلية موسمي 2015/2014 و 2016/2015 وذلك لتقدير تأثير إضافة حمض الهيوميك إلى التربة على خصائص التربة الكيماوية و النشاط الميكروبي و النمو و المحصول و جودة الثمار و محتوى الأوراق من العناصر و ذلك على أشجار الليمون البلدي في مركز بلطيم محافظة كفر الشيخ. أظهرت النتائج أن إضافة حمض الهيومك إلى التربة أدى إلى تحسين خصائص التربة المالحة حيث إنخفض زادت السعة التبادلية الكاتيونية والعناصر الميسرة والنشاط الميكروبي، بالإضافة إلى تحسن معنوي في نمو الأشجار و المتمثل في طول النموات و عدد الأوراق على النمو و مساحة الأوراق و حجم الشجرة. أدت المعاملة بحمض الهيوميك إلى زيادة محصول الأشجار من الثمار وزنا وعددا و ذلك بالمقارنة بالأشجار التي لم تعامل. بالإضافة إلى ذلك حدثت زيادة معنوية في وزن و حجم الثمار و النسبة المئوية للعصير و المواد الصلبة الكلية بينما حدث إنخفاض طفيف في قيم الحموضة الكلية . إضافة حمض الهيوميك إلى التربة زاد من إمتصاص أشجار الليمون البلدي للعناصر الغذائية مثل النتروجين و الفوسفور و البوتاسيوم و الحديد و المنجنيز و الزنك. و واضح من خلال مناقشة نتائج البحث أنة يمكن أن نوصى باستخدام المعاملة 20 مل لكل شجرة على ثلاث دفعات في مارس و مايو و يوليو و ذلك لتأثيرها الايجابي على نمو و إنتاج أشجار الليمون المصري تحت ظروف التربة المالحة.