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Effect of humic acid on some vegetative traits and ion concentrations of Mexican Lime (*Citrus aurantifolia* Swingle) seedlings under salt stress

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Abstract
Effects of humic acid on some vegetative characteristics and mineral concentrations of Mexican lime were investigated under different salinity concentrations. Four doses of humic acid (0, 1500, 3000 and 4000 mg/kg soil) and four salinity levels (1500, 2500, 3500 and 4500 µmos) were applied on Mexican lime seedlings. Experiment was conducted as a factorial arrangement based on a completely randomized design. The results showed that humic acid had a positive influence on plant height under salinity stress. Shoot number was significantly increased by application of humic acid under salinity stress conditions. In 4500 µmos salinity, application of 4500 mg/kg humic acid caused an increase in shoot fresh and dry weights, root dry weight and shoot potassium concentration. Percentage of sodium was decreased by application of 3000 mg/kg humic acid in the shoot of plants that were exposed to 4500 µmos salinity level. humic acid at 1500 mg/kg caused a reduction in shoot sodium percentage under 3500 µmos salinity level. When compared to the non-treated plants, application of humic acid led to a decline in chloride percentage in Mexican lime seedlings. In conclusion, 3000 and 4000 mg/kg humic acid can be used to reduce the toxic effects of salinity and also to decrease the uptake of toxic elements such as sodium and chloride in Mexican lime seedlings.

Keywords: chloride, potassium, sodium, vegetative traits.

Abbreviations: Cl, Chloride; **CRD**, Completely Randomized Design; **K,** Potassium; **Na,** Sodium**.**

Introduction

Mexican lime (*Citrus aurantifolia* Swingle) is native to the Indo-Malayan region. The Mexican lime continues to be cultivated more or less on a commercial scale in India, Egypt, Mexico, Iran and the West Indies, tropical America, and throughout the tropics of the Old World (Morton, 2013). Salt stress is described by high amounts of Na^{+}, Mg^{+2}, Ca^{+2}, Cl^{-}, HCO^{3-}, SO_2^{4-}, and B ions which have negative impacts on the vegetative and generative growth. Generally, NaCl is the main source of salt stress for plants. The salinity has mainly two negative impacts: possible toxic effects of excessive ions and increasing osmotic potential. Physiological reactions to salt stress comprise increase in osmotic potential, dehydration of shoot, and/or a loss of turgor potential, decrease

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in growth rate (Mahmoudi and Aryaee, 2015). In many studies, organic acids were reported to improve the physiological traits (Ahmad *et al*., 2010).

In brief, high salt concentrations in the soil reduce the physiological activities of plants and negatively affect plant growth and development (Khaled and Fawy, 2011). Humic acid basically improves soil conditions for growth and quality of the crop. Many studies have indicated the practical importance of humic acid in agriculture. For example, Nardi *et al*. (2002), Buyukkeskin and Akinci (2011), Celik *et al*. (2011), Tahir *et al*. (2011) have described beneficial effects of humic substances on plant growth, mineral nutrition, seed germination, seedling growth, root initiation, root growth, shoot development and macro-and microelements uptake.

Masciandaro *et al*. (2002) have indicated that humic substances might counteract with abiotic stress conditions such as unfavorable temperatures, pH, and salinity which promote uptake of nutrients and reduce uptake of some toxic elements. Mahmoudi and Barzegar-zoghalchali (2015) reported that humic acid can decrease negative soil properties and can improve physiological properties of Thomson navel cultivar of sweet orange. They suggested that humic acid can be used to decrease the negative effect of lime on plant growth and development.

Humic acids can significantly reduce water evaporation and increase its use by plants in dry and sandy soils. Furthermore, they increase the water holding capacity of soil. Humic acids can help to recover plant chlorosis, increase permeability of plant membranes and promote plant enzyme systems. They accelerate cell division, induce root development, and decrease negative effects of stresses on plants. Under the influence of humic acids, plants grow stronger and strengthen plant resistance against diseases. Humic acids reduce soil erosion by increasing the cohesive forces of the very fine soil particles. They improve physical properties of soil by increasing the exchange capacity and buffering qualities as a result improve soil structure. Humic acids promote the chelation of many elements and making them available to plants. Through improvement of soil properties, humic substances can improve nutrient uptake and plant growth (Khaled and Fawy 2011).

Azeem *et al*. (2014) studied phenology, growth and productivity of maize following treating them with humic acid and nitrogen and they found that using three kg humic acid and 160 kg nitrogen per hectare can result in positive responses of growth, phenology and productivity in maize plants. Abbas *et al*. (2013) reported that humic acid is emerging as the most prominent bio-fertilizers in enhancing morpho-physiological and biochemical aspects of plant growth. Split application of humic acid at three different growth phases of Kinnow mandarin induced reproductive vigor and physio-biochemical attributes, as a result it was useful to obtain high yields. Therefore, in the present study potential of humic acid to improve vegetative growth and distribution of some mineral elements in Mexican lime seedlings was investigated when plants exposed to different levels of salinity stress.

Material and Methods

To evaluate the influence of humic acid on reducing negative effects of salinity stress different concentrations of humic acid were used in the culture medium of Mexican lime (*Citrus aurantifolia* Swingle) seedlings. Ten kg plastic pots containing loamy soil were used for culturing the plants. Big gravels were used at the bottom of the pots. One-year-old Mexican lime seedlings were cultured in the pots and non-saline water (S1) was used for irrigation of the plants in all pots until the establishment of the seedlings. After establishment of Mexican lime seedlings, they were irrigated by saline solutions. The experiment was conducted as a factorial arrangement based on a complete randomized design (CRD) with four replications. The first factor was salinity with four levels including: S1) non-saline water with EC=1500 µmos, S2) tap water with EC=2500 µmos, S3) saline water with EC=3500 and S4) saline water with EC=4500 µmos. Salinity was imposed to the plant using different concentrations of NaCl. The second factor was different concentrations of humic acid with four levels (0, 1500, 3000 and 4500 mg/kg soil). To prevent salt accumulation in the root medium, plants were irrigated by non-saline water each two weeks. In 15, 30 and 45 days after beginning of the experiment, liquid humic acid was applied to the pots. Before applying the treatments and in the end of the experiment (three months), vegetative parameters such as plant height, crown diameter and number of lateral shoots were measured. At the end of the experiment other parameters such as shoot and root fresh and dry weights and concentration of Na, Cl and K were also measured in the plant shoots. Potassium and sodium concentrations were determined by flame emission (Horneck and Hanson 1998) (Ependorf Elex6361). Chloride concentration was measured using a chloride meter (Jenway, Pclms). The obtained data were analyzed by SAS (version 9.1) software and the means were compared using LSD ($P<0.05$). Correlation coefficients between parameters were estimated using Pearson's method by SPSS (version 16) software.

Results and Discussion

Significant ($P<0.01$) interactions were observed between salinity and humic acid factors for the all evaluated parameters except for root fresh weight. Salinity had significant influence on root fresh weight, no significant influence of humic acid had on root fresh weight was observed.

Plant height

Plant height was increased by increasing humic acid concentration from 0 to 3000 mg/kg in non-saline condition (EC= 1500 µmos). The highest percentage of increase in plant height was observed in plants treated with 1500 mg/kg humic acid under 2500 µmos salinity. Inducing effects of humic acid on plant height was more emphasized under 3500 µmos salinity (Table 1). The highest increase in plant height (9.5%) was observed in plants that simultaneously exposed to 2500 µmos salinity and treated with 1500 mg/kg humic acid, while the lowest plant height was observed in plants exposed to 4500 µmos salinity and treated with 3000 mg/kg humic acid (without change). Application of 3000 and 4500 mg/kg humic acid had a positive effect on plant height under 3500 µmos salinity, but no effect of humic acid on plant height was observed following increasing salinity level. Significant increase in plant height was observed following application of 3000 and 4500 mg/kg humic acid under non-saline condition (Table 1).

Table 1. Increasing percentage (%) in height of Mexican lime seedling treated with different levels of humic acid and salinity

Salinity (µmos) Humic acid (mg/kg)	1500	2500	3500	4500
0	$^{c}1.7^{D} \pm 0.08$	$^{b}6.1^{B} \pm 0.12$	$^{c}4.7^{C} \pm 0.28$	$^{a}9.2^{A} \pm 0.07$
1500	$^{c}1.7^{C} \pm 0.27$	$^{a}9.5^{A} \pm 0.45$	$^{d}3.5^{B} \pm 0.20$	$^{b}2.1^{C} \pm 0.17$
3000	$^{a}8.5^{A} \pm 0.20$	$^{d}2.8^{C} \pm 0.14$	$^{a}6.2^{B} \pm 0.10$	$^{c}0.0^{D} \pm 0.00$
4500	$^{b}5.4^{B} \pm 0.15$	$^{c}3.9^{C} \pm 0.36$	$^{a}6.8^{A} \pm 0.31$	$^{b}2.5^{D} \pm 0.36$

Means with same letters have not significant difference according to LSD ($P<0.05$).
Minuscules are relative to comparison of humic acid levels in each salinity level (column comparison) and capitals are relative to comparison of salinity levels in each humic acid level (row comparison).

Number of shoots

Number of shoots was increased by increasing humic acid concentration from 0 to 3000 mg/kg in non-saline condition (EC= 1500 µmos). The highest percentage of increase in number of shoots was observed in plants treated with 2500 µmos salinity without humic acid applications. Percentage of increase in number of shoots was declined by increasing humic acid concentration up to 3000 mg/kg under salinity 3500 µmos. The highest number of shoots was observed in plants treated with 3000 mg/kg humic acid or without application of humic acid. Minimum number of shoots was observed in plants treated with 4500 mg/kg of humic acid (Table 2). In plants treated with 1500 mg/kg humic acid, number of shoots was increased by increasing salinity level from 1500 to 4500 µmos. By increasing salinity level, no distinct trend was observed for the number of shoots in plants treated with 3000 and 4500 mg/kg humic acid (Table 2). The most and least percentage of number of shoot increase were observed in plants under 4500 µmos salinity and treated with 3000 and 4500 mg/kg humic acid (65.0% and 8.3%), respectively. Application of 3000 mg/kg humic acid led to a significant increase in number of shoots under 4500 µmos salinity (Table 2). According to the correlation coefficients, there was a positive and significant relationship between salinity concentration and number of shoots. Number of shoots was significantly increased by higher salinity levels. On the other hand, there was a negative correlation between humic acid levels and number of shoots. Number of shoots was decreased by increasing humic acid concentrations (Table 11). Manas *et al.* (2014) observed increasing in the number of branches using humic acid + Zn + B in pungent pepper. El-Hak *et al.* (2012) reported that under humic acid treatment, the number of branches/plant significantly increases in pea plants.

Crown diameter

By increasing humic acid levels, no distinct trend was observed for crown diameter in plants treated with 1500 and 2500 µmos salinities. Crown diameter remained constant by increasing humic acid under 3500 µmos salinity (Table 3). The highest percentage of increase in crown diameter (17.5%) was observed under 2500 µmos salinity and application of 3000 mg/kg humic acid. Application of 1500 and 3000 mg/kg humic acid caused an increase in crown diameter under 2500 and 4500 µmos salinities, respectively (Table 3). There was a negative and significant relationship between salinity and humic acid concentration for the crown diameter. Crown diameter was significantly (P<0.05) decreased by increasing salinity and humic acid levels (Table 11). Gulser *et al.* (2010) revealed that pepper crown diameter increased by application of 4000 mg/kg humic acid. Webb *et al.* (1988) reported that cross-sectional stem area of Hamlin sweet orange and Star Ruby grapefruit increased at all humate rates after 11 and 10 months, respectively. The 1 lb rate was associated with the greatest increase in cross-sectional stem area. Rates less than or greater than 1 lb per tree resulted in smaller increases in cross-sectional areas.

Table 2. Increasing percentage (%) in number of shoot of Mexican lime seedling treated with different levels of humic acid and salinity

Salinity (µmos) Humic acid (mg/kg)	1500	2500	3500	4500
0	$^{b}17.5^{D} \pm 3.06$	$^{a}49.3^{B} \pm 3.30$	$^{a}34.6^{C} \pm 6.51$	$^{a}57.1^{A} \pm 2.92$
1500	$^{b}9.8^{C} \pm 1.90$	$^{b}23.0^{B} \pm 1.74$	$^{ab}28.4^{B} \pm 3.44$	$^{b}37.2^{A} \pm 1.13$
3000	$^{a}35.2^{B} \pm 0.07$	$^{bc}15.4^{C} \pm 0.16$	$^{ab}30.0^{B} \pm 0.00$	$^{a}65.0^{A} \pm 6.12$
4500	$^{b}16.1^{B} \pm 0.04$	$^{c}11.8^{B} \pm 0.31$	$^{b}24.8^{A} \pm 2.45$	$^{c}8.3^{B} \pm 0.68$

Means with same letters have not significant difference according to LSD (P<0.05).
Minuscules are relative to comparison of humic acid levels in each salinity level (column comparison) and capitals are relative to comparison of salinity levels in each humic acid level (row comparison).

Table 3. Increasing percentage (%) in crown diameter of Mexican lime seedling treated with different levels of humic acid and salinity

Salinity (µmos) Humic acid (mg/kg)	1500	2500	3500	4500
0	$^{c}10.8^{B} \pm 0.70$	$^{b}0.0^{C} \pm 0.00$	$^{a}0.0^{C} \pm 0.00$	$^{a}13.3^{A} \pm 1.36$
1500	$^{a}16.7^{A} \pm 0.27$	$^{b}0.0^{C} \pm 0.00$	$^{a}0.0^{C} \pm 0.00$	$^{a}12.8^{B} \pm 0.33$
3000	$^{b}13.7^{B} \pm 0.29$	$^{a}17.5^{A} \pm 0.20$	$^{a}0.0^{C} \pm 0.00$	$^{b}0.0^{C} \pm 0.00$
4500	$^{d}0.0^{A} \pm 0.00$	$^{b}0.0^{A} \pm 0.00$	$^{a}0.0^{A} \pm 0.00$	$^{b}0.0^{A} \pm 0.00$

Means with same letters have not significant difference according to LSD (P<0.05).
Minuscules are relative to comparison of humic acid levels in each salinity level (column comparison) and capitals are relative to comparison of salinity levels in each humic acid level (row comparison).

Table 4. Shoot fresh weight (g) of Mexican lime seedling treated with different levels of humic acid and salinity

Salinity (µmos) Humic acid (mg/kg)	1500	2500	3500	4500
0	$^{b}59.2^{A} \pm 5.74$	$^{a}64.0^{A} \pm 3.74$	$^{b}38.0^{B} \pm 1.83$	$^{b}56.0^{A} \pm 1.63$
1500	$^{a}70.0^{A} \pm 1.63$	$^{ab}59.2^{B} \pm 3.09$	$^{a}62.0^{AB} \pm 4.08$	$^{a}69.0^{A} \pm 3.67$
3000	$^{b}55.2^{A} \pm 4.03$	$^{ab}55.2^{A} \pm 4.11$	$^{b}43.2^{B} \pm 4.19$	$^{ab}62.8^{A} \pm 2.87$
4500	$^{a}73.2^{A} \pm 4.50$	$^{b}50.0^{B} \pm 0.00$	$^{a}53.2^{B} \pm 2.50$	$^{a}66.0^{A} \pm 0.00$

Means with same letters have not significant difference according to LSD (P<0.05).
Minuscules are relative to comparison of humic acid levels in each salinity level (column comparison) and capitals are relative to comparison of salinity levels in each humic acid level (row comparison).

Shoot fresh weight

Shoot fresh weight decreased by increasing humic acid concentrations under 2500 µmos salinity. Under 3500 and 4500 µmos salinities, application of humic acid led to an increase in shoot fresh weight compared to the plant without humic acid treatments (Table 4). The highest shoot fresh weight was observed under 1500 µmos salinity and application of 1500 mg/kg humic acid (70.00 g) and the lowest shoot fresh weight was observed under 3500 µmos salinity without application of humic acid (38.00 g). Shoot fresh weight increased by application of 4500 mg/kg humic acid under 4500 µmos salinity (Table 4).

Shoot dry weight

In the all salinity levels except 4500 µmos, shoot dry weight decreased by increasing humic acid concentration up to 3000 mg/kg. Humic acid had a positive effect on shoot dry weight under 3500 and 4500 µmos salinities. Application of humic acid increased shoot dry weight under salinity stress (Table 5). The highest shoot dry weight was obtained under 4500 µmos salinity and application of 1500 mg/kg humic acid (31.00 g) and the least shoot dry weight was observed under 3500 µmos salinity in the absence of humic acid (16.52 g). Application of 4500 mg/kg humic acid led to an increase in shoot dry weight under 4500 µmos salinity (Table 5). Gulser *et al*. (2010) observed that highest shoot fresh and dry weights of pepper can be obtained by application of 4500 mg/kg humic acid. Khaled and Fawy (2011) reported that the highest dry weight was obtained by application of 0.1% dose of humic acid. Nevertheless, the dry weight was decreased at 0.2% dose of humic acid. They found that although application of NaCl caused a decrease in dry weight, application of soil humus can limit this decrease especially in 60 mM treatment of NaCl. Foliar application of humic acid can also enhance dry weight of plants exposed to NaCl salinities. However, the highest dry weight was obtained with 0.1% of humic acid in NaCl-treated plants.

Table 5. Shoot dry weight (g) of Mexican lime seedling treated with different levels of humic acid and salinity

Salinity (µmos) Humic acid (mg/kg)	1500	2500	3500	4500
0	$^{a}30.4^{A} \pm 2.9$	$^{a}26.8^{AB} \pm 0.2$	$^{c}16.5^{C} \pm 0.1$	$^{b}26.0^{B} \pm 0.4$
1500	$^{b}25.9^{B} \pm 1.4$	$^{b}22.6^{BC} \pm 1.9$	$^{b}21.4^{C} \pm 0.7$	$^{a}31.0^{A} \pm 1.5$
3000	$^{c}17.3^{B} \pm 0.4$	$^{b}20.6^{B} \pm 1.3$	$^{a}26.8^{A} \pm 2.0$	$^{b}25.8^{A} \pm 0.2$
4500	$^{ab}26.8^{AB} \pm 0.1$	$^{ab}23.0^{B} \pm 0.6$	$^{bc}18.8^{C} \pm 0.6$	$^{a}30.7^{A} \pm 3.5$

Means having same letter, have not significant difference according to LSD ($P<0.05$).
Minuscules are relative to comparison of humic acid levels in each salinity level (column comparison) and capitals are relative to comparison of salinity levels in each humic acid level (row comparison).

Table 6. Root dry weight (g) of Mexican lime seedling treated with different levels of humic acid and salinity

Salinity (µmos) Humic acid (mg/kg)	1500	2500	3500	4500
0	b9.8A ± 0.85	a9.2A ± 0.04	c7.5B ± 0.09	b9.0A ± 0.37
1500	b9.8B ± 0.29	a8.7B ± 0.68	b9.8B ± 0.13	a11.1A ± 0.39
3000	c7.6C ± 0.11	a8.2C ± 0.09	a11.2A ± 0.26	b9.7B ± 0.27
4500	a11.7A ± 0.36	a8.7B ± 0.02	c6.6C ± 0.12	b9.8B ± 1.07

Means with same letters have not significant difference according to LSD ($P<0.05$).
Minuscule are relative to comparison of humic acid levels in each salinity level (column comparison) and capitals are relative to comparison of salinity levels in each humic acid level (row comparison).

Root dry weigh

Different concentrations of humic acid caused various effects on root dry weight under each salinity levels. By incresaing salinity levels, root dry weight was increased by application of 1500 and 3000 mg/kg humic acid (Table 6). The highest (11.74 g) and the lowest (6.56 g) root dry weights were observed in plants treated with 4500 mg/kg humic acid under 1500 and 3500 µmos salinities, respectively. Root dry weight was increased by application of 4500 mg/kg humic acid under 4500 µmos salinity treatment (Table 6). It has been also suggested that the positive effects of humic acid application on the negative effects of salinity stress is via enhancement in root system spreading (Behzad-Amiri *et al*., 2009). Gulser *et al*. (2010) found that highest root fresh and dry weights of pepper can be obtained by application of 4000 mg/kg humic acid. When applied to soils, humic acids add essential organic material necessary for water retention; therefore can improve the growth of the roots (Khaled and Fawy, 2011).

Shoot potassium content

In all salinity levels except 4500 µmos, application of humic acid caused an increase in shoot K content. Application of 4500 mg/kg humic acid enhanced shoot K content even though salinity levels increased (Table 7). The greatest shoot K content (1.791%) was observed in plants treated with 1500 mg/kg humic acid under salinity 1500 µmos and the lowest shoot K content (1.087%) obtained under 2500 µmos salinity without application of humic acid. Shoot K content increased by application of 4500 mg/kg humic acid under 4500 µmos salinity (Table 7). There was a positive and significance relationship between humic acid concentration and shoot K content. Shoot K content was significantly increased by higher humic acid concentrations (Table 11). Asik *et al*. (2009) found that application of humus can increase potassium uptake in wheat. Demir *et al*. (1993) revealed that humic acid enhance foliar potassium content in the cucumber plants exposed to salinity stress. Khaled and Fawy (2011) reported that foliar application in 0.1% humic acid can increase amount of K in plants that exposed to 60 mM NaCl treatment when compared with the control and 0.2% humic acid treatment. El-Mohamedy and Ahmed (2009) found that humic acid + phosphorien treatment increases the K content when compared to K content in leaves of untreated plants (control) during two successive years. During the past several years humic acid and /or bio-fertilizers have been promoted by many investigators as plant growth amendments to increase plant nutrient uptake and as a result to improve plant growth (El-Monem *et al*., 2008). Furthermore, Khaled and Fawy (2011) found that foliar application of humic acids can increase the uptake of K. Increase in K content following application of high concentrations of humic acid has been reported in pearl millet (Daur, 2014) and Maize (Daur and Bakhashwain, 2013).

Shoot sodium content

In the all salinity levels except 3500 µmos, application of humic acid caused a reduction in shoot Na content when compared to control treatment. Application of 3000 mg/kg humic acid caused a reduction in shoot Na content under 4500 µmos salinity (Table 8). The highest shoot Na content (1.942%) was observed under 4500 µmos salinity without application of humic acid and the lowest shoot Na content (1.492%) was obtained under 1500 µmos salinity and application of 1500 mg/kg humic acid. Shoot Na content decreased by application of 3000 mg/kg humic acid under 4500 µmos salinity. Among humic acid treatments, application of 1500 mg/kg humic acid had a better influence on the reduction of shoot Na content under salinity 3500 µmos (Table 8). There was a positive and significance relationship between salinity levels and shoot Na content. Conversely, shoot Na content decreased by enhancing humic acid concentration (Table 11). However, Asik *et al*. (2009) reported increase in sodium uptake in wheat by application of humic acid. Khaled and Fawy (2011) found that foliar application of humic acids can increase the uptake of Na. They revealed that the interaction effect between soil humus and NaCl treatment was statistically significant for Na uptake of corn.

Table 7. Shoot potassium content (K %) in Mexican lime seedling treated with different levels of humic acid and salinity

Salinity (µmos) Humic acid (mg/kg)	1500	2500	3500	4500
0	c1.22B ± 0.06	c1.09C ± 0.04	b1.12BC ± 0.02	b1.42A ± 0.11
1500	a1.79A ± 0.04	b1.39B ± 0.01	a1.31B ± 0.01	b1.31B ± 0.02
3000	b1.44B ± 0.03	a1.57A ± 0.03	a1.27C ± 0.02	b1.35BC ± 0.01
4500	b1.54B ± 0.05	b1.38C ± 0.06	a1.27C ± 0.00	a1.70A ± 0.02

Means having same letter, have not significant difference according to LSD ($P<0.05$).
Minuscule are relative to comparison of humic acid levels in each salinity level (column comparison) and capitals are relative to comparison of salinity levels in each humic acid level (row comparison).

Table 8. Shoot sodium content (Na %) in Mexican lime seedling treated with different levels of humic acid and salinity

Salinity (µmos) Humic acid (mg/kg)	1500	2500	3500	4500
0	a1.71B ± 0.00	a1.90A ± 0.04	a1.76B ± 0.06	a1.94A ± 0.04
1500	b1.49B ± 0.06	b1.63A ± 0.02	b1.63A ± 0.06	b1.67A ± 0.06
3000	b1.59B ± 0.02	b1.60B ± 0.04	a1.82A ± 0.05	b1.61B ± 0.00
4500	b1.54A ± 0.05	b1.53A ± 0.00	b1.62A ± 0.01	b1.64A ± 0.01

Means having same letter, have not significant difference according to LSD ($P<0.05$).
Minuscule are relative to comparison of humic acid levels in each salinity level (column comparison) and capitals are relative to comparison of salinity levels in each humic acid level (row comparison).

Shoot chloride content

In all salinity levels, application of humic acid led to a reduction in shoot Cl content when compared to Cl levels in control treatment. Under non-saline condition (EC= 1500 µmos) and 2500 µmos salinity, increasing humic acid concentration reduced shoot Cl content. Application of 4500 mg/kg humic acid led to a reduction in shoot chloride content under 3500 and 4500 µmos salinities. In comparison with control plants, Application of humic acid caused a reduction in shoot chloride contents (Table 9). The highest shoot Cl content (2.979%) was observed under salinity 4500 µmos without application of humic acid and the least shoot Cl content (1.232%) was obtained under salinity 1500 µmos and application of 4500 mg/kg humic acid. Shoot Cl content remained constant after application of 4500 mg/kg humic acid under 4500 µmos salinity rather than 1500 µmos salinity. Application of humic acid caused a reduction in shoot Cl content than non-application of humic acid (Table 9). There was a positive and significance relationship between salinity levels and shoot Cl content. Shoot Cl content decreased by increasing humic acid concentrations (Table 11). It has been reported that humic substances may enhance the uptake of some nutrients, reduce the uptake of toxic elements, and improve the plant responses to salinity. However, there is lack of information regarding humic acids application and its effects on plant salinity tolerance (Khaled and Fawy, 2011).

Root fresh weight

The highest root fresh weight was observed in 4500 µmos salinity and the lowest was observed under 2500 µmos salinity. Root fresh weight was significantly decreased by increasing salinity from 1500 to 2500 µmos, but root fresh weight was increased by increasing salinity level from 2500 to 4500 µmos (Table 10).

Table 9. Shoot chloride content (Cl %) in Mexican lime seedling treated with different levels of humic acid and salinity

Salinity (µmos) Humic acid (mg/kg)	1500	2500	3500	4500
0	a1.82B ± 0.09	a2.62A ± 0.08	a2.19B ± 0.11	a2.98A ± 0.41
1500	ab1.57AB ± 0.14	b1.46B ± 0.01	bc1.79AB ± 0.00	c1.88A ± 0.03
3000	ab1.45C ± 0.00	b1.43C ± 0.07	ab2.12B ± 0.12	b2.57A ± 0.34
4500	b1.23A ± 0.03	b1.46A ± 0.10	c1.41A ± 0.00	c1.49A ± 0.10

Means with same letters have not significant difference according to LSD (P<0.05).
Minuscule are relative to comparison of humic acid levels in each salinity level (column comparison) and capitals are relative to comparison of salinity levels in each humic acid level (row comparison).

Table 10. Effect of salinity levels on root fresh weight

Salinity (µmos)	1500	2500	3500	4500
Root fresh weight (g)	28.62ab ± 1.9	24.62b ± 1.6	27.38ab ± 1.8	31.50a ± 2.0

Means with same letters have not significant difference according to LSD (P<0.05).

Conclusion

Based on the obtained results, application of 3000 and 4500 mg/kg humic acid had a positive effect on improving plant height under 3500 µmos salinity. By increasing salinity, humic acid had no influence on plant height. Plant height significantly increased by application of 3000 and 4500 mg/kg humic acid under non-saline condition. Application of 3000 mg/kg humic acid significantly increased number of shoots under 4500 µmos salinity. Application of 3000 and 1500 mg/kg humic acid caused increase in crown diameter under 2500 and 4500 µmos salinities. No change was observed in the crown diameter under 3500 µmos salinity. Shoot fresh and dry weights, root dry weight and shoot potassium content were increased by application of 4500 mg/kg humic acid under 4500 µmos salinity. Application of 3000 mg/kg humic acid decreased shoot sodium content under 4500 µmos salinity. Application of 1500 mg/kg humic acid had a significant influence on reduction of shoot sodium content under 3500 µmos salinity. Shoot Cl content remained constant under 4500 µmos salinity in comparison with 1500 µmos salinity following application of 4500 mg/kg humic acid. Application of humic acid kept shoots Cl content lower than non-application of humic acid. Finally, in the present study, application of 3000 and 4500 mg/kg humic acid could be a useful tool to decrease detrimental effects of salinity and to reduce uptake of toxic ions such as sodium and chloride.

Table 11. Correlation coefficient between salinity, humic acid and the measured parameters

	Salinity	Humic acid	Height increasing	shoot no. increasing	Crown diameter increasing	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	K	Na	Cl
Salinity	1											
Humic acid	0.000	1										
Height increasing	-0.104	-0.085	1									
shoot no. increasing	0.467**	-0.402**	0.146	1								
Crown diameter increasing	-0.255*	-0.281*	-0.109	-0.058	1							
Shoot fresh weight	-0.117	0.082	-0.260*	-0.096	0.155	1						
Root fresh weight	0.162	-0.039	-0.280*	0.107	-0.081	0.386**	1					
Shoot dry weight	0.157	-0.063	-0.351**	-0.039	0.050	0.492**	0.421**	1				
Root dry weight	0.036	0.024	-0.309*	-0.063	-0.051	0.444**	0.554**	0.802**	1			
K	-0.154	0.416**	-0.193	-0.471**	0.384**	0.390**	0.011	0.158	0.171	1		
Na	0.380**	-0.540**	0.333**	0.534**	-0.069	-0.333**	0.026	0.141	0.042	-0.473**	1	
Cl	0.449**	-0.552**	0.046	0.675**	-0.014	-0.180	0.054	0.137	0.050	-0.328**	0.644**	1

*, ** significant at $P<0.05$ and $P<0.01$, respectively.

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