

## The Effects of Humic Acid and Calcium on Morpho-Physiological Traits and Mineral Nutrient Uptake of Pistachio Seedling under Salinity Stress

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Received: 2 April 2016

Accepted: 20 July 2016

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### Abstract

The study was carried out to evaluate the effects of soil application of humic acid and calcium on morpho-physiological traits and Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup> uptake of Pistachio seedling (Akbari) grown under salt stress. A completely randomized design was used with four replications in greenhouse conditions. The experimental treatment consisted of four levels of humic acid (Bis humic) (0, 4, 8 and 12 gr kg<sup>-1</sup> soil) and anti-salt as a source of Ca<sup>+2</sup> (0, 1, 2 and 3 gr kg<sup>-1</sup> soil). Results indicated that humic acid and anti-salt significantly increased vegetative growth, such as root fresh and dry weight, stem diameter, leaf number and leaf area, of pistachio seedling in comparison with the control group (p<0.05). However, no significant differences in fresh and dry weight of shoot of pistachio seedling were detected in all treatments except in humic acid 8 gr kg<sup>-1</sup> soils. The results indicated that the application of humic acid and anti-salt decreased Na<sup>+</sup> absorption in pistachio seedling in all experimental treatments (p<0.05). No significant differences in Ca<sup>+2</sup> and Mg<sup>+2</sup> absorption were detected in all treatments. Results showed a significant reduction of the electrical conductivity (EC) and sodium adsorption ratio (SAR) in all levels of humic acid and anti-salt in soil. However, in these treatments, there were no statistically significant differences in the pH. The best concentration of humic acid and anti-salt was 8 gr kg<sup>-1</sup> soil and 3 gr kg<sup>-1</sup> soil, respectively. This study revealed the relative importance and efficiency of humic acid and anti-salt to salinity stress tolerance in pistachio seedling.

**Keywords:** Calcium, Humic acid, Pistachio, Salinity, Sodium adsorption ratio.

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### Introduction

Salinity is one of the most important problems that restrict cultivation of crops in arid and semi-arid regions (Ashraf and Foolad, 2007). The most important problem facing the economic crop production in arid regions are high concentration of ions, especially NaCl present in either soil or water (Ozdener and Kutbay, 2008; Wang *et al.*, 2003; Mostafazadeh- Fard *et al.*, 2007; Moeinrad, 2008). Calcium has been reported to inhibit Na<sup>+</sup> uptake and thereby reduce its adverse effect on seed germination (Bonilla *et al.*, 2004; Nayyar, 2003, Sabir & Ashraf, 20 07) as well as the increase plants growth (Munns, 2002; Tobe *et al.*,

2001). There was a competition between Na<sup>+</sup> and Ca<sup>+2</sup> ions to enter the cell membrane. Therefore, it has been claimed that higher calcium levels in soil protect cell membrane from the negative effects of salinity (Türkmen *et al.*, 2004). Natural and technological methods have been studied in recent years to reduce salt damage in agricultural products (Walker and Bernal, 2008). Until now, the beneficial effects of humic substances have been mentioned. Recent literature has shown that humic substances as the major component of soil organic matter could be used as a growth regulator to regulate hormone levels, improve

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plant growth, micronutrient uptake, biochemical processes in plants (respiration, photosynthesis and chlorophyll content) and enhance stress tolerance (Pena-Mendez *et al.*, 2005; Delfine *et al.*, 2005; Sangeetha *et al.*, 2006; Pan *et al.*, 2009; Cimrin *et al.*, 2010; Khaled and Fawy, 2011). Moghaddam and Soleimani (2012) studied compensatory effects of humic acid on physiological characteristics of pistachio seedlings (*Pistacia vera*) under salinity stress. Results showed that humic acid treatments increased shoot growth under salinity stress. Dobbss *et al.*, 2010 studied bioactivity of chemically transformed humic matter from vermincompost on plant root growth. Results showed that lateral root emergence increased and H<sup>+</sup>-ATPase activity in root vesicles increased. Jindo *et al.*, 2012 studied root growth promotion by humic acids from composted and non-composted urban organic wastes. Results showed that root growth increased, the number of mitotic sites on roots increased and the number of mitotic sites and proton pump activity in roots increased. Tahir *et al.*, 2011 studied lignite-derived humic acid effect on growth of wheat plants in different soils. Results showed that plant height and dry weight of roots and shoots increased and the uptake of N was enhanced. El-Nemr *et al.*, (2012) investigated the response of growth and yield of cucumber plants (*Cucumis sativus* L.) to different foliar applications of humic acid and bio-stimulators. The results showed that plant growth and yield increase and the uptake of Nitrogen, Phosphate, K<sup>+</sup>, Ca<sup>+2</sup> and Mg<sup>+2</sup> were enhanced. Due to the positive effect of humic substances on the visible growth of plants, humic acid is a promising natural resource that can be used as an alternative to synthetic fertilizers to increase crop production. Pistachio (*Pistacia vera* L.), one of the most important horticultural products of Iran, has been planted widely in the province of Kerman (Talaie & Panahi, 2002). Although it is classified as a salt-tolerant crop, salinity stress causes accumulation of sodium and chloride in trees, leading to a reduction in growth, photosynthesis and also caused morphological changes in the plants (Ferguson *et al.*, 2002). Humic acid is one of the ben-

eficial strategies to improve yield in saline fields (Delfine *et al.*, 2005). Therefore, the present study was conducted to evaluate the effects of soil application of humic acid and anti-salt on morphophysiological traits and element uptake of pistachio seedling (Akbari) grown under salinity stress.

## Materials and Methods

The greenhouse experiments were carried out to determine the effect of different levels of humic acid used in soil on the morpho-physiological traits and uptake of Na<sup>+</sup>, Mg<sup>+2</sup> and Ca<sup>+2</sup> ions of pistachio seedling (Akbari) during the 2012-2013 growing season in Iran, Rafsanjan (Elevation 1469 m, Latitude East 56, Longitude North 30). The soil used for this study was collected from 0–30 cm depth of the pistachio orchards located in Rafsanjan. Soil was air-dried and passed through of 4 mm sieve. The salinity factors of the soil in the experimental treatment were pH: 7.55 and EC: 38dSm<sup>-1</sup>. About 2 kg of the soil placed into clay pots and then, various concentrations of humic acid (0, 4, 8 and 12 gr kg<sup>-1</sup> soil) and anti-salt commercial matter as a source of humic acid and calcium (0, 1, 2 and 3 gr kg<sup>-1</sup> soil) were added to the pots and mixed into the soil. Before planting, pistachio seeds were sterilized with 10% sodium hypochlorite for 10-12 minutes three times and washed in each stage and soaked in distilled water for 24 hours. Seeds of pistachio, cultivar Akbari were sown in experimental pots in the greenhouse condition. For leaching and saline soil reclamation, pots were irrigated with municipal water every two days (approximately 300 ml). After germination, the seedling pots were irrigated every four days. After three months of vegetative growth, the plants were harvested. Morphological indices such as length of shoot, stem diameter, leaf number and leaf area (Leaf Area Meter, AM200, ADC, Bioscientific LTD, UK) were recorded. Plant samples dried at 65°C and fresh and dry weight of root and shoot were determined. In order to determine absorption of the element, the plant samples were dry digested by using of HCl (Ryan *et al.*, 2001). The Mg<sup>+2</sup>, Na<sup>+</sup> and Ca<sup>+2</sup>

ions were determined by flame emission (APHA, 1992). After the harvesting stage, the electrical conductivity (EC meter) and pH value (pH meter) (Richards, 1954) were determined.

The formula to calculate sodium absorption ratio (SAR) is given below, with concentrations expressed in milliequivalents per liter (meq/L) analyzed from a saturated paste soil extract.

$$SAR = [Na^+] / \sqrt{([Ca^{+2}] + [Mg^{+2}]) / 2}$$

**Statistical analysis**

The experiment was conducted in a completely randomized design (CRD) with four replications. Data were analyzed using MSTATc software. Mean comparisons were grouped by Duncan’s multiple range tests at the probability level of 5 percent.

**Results**

**The effect of Anti-salt and humic acid on the growth of pistachio seedlings**

In this study, the effect of anti-salt and humic acid on height, stem diameter, fresh and dry weight of shoot and root were statistically significant at a 5% level (Table1). The comparison of means by the Duncan's method showed that the fresh and dry weight of root increased by increasing in concentration of anti-salt (Fig. 1). Humic acid application resulted in a significant increase on growth plant. The comparison of means by Duncan's method did not show any significant effect on the fresh and dry weight of shoot by increasing in concentration of anti-salt (Fig. 2). Humic acid application significantly increased fresh and dry weight of shoot compared to all treatments.

**Table 1. The effect of humic acid and Anti-salt on growth parameters of pistachio seedlings: results of ANOVA**

Means Square								
Source of variance	df	Number of leaves	Stem diameter	Fresh weight of root(g)	Dry weight of root (g)	Fresh weight of shoot(g)	Dry weight of shoot(g)	Leaf area (cm <sup>2</sup> )
Treatment	6	158.857*	1.811*	0.606*	0.201*	0.678*	0.237*	0.000*
Error	21	43.250	1.581	6.260	2.018	4.760	1.130	0.001
Total	-	202.107	3.392	6.866	2.229	5.438	1.386	0.001

Significant F-test at \*\*P < 0.01, at \*P < 0.05 and non-significant (NS)

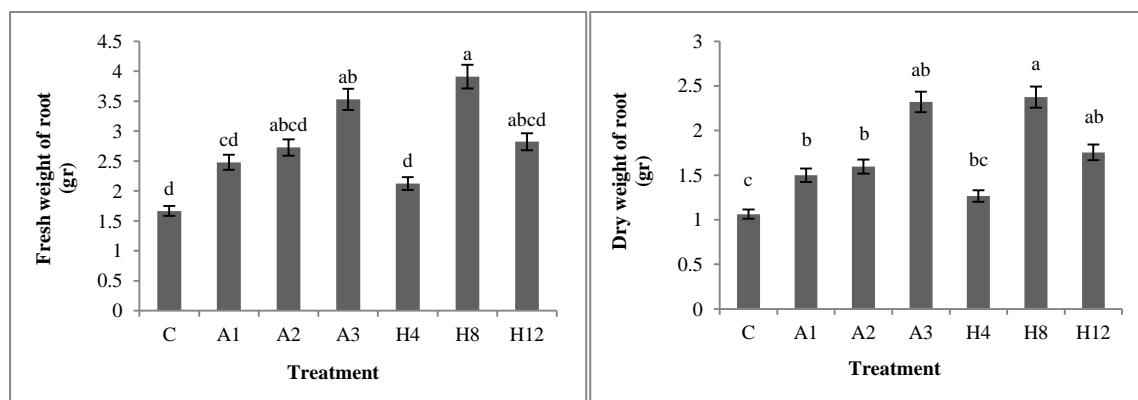


Fig. 1. The effect of humic acid and Anti-salt on weight of root and shoot on pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

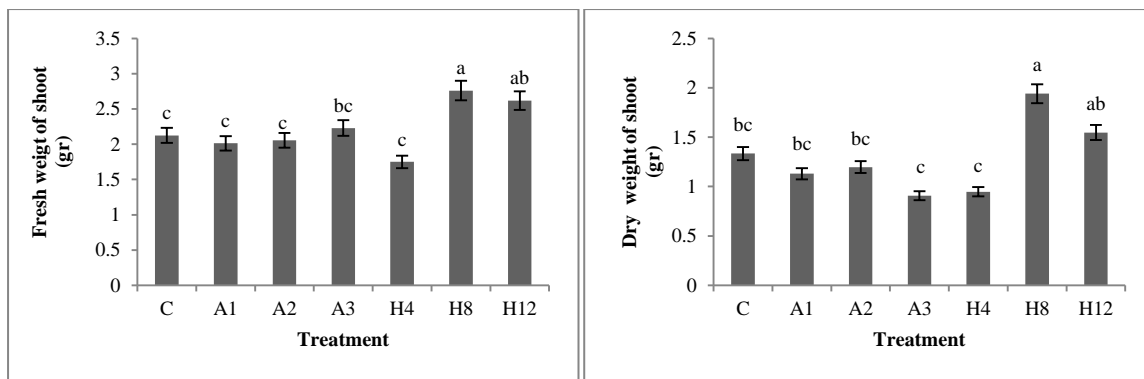


Fig. 2. The effect of humic acid and Anti-salt on weight of root and shoot on pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

The comparison of means by Duncan's method showed that the plant height decreased in the control group. The highest effect of anti-salt and humic acid

treatments on plant height were include 2gr and 8, 12, respectively (Fig. 3).

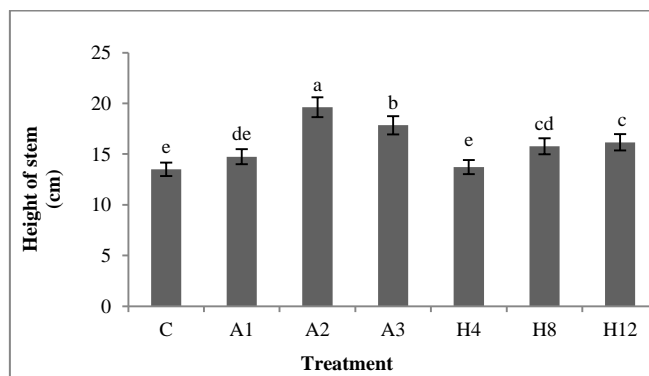


Fig. 3. The effect of humic acid and Anti-salt on plant height in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

The application of anti-salt significantly increased stem diameter, and the maximum diameter was in 3gr. However, in humic acid treatments didn't show any significant differences compared to the control group (Fig. 4). Similar observations were also reported by

zucchini (Mora *et al.*, 2010), tomato (Adani *et al.*, 1998; Lulakis and Petsas., 1995), wheat (Tahir *et al.*, 2011), corn (Eyheraguibel *et al.*, 2008) and pepper (Cimrin *et al.*, 2010).

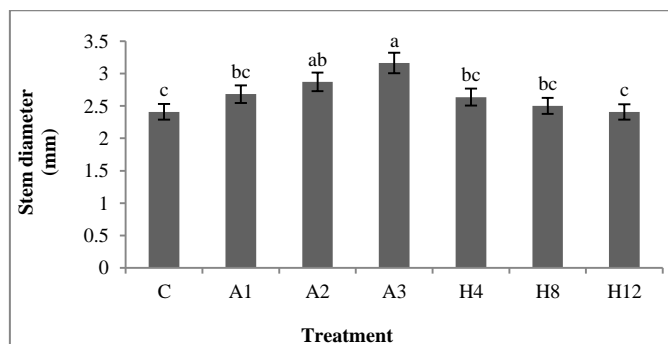


Fig. 4. The effect of humic acid and Anti-salt on stem diameter in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test).

Results showed that anti-salt and humic acid treatments on leaf number and leaf area in pistachio seedlings were statistically significant at the 5% level (Table 1). The comparison of means by the Duncan's

method showed that the application of humic acid and anti-salt increased the number of leaves in all treatments (Fig. 5).

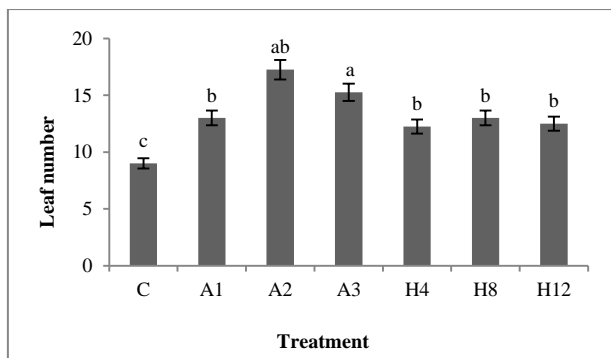


Fig. 5. Effect of Anti-salt and humic acid on leaf number in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

However, leaf area didn't show any significant differences compared to control except in humic acid 8 grams (Fig. 6).

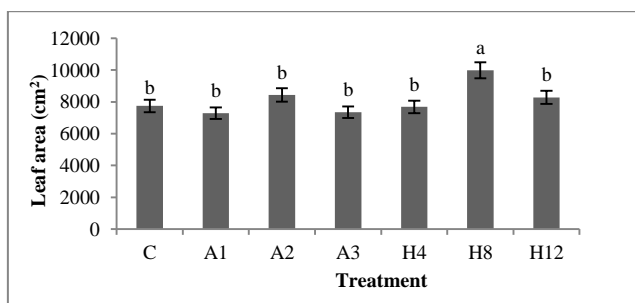


Fig. 6. Effect of Anti-salt and humic acid on leaf number and leaf area in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

**The effect of anti-salt and humic acid on the Na<sup>+1</sup>, Mg<sup>+2</sup>, Ca<sup>+2</sup> uptakes of pistachio seedlings**

The results of the ANOVA showed that the sodium, potassium and calcium uptake of humic acid and anti-salt treatments were statistically significant at the 5% level (Table 2). The comparison of means by the Duncan's method showed that with increasing the

concentration of anti-salt, sodium uptake in shoot significantly decreased. In Fig. 7 the effect of anti-salt and humic acid on sodium uptake in pistachio seedlings have been shown.

**Table 2. The effect of humic acid and Anti-salt on Mg<sup>+2</sup>, Na<sup>+</sup>, Ca<sup>+2</sup> uptake, pH and EC of pistachio seedlings: results of ANOVA.**

Means Square						
Source of variance	DF	Mg <sup>+2</sup>	Na <sup>+1</sup>	Ca <sup>+2</sup>	Ec	SAR
Treatment	6	0.118 <sup>ns</sup>	1.422*	0.838 <sup>ns</sup>	80.260*	242.693*
Error	21	0.666	0.236	2.711	2.986	5.081
Total	27	0.783	1.658	3.550	83.246	247.774

Significant F-test at \*\*P < 0.01, at \*P < 0.05 and non-significant (NS)

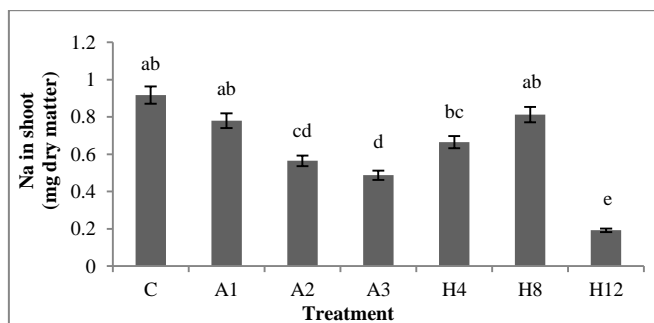


Fig. 7. Effect of Anti-salt and humic acid on sodium uptake in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

The comparing mean by Duncan's method showed that with increasing the concentration of anti-salt and humic acid, magnesium uptake in shoot did not show any statistically significant difference in all treatments (Table 2). The results of the ANOVA didn't show any significant differences in all treatments compared to control.

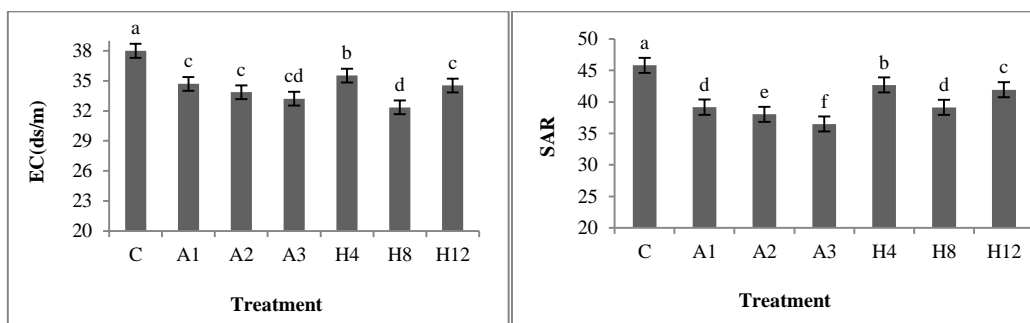


Fig. 8. Effect of Anti-salt and humic acid on EC and SAR  
Means followed by same letter are not significantly different at 5% probability using Duncan's test)

The results from the analysis of variance showed that humic acid and anti-salt had a statistically significant difference in electrical conductivity (EC) and sodium adsorption ratio (SAR) at the 5% level (Table 2). The use of anti-salt and humic acid did not show any significant effect on the pH compared with the control treatments. As the results showed, the electrical conductivity in all treatments compared to the control significantly reduced. Minimum EC of humic acid and anti-salt treatments were recorded in 8 gr and 3 gr, respectively. As well as electrical conductivity, the sodium adsorption ratio showed a significant decrease in all treatments. The anti-salt treatments showed a greater reduction compared to humic acid treatments (Fig. 8).

## Discussion

This study showed a significant reduction in the growth of pistachio seedlings. Many researchers have reported that the treatment of low levels of NaCl reduced the dry weight of experimental plants (Ashraf *et al.*, 2008; Shariat jafari *et al.*, 2009). The results obtained in our study were similar to these finding. Humic acid and anti-salt application resulted in a significant increase on growth plant. According to the results, increase in the humic acid and anti-salt level

## The effect of Anti-salt and humic acid on EC and SAR after harvesting in treated soil

Changes in pH, electrical conductivity (EC) and sodium adsorption ratio (SAR) in treated soil after harvesting with humic acid and anti-salt are shown in Fig. 8.

gradually increased the growth, expressed as fresh and dry weight of root, plant height and stem diameter. These results are in agreement with those obtained by Canellas *et al.*, 2011; Adani *et al.*, 1998 (tomatoe), Tahir *et al.*, 2011; Peng *et al.*, 2001(wheat), Jindo *et al.*, 2012; Canellas *et al.*, 2009; Eyheraguible *et al.*, 2008; Canellas *et al.*, 2002 (corn), Cimrin *et al.*, 2010 (pepper), Dobbss *et al.*, 2010; Canellas *et al.*, 2010 (Arabidopsis), Costa *et al.*, 2008 (ornamental plants). They reported humic acid significantly increased secondary roots and root growth. In general, some of the humic acid increased root length, while others are increasing root density. Root growth was significantly related to the hydrophobic humic acids, and these features, especially for plant adaptation to various conditions, including salinity of the soil, was important (Romheld and Neumann, 2006). The results indicated that humic acid increased resistance to salinity. Similar results were obtained by Zidan *et al.*, 1990; Garcia *et al.*, 2000. Moghaddam and Soleimani, 2012 reported that humic acid improved the negative effects of plant growth as a result of irrigating trees with water in a ratio of sodium chloride on the pistachio (*Pestacia vera*). This was related to a reduction of accumulation of proline and the level of abscisic acid in humic acid treatment compared to the control.

Aydin *et al.*, 2012 studied different concentrations of salt and humic acid on common beans. Results showed that humic acid affected saline soil, reduced electrical conductivity into soil and proline leakage from the plant and increased resistance to salinity. A similar observation was obtained by Mohamed, 2012 on maize. Cimrin *et al.*, (2010) showed that humic acid treatments had positive effects of the root growth, changes in mineral absorption and decreased membrane damage and thus, improving salt tolerance in plants. The amount of calcium can reduce the harmful effects of high salinity on pistachio trees and calcium alone increased the growth parameter under salt stress in plants. This was also reported by Hojjat nooghi and mozafari (2012), Zidan *et al.*, (1990). Salt stress leads to morphological changes in smaller leaves, reduces the number of openings and changes the mesophilic surface (Javed *et al.*, 2000; Manns, 2002; Ranjbar *et al.*, 2002). In research by Ghorbani *et al.*, (2010), humic acid (3500 and 4500 grams per hectare) increased durability, leaf area and economic performance on the corn. In addition, Albayrak and Camas (2005) reported that treatment with 1200 mg.l increased leaf area. In this study, sodium, potassium and calcium ions uptake was affected by humic acid and anti-salt treatments. Reducing sodium absorption by the use of humic acid in various plants has been reported (Sanchez- Sanchez *et al.*, 2006; Cirmin *et al.*, 2010). Calcium reduced the harmful effects of salinity on the growth of plants and is known to increase potassium. Calcium can reduce the sodium content in plant tissues (Dabuxilatu ikeda, 2005). A research conducted on pistachio and almond seedlings of two cultivars of Qazvin and Zarand under 150 mM NaCl and different concentrations of calcium sulfate showed calcium reduced the concentration of sodium in the roots and leaves of pistachio (Tavallali *et al.*, 2008), which are consistent throughout the reports on the anti-salt. Competition between  $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$  for uptake by crops has been reported. The link on the plasma membrane surface in roots was less likely to bond with hydrated magnesium than calcium. The

high concentration of calcium in the leaves increased calcium and reduced magnesium concentration (Bernstein, 1975). Bernstein *et al.*, (1974) studied six plants in salinity stress (sodium chloride and calcium total), and the results showed that magnesium concentration was reduced in plants, though this has been not thoroughly studied. The results of this study didn't show any significant differences in all treatments compared to control. Humic acid as chelate can hold sodium ions and prevent their absorption by the plant (Pena-Mendez *et al.*, 2005; Wang *et al.*, 2001). Calcium plays an important role in crop production, especially in terms of salinity stress. According to many researchers, there is competition between sodium and calcium ions to enter the cell membrane and calcium protects the cell membrane from the negative effects of salt (Cramer *et al.*, 1986; Lynch & Lauchli, 1988; ashraf, 2004; Mahmood, 2009).

Soil salinity is a serious environmental problem on plant growth and yield. Soil salinity is measured as the salt concentration of the soil solution in terms of g/l or electric conductivity (EC) in dS/m. High SAR reduces water infiltration in the soil. Hence, we have to decrease SAR with fertilizers and materials, which have a positive effect on the reduction in SAR value. Sabzevari *et al.*, (2009) stated that a very small amount of organic acids significantly improved the effects of physical, chemical and biological soil and due to beneficial hormone compounds effect of increasing yield and improving the quality of agricultural products. The results of this study showed that humic acid and anti-salt reduced the adverse effects of salinity on pistachio seedling. Application of humic acid and anti-salt had a significant effect on plant growth parameters, including, root dry weight, height and diameter, number leaf and leaf area and caused them to increase. Humic acid and anti-salt significantly reduced sodium absorption and did not show any significant uptake of magnesium and calcium. In general, the best concentration of humic acid and anti-salt used in this experiment was 8 g  $\text{kg}^{-1}$  of soil and 3g  $\text{kg}^{-1}$  soil, respectively.

## Acknowledgements

Special thanks to Pistachio Research Center in Rafsanjan for financial support of this research.

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