Effect of Different Levels of Humic Acids on the Nutrient Content, Plant Growth, and Soil Properties under Conditions of Salinity

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Abstract: In this study, the effects were investigated of salinity, foliar and soil applications of humic substances on the growth and mineral nutrients uptake of Corn (Hagein, Fardy10), and the comparison was carried out of the soil and foliar applications of humic acid treatments at different NaCl levels. Soil organic contents are one of the most important parts that they directly affect the soil fertility and textures with their complex and heterogenous structures although they occupy a minor percentage of the soil weight. Humic acids are an important soil component that can improve nutrient availability and impact on other important chemical, biological, and physical properties of soils. The effects of foliar and soil applications of humic substances on the plant growth and some nutrient elements uptake of Corn (Hagein, Fardy10) grown at various salt concentrations were examined. Sodium chloride was added to the soil to obtain 20 and 60mM saline conditions. Solid humus was applied to the soil one month before planting and liquid humic acids were sprayed on the leaves twice on 20th and 40th day after seedling emergence. The application doses of solid humus were 0, 2 and 4 g/kg and those of liquid humic acids were 0, 0.1 and 0.2%. Salinity negatively affected the growth of corn; it also decreased the dry weight and the uptake of nutrient elements except for Na and Mn. Soil application of humus increased the N uptake of corn while foliar application of humic acids increased the uptake of P, K, Mg,Na,Cu and Zn. Although the effect of interaction between salt and soil humus application was found statistically significant, the interaction effect between salt and foliar humic acids treatment was not found significant. Under salt stress, the first doses of both soil and foliar application of humic substances increased the uptake of nutrients.

Keyword: humic acids; nutrient content; plant growth; salinity, soil properties

In recent years, many advances have been achieved towards improvement on the quality and quantity in agriculture. The advances and development in agriculture depend not only on mechanisation and new hybrid seeds but also on the improvement on the soil properties which also help to increase the crop productivity. Unsuitable soil conditions for the plant development generally arise from the lack of organic contents in the soil. To solve this problem, humic substances have started to be given to the soil in Egypt and in other parts of the world as well to improve the crop yield. For example, humic and fulvic acids preparations have been commonly used in many greenhouses. Majority of them are produced domestically although some of them are imported.

The effect of organic matter on the soil properties such as physical, chemical, and biological ones has been well known for a long time. The organic matter content of soil in Turkey is generally low (Eyupoglu 1998). Soil organic matter contains residues of plants and animals and primary and high polymer organic compounds formed by their decomposition. Soil organic matter has not certain chemical formula due to its dynamic structure.Soil organic matter mainly consists of humic and fulvic acids which are called humin materials (Schnitzer 1982; Andriesse 1988). They are mainly produced from nitrogenous compounds containing decomposed amino acids and aromatic complexes (Andriesse 1988). Those organic complexes affect the soil properties and physiological properties of plants due to carboxyl ($COOH^-$) and phenolic (OH^-) groups (Lee & Barlett 1976; Schnitzer 1992). It was reported that humic acids affect physical and chemical properties of soils (Vaughan & Linehan 1976; Boyle *et al.* 1989; Schnitzer 1992). However, their affects on soil have not yet been clearly elucidaded.

In many studies, humic and fulvic acids preparations were reported to increase the uptake of mineral elements (Maggioni *et al.* 1987; De Kreij & Basar 1995; Mackowiak *et al.* 2001), to promote the root length (Vaughan & Malcolm 1979; Canellas *et al.* 2002), and to increase the fresh and dry weights of crop plants (Kauser *et al.* 1985; Chen *et al.* 2004a, b). Due to the positive effect of humic substances on the visible growth of plants, these chemicals have been widely used by the growers instead of other substances such as pesticides etc. This, however, has led to growers using higher-amounts of these substances.

The plant growth and yield are reduced in salt-affected soils because of the excess uptake of potentially toxic ions (Grattan & Grieve 1999). Soil salinity is characterised by high amounts of Na^+, Mg^{+2}, Ca^{+2}, Cl^-, HCO_3^-, SO_4^{-2}, and B ions which have negative effects on the plant growth. Generally, NaCl causes salt stress in nature. The general effect of soil salinity on plants is called the physiological drought effect. A high salt content decreases the osmotic potential of the soil water and, consequently, this reduces the availability of the soil water for plants. Briefly, the uptake of water by plant roots is limited by increased amounts of Na and Cl. Eventually; high salt concentrations in the soil reduce the absorption of nutrients by plants which negatively affects the fertility of the soil.

MATERIAL AND METHODS

The soil used in this study was collected from 0–20 cm depth of the field located in 6th October goveronate. Some physical and chemical properties of the soil were determined; the texture was determined using the pipette method. pH and EC were measured in 1:2.5 water extract, lime was determined according to Richards (1954). Organic matter content was analyzed according to the modified Walkley-Black method (Nelson & Sommers 1982). Total nitrogen was determined by means of Buchi K-437/K-350 digestion/distillation unit according to the Kjeldahl method (Bremmer 1965). Available P was determined with Shimadzu UV 1208 model spectrophotometer according to the Watanabe and Olsen (1965). Exchangeable cations (Na, K, Ca and Mg) were extracted with ammonium acetate at pH 7.0 (Jackson 1973) and determined using Eppendorf Elex 6361 model Flame photometer. Available Fe, Cu, Zn, Mn were extracted with DTPA (0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA pH 7.3) (Lindsay & Norwell 1978) and determined with Philips PU9200x model Atomic Absorption Spectrophotometer. Some chemical and physical properties of the soil used in the research are shown in Table 1. The soil used in the experiment was sandy clay and of neutral pH. It was low

Table 1. Some chemical and physical properties of the soil studied (6th October goveronate)

Physical properties (%)	
Coarse sand	10.6
Fine sand	34.55
Silt	15.22
Clay	39.63
Texture	sandy clay
Chemical properties	
pH (–)	8.1
Electric conductivity (dS/m)	0.25
Organic matter (%)	0.15
CEC (cmol/kg)	55.0
P (mg/kg)	3.2
N (%)	0.01
$CaCO_3$ (%)	0.25
Water soluble ions (me/l)	
Ca	0.15
Mg	0.01
Na	0.05
K	0.04
HCO_3	0.15
Cl	0.1
SO_4	0.02
Available nutrients (ppm)	
Fe	0.44
Cu	0.25
Zn	0.15
Mn	0.05

CEC – Cation exchange capacity

in terms of lime, salt, and organic matter contents. The soil was not adequate in terms of nitrogen, phosphorus, and zinc. The experiment was conducted in a greenhouse in completely randomised factorial design with three soil application doses of humus 0 (control), 2, and 4 g/kg, three foliar application doses of humic acids (0, 0.1, and 0.2%) and three NaCl doses 0 (control), 20, and 60mM. Each application consists of three replications. The soil applied humus was obtained from solid Leilihumus (70% w/w, pH 5.17, EC: 4.80 mS/cm) and foliar applied humic acid was obtained from liquid Leilihumat (humic acid: 15% w/v, pH 10.66, EC: 28.8 mS/cm). Air-dried soil samples were passed through 2 mm sieve. For solid humus applications, Leilihumus was put into a large bowl according to the application doses and the total weight of the soil was adjusted to 7 kg.The mixture was homogenised and put into polyethylene covered plastic pots. For foliar humic acid applications, 7 kg of soil was put into polyethylene covered plastic pots. NaCl was added to the pots both for the soil and foliar treatments according to the application doses. The pots were exposed to the incubation period of 30 days. As a basal fertilizer, nitrogen (100 mg/kg as NH_4NO_3), phosphorus (80 mg/kg), potassium (100 mg/kg as KH_2PO_4), and zinc (0.5 mg/kg as $ZnSO_4$) were applied to the pots before planting. The corn (Hagein, Fardy10) cultivar was grown in pots of 20 cm diameter and 18 cm hight. All pots were irrigated with deionised water during the experiment. Leilihumat was sprayed twice in 5 l of deionised water, 20 and 40 days after seedling emergence, same as with the foliar treatment. After two months vegetation period, the plants were harvested, dried at 65°C, dry weights were determined, and the plant samples were wet digested by using HNO_3 + $HClO_4$ (4:1) mixture. Nitrogen was determined by the Kjeldahl method (Bremmer 1965) (Buchi K-437, K-350), P was determined by the Vanadomolybdophosphoric method (Kacar & Intal 2008) (Shimadzu UV 1208), K, Na, and Ca were determined by flame emission (Horneck & Hanson 1998) (Ependorf Elex 6361), and Mg, Fe, Mn, Zn, and Cu nutrients were determined by atomic absorption spectrometry (Hanlon 1998) (Philips PU 9200x, Pye Unicam Ltd. GB). All data obtained were subjected to statistical analysis. This analysis was performed using Tarist, a statistical software (Tarist 1994), and mean values were grouped with LSD multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Humic acids are technically not a fertiliser, although in some walks people do consider it. Humic acids are an effective agent to use as a complement to synthetic or organic fertilisers. In many instances, regular humic acids use will reduce the need for fertilisation due to the soil and plant ability to make better use of it. In some instances, fertilisation can be eliminated entirely if sufficient organic material is present and the soil can become self sustaining through microbial processes and humus production.

Effects of NaCl on the plant growth and nutrients uptake: As mentioned above, one way the plant growth can be improved is through the structural improvement of sandy clay soil allowing for a better root growth development. The plant growth is also improved by the ability of the plant to uptake and receive more nutrients. Humic acids are especially beneficial in freeing up nutrients in the soil so that they are made available to the plant as needed. For instance, if an aluminium molecule is bound with one of phosphorus, humic acids detach them making the phosphorus available for the plant. Humic acids are also especially important because of their ability to chelate micronutrients, thus increasing their bio-availability. According to the analysis results, the application of 20mM NaCl increased the dry weight, N, P, K, Ca, Mg, Fe, Cu, and Mn contents of the plants, but the amounts decreased with the application of 60mM NaCl (Figure1). Particularly, the effect of NaCl application at a dose of 60mM had a negatively significant effect on the dry weight and mineral elements uptake of corn.

Effects of soil application of humus on the plant growth and nutrients uptake: The soil applications of humus had a significant effect on the uptake of N in corn. When compared with the control treatment, the dry weight and mineral nutrients uptake of corn were found higher at both application doses of humus (Figure 2). The highest dry weight and nutrients uptake were obtained with 2 g humus/kg treatment. The dry weight and nutrients uptake were negatively affected by the application of 4 g humus/kg.

Effects of foliar application of humic acid on the plant growth and nutrients uptake: Foliar applications of humic acid had a significant effect on the dry weight and mineral elements uptake in Corn (Figure 3). When compared with the control

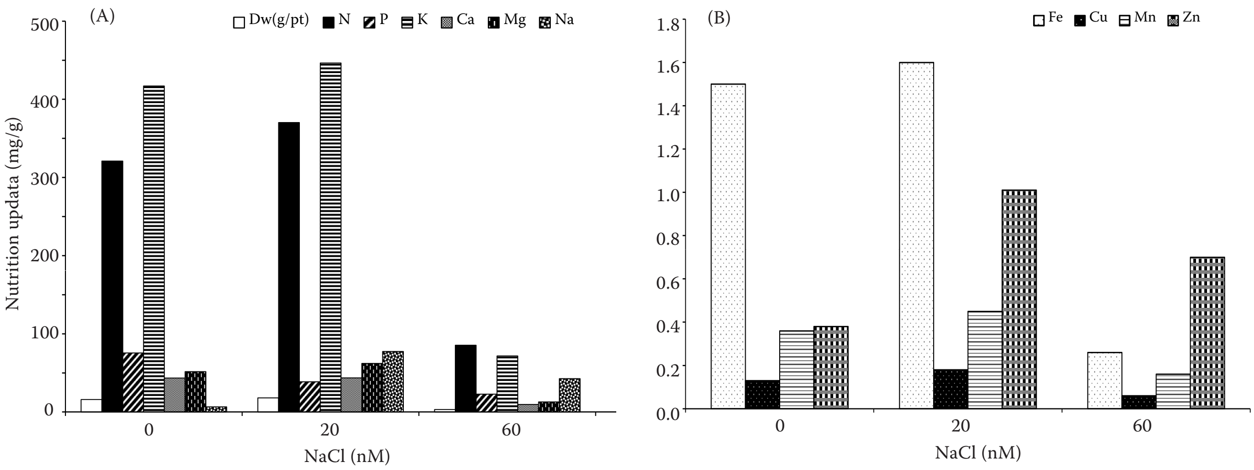

Figure 1. Effect of NaCl on dry weight and plant nutrients uptake

treatment, the dry weight and the uptake of nutrients were found higher on humic acid applications. Foliar application of humic acid affected the uptake of P which was statistically significant in the uptake of Na, K, Cu, and Zn. However, its amounts were not found statistically significant with other nutrients. The highest dry weight and nutrients uptake were obtained with 0.1% dose of humic acid. Nevertheless, the dry weight and nutrients uptake were decreased at 0.2% dose of humic acid, but the amounts except for Fe, Cu, and Mn were found higher than in the control.

Effects of interaction between humic substances and NaCl treatment: When applied to clay soils, humic acids can help break up compacted soils, allowing for enhanced water penetration and better root zone growth and development. When applied to sandy soils, humic acids add essential organic material necessary for water retention, thus improving the root growth and enhancing the sandy soil ability to retain and not leach out vital plant nutrients. The effects of foliar and soil applications of humic substances on the growth and mineral nutrients uptake and their interactions with salt doses are presented in Table 2. The interaction effect between soil humus and NaCl treatment was found statistically significant as to Mn, Zn, and Na uptake of corn. The interactions in dry weight and the interaction of the amount of N, P, K, Ca, Mg, Fe and Cu in wheat were found to be insignificant. Although the application of NaCl decreased the dry weight and nutrients uptake, humus soil application of soil humus limited the decrease especially with 60mM treatment of NaCl; it was found that the soil application of humus generally enhanced the uptake of plant nutrients, especially with 60mM NaCl treatment. This is seen when compared with the other applications (Figure 3).The interaction effect between the foliar

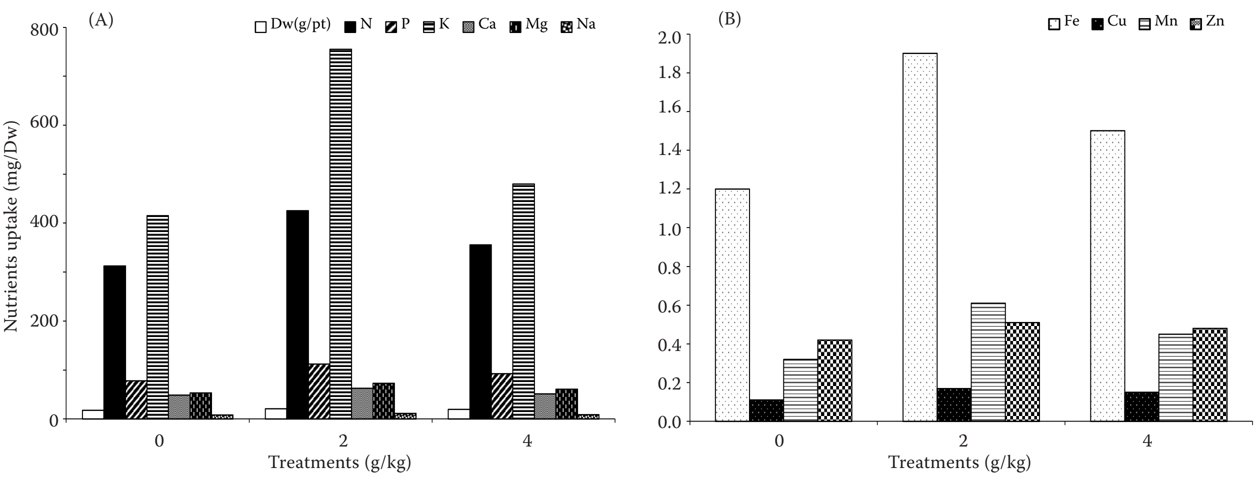

Figure 2. Effect of soil application of humic substances on dry weight and plant nutrients uptake

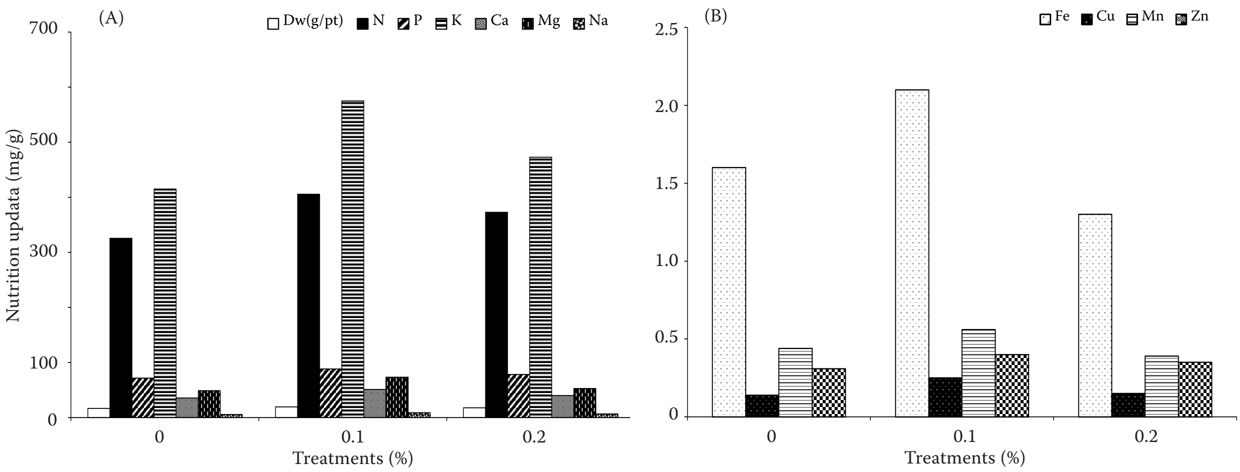

Figure 3. Effect of foliar application of humic acid on dry weight and plant nutrients uptake

application of humic acid and NaCl was not found statistically significant. Foliar application of humic acid enhanced the dry weight and mineral elements uptake in NaCl treatments. However, the highest dry weight and nutrients uptake were obtained with 0.1% of humic acid in NaCl treatment.

Small amounts of salt may cause a stimulative effect on the growth and uptake of nutrients, but a toxic effect appears when the concentration rises. Khan *et al.* (2000) reported that the total dry weight content of plants was not inhibited at low salinities, but the dry weight production was significantly inhibited at high NaCl amounts. Kurban *et al.* (1999) also pointed out that the optimum growth of plants increased at a low salinity but decreased at a high salinity. The negative effect of salt on the dry weight and mineral elements uptake can be attributed to the lower osmotic potential of the soil solution due to the increased concentration of NaCl. Many laboratory and glasshouse studies have shown that salinity can reduce N accumulation in plants (Alam 1994), P concentrations (Navarro *et al.* 2001) and the uptake of K in plants due to the competitive process by Na (Lopez & Satti 1996). A high Na content in soil solution has also an antagonistic effect on the uptake of Ca and Mg (Bernstein 1975). This is most likely caused by displacing Ca in the membranes of the root cells (Yeomiyahu *et al.* 1997).

Furthermore, a reduced uptake of minerals has been observed in several species of plants grown in saline conditions (Francois & Maas 1999). In saline soil, the solubility of micronutrients is particularly low and the plants grown in these soils often show deficiencies of these elements (Page *et al.* 1990).

Chen and Aviad (1990), Fagbenro and Agboda (1993), David *et al.* (1994) have reported promoted growth and nutrient uptake of plants due to the addition of humic substances. The plants take more mineral elements due to better-developed root systems. In addition, the stimulation of ions uptake in the applications of humic materials led many investigators to proposing that these materials affect membrane permeability (Zientara 1983). This is related to the surface activity of humic substances resulting from the presence of both hydrophilic and hydrophobic sites (Chen & Schnitzer 1978). Therefore, the humic substances may interact with the phospholipid structures of the cell membranes and react as carriers of nutrients through them. The features discussed were negatively affected with the application of 2 g humus/kg level. This result might be related to the application levels. On the other hand, the application of very high doses of humic acids is less effective (Lee & Bartlett 1976). According to several researches, the results change due to the levels of treatment, growing media, and origin of humic substances (Chen & Aviad 1990; Arancon *et al.* 2006).There are few researches into the use of humic substances for foliar application. Cooper *et al.* (1998) applied humic substances for creeping bent grass in sand culture at rates of 100, 200 and 300 mg/l and found that the rate of application did not have any effect on the plant growth. Fernandez *et al.* (1996) pointed out that under field conditions, foliar application of leonardite extracts stimulated the shoot growth and promoted the accumulation of K, B, Mg, Ca, and Fe in leaves. However, when leaf N and leaf K values were below the sufficiency range, the foliar

Table 2. Effect of soil and foliar application of humic substances to plant nutrients uptake (mg/g) under NaCl salt conditions (mM)

NaCl	Soil application (g/kg)			Foliar application (%)		
	0	2	4	0	0.1	0.2
Dry weight (g/pot)						
0	17.10	20.20	19.80	15.80	19.41	18.00
20	19.30	20.10	19.60	18.10	18.10	15.80
60	1.51	4.90	5.50	3.80	6.50	4.95
Nitrogen						
0	315.10	425.10	354.10	326.50	405.50	375.20
20	352.00	430.20	350.50	379.10	361.20	345.10
60	55.10	131.50	140.60	111.25	156.90	113.91
Phosphorus						
0	75.70	105.60	90.70	70.80	88.50	78.60
20	88.50	90.30	86.20	73.70	77.80	70.60
60	15.60	30.10	35.30	24.50	35.30	29.90
Potassium						
0	411.50	650.10	475.00	415.11	575.11	470.60
20	465.20	503.60	443.90	420.20	455.10	355.80
60	45.60	111.90	120.90	90.50	150.30	100.90
Calcium						
0	45.50	60.90	50.90	35.60	49.50	41.20
20	48.90	51.90	42.30	34.50	38.60	30.70
60	6.60	13.50	15.50	10.90	15.10	11.50
Magnesium						
0	52.20	70.60	60.00	45.30	71.60	53.10
20	63.90	58.30	62.10	55.60	60.90	44.20
60	7.88	15.80	19.30	14.20	22.20	13.80
Sodium						
0	7.30	10.20	8.00	5.21	8.50	6.40
20	84.00	90.10	68.9	65.50	64.30	60.60
60	24.30	58.10	73.50	57.50	80.50	57.90
Iron						
0	1.21	1.91	1.51	1.50	1.85	1.27
20	1.51	1.71	1.31	1.60	1.92	1.04
60	0.11	0.40	0.60	0.36	0.80	0.35
Copper						
0	0.12	0.17	0.15	0.14	0.20	0.13
20	0.17	0.18	0.17	0.17	0.19	0.13
60	0.03	0.06	0.07	0.05	0.05	0.04
Manganese						
0	0.32	0.58	0.40	0.43	0.57	0.38
20	10.37	0.96	0.78	0.83	1.10	0.81
60	0.29	0.98	1.35	0.95	1.20	0.95
Zinc						
0	0.37	0.48	0.48	0.26	0.34	0.30
20	0.53	0.55	0.45	0.33	0.40	0.34
60	0.11	0.26	0.37	0.20	0.27	0.20

application of humic substances was ineffective in promoting the accumulation of these nutrients in leaves. In a field experiment Govindasmy and Chandresakaran (1992) sprayed humic acids extracted from lignite onto sugarcane and they found that the addition of humic acids improved sugar yield and nutrient concentration in leaf blades and sheaths. Defline *et al.* (2005) investigated the effect of foliar application of N and humic acids on the growth and yield of corn. Moreover, they specified that the foliar application of humic acids caused a transitional production of plant dry mass with respect to the unfertilised control. In contrast to Defline *et al.* (2005), Pavlikova *et al.* (1997) studied the effect of potassium humate. Humic acids were applied by spray during the growth season of cultivated crops at a dose of 20 mg/l. The yields of the cultivated crops were not affected significantly by the application of potassium humate due to the high amounts of humic substances.

According to many researchers, humic substances may enhance the uptake of some nutrients, reduce the uptake of toxic elements, and improve the plant response to salinity. However, there are not many researches into humic acids application and their effects on plant salinity tolerance. Liu (1998) found out that the application of humic acids during salinity stress did not increase the uptake of N, P, K or Ca. Also, in the present study; foliar application in 0.1% humic acid treatment increased the dry weight, N, P, K, Ca, Mg, Na, Fe, Zn, and Mn amounts in plants which 60mM NaCl treatment when compared with the control and 0.2% humic acid treatment. Chavan and Karadge (1980) also reported an increase of Mn contents in all the part of plant that have been treated with salt.

CONCLUSION

Humic acids can significantly reduce water evaporation and increase its use by plants in non-day, arid, and sandy soils. Furthermore, they increase the water holding capacity of soils. Humic acids aid in correcting plant chlorosis, increase the permeability of the plant membranes and intensify enzyme systems of plants. They accelerate cell division, show greater root development, and decrease stress deterioration.Under the influence of humic acids, plants grow stronger and they better resist plant diseases. Humates reduce soil erosion by increasing the cohesive forces of the very fine soil particles. They improve the soil structure and improve physical properties of soil by increasing the exchange capacity and buffening qualities, promoting the chelation of many elements and making these available to plants. Humic substances can ameliorate negative soil properties; improve the plant growth and nutrients uptake. They may be used in the case of the negative effect of salt that would inhibit the plant growth and nutrient elements uptake. Overall, we found out that the application doses are important for deriving benefit from humic substances under salt conditions. Economical levels of application should be determined and should not exceed 2 g humus/kg in soil and 0.1% in foliar.

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