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Full Length Research Paper

Phosphorus and humic acid application alleviate salinity stress of pepper seedling

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Humic acid is a commercial product that contains many elements which improve the soil fertility and increase the availability of nutrient elements. It consequently affects plant growth and yield and ameliorates the deleterious effects of salt stress. The objective of the study was to determine the effect of humic acids and phosphorus on growth and nutrient content of pepper seedlings (cv. Demre) grown under moderate salt stress in growth chamber conditions. Applications of different levels of phosphorus [0 (P_0), 50 (P_1), 100 (P_2) and 150 (P_3) mg kg^{-1}] and humic acid [0 (HA_0), 750 (HA_1) and 1500 (HA_2) mg kg^{-1}] to growing media containing moderate salt dose (8 mM NaCl treatment) were studied. The study was replicated four times with 20 plants in each replicate. Humic acid (HA) and phosphorus applications increased the growth and growth parameter of plants. In company, effects of HA and P application was more effective on growth and growth parameter than each separate effect. The optimum total yield was obtained from 69 mg kg^{-1} P application with HA_2 doses according to regression analysis. Humic acid application significantly increased N, P, K, Ca, Mg, S, Mn and Cu contents of shoot of pepper seedling. Also, N, P, K, Ca, S, Fe, Mn, Zn and Cu contents of root were increased with humic acid application. Na contents of both shoot and root of pepper decreased with increased humic acid doses. It can be concluded that high humic acid doses has positive effects on salt tolerance based on the plant growth parameters and nutrient contents. The present study suggests that HA treatments can ameliorate the deleterious effects of salt stress on pepper plants and HA could offer an economical and simple application to reduce problems of pepper production in moderately saline soil.

Key words: Nutrient uptake, plant growth parameter, salt stress, pepper.

INTRODUCTION

Salinity is a major abiotic stress reducing the yield of wide variety of crops all over the world (Tester and Davenport, 2003; Ashraf and Foolad, 2007). Worldwide, 100 million ha or 5% of the arable land is adversely affected by high salt concentration which reduces crop growth and yield (Heuer, 1994; Ghassemi et al., 1995). The restriction of plant growth and productivity due to salinity is especially acute in arid and semi-arid regions around the world (Kuznetsov and Shevyakova, 1997). Salinity may occur when there is irregular irrigation, inadequate drainage, wrong fertilizer application, and it extremely increases particularly in a cultivation that is protected (Tekinel and Çevik, 1983; George et al., 1997; Wang et al., 2003). Plants growing in saline media come across generally with major drawbacks. The first is the increase in the osmotic stress due to high salt concentration of soil solution that decrease water potential of soil. The second is the increase in concentration of Na and Cl, exhibiting tissue accumulation of Na and Cl, and inhibition of mineral nutrients uptake (Marschner, 1995). For overcoming the negative effect of salinity, the addition of supplemental organic matter (Walker and Bernal, 2004; Walker and Bernal, 2008), different sources of nitrogen (Frechilla et al., 2001), calcium (Tuna et al., 2007) and potassium (Türkmen et al., 2000) to growth as an ameliorative agent

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could be necessary.

The major functional groups of humic acid include carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone and quinoid (Russo and Berlyn, 1990). The mechanism of humic acid in promoting plant growth is not completely known. In addition, increasing cell membrane permeability, oxygen uptake, respiration, photosynthesis, phosphate uptake and root cell elongation of plant growth factors have been proposed by some authors to explain positive effect of humic acid (Vaughan, 1974; Cacco and Dell Agnolla, 1984; Russo and Berlyn, 1990). On the other hand, humic acid has beneficial effects on nutrient uptake by plants and was particularly important for transportation and availability of micro nutrient (Böhme and Thi Lua, 1997). Some studies reported that humic acid can be used as a growth regulator to regulate hormone level, improve plant growth and enhance stress tolerance (Piccolo et al., 1992). Humic acids may stimulate shoot and root growth, and improve resistance to environmental stress in plant, but the physiological mechanism has not been well established (Delfine et al., 2005). Türkmen et al. (2005) suggested that humic acid may promote much growth of pepper seedlings in salty condition.

Therefore, the present study aimed to investigate both effects of different humic acids and phosphorus applications to decrease the negative effect of NaCl on the growth and nutrient contents of pepper seedlings (cv. Demre) grown under moderate salt stress which is an important environmental problem.

MATERIALS AND METHODS

Growth conditions and plant materials

The study was conducted in Yüzüncü Yıl University in Van under growth chamber conditions in Turkey, 2007. Pepper seedlings (cv. Demre) plants were maintained under growth chamber at the temperatures of 22 ± 1°C, with 12 h fluorescent illumination and 8000 lux light intensity. As a result, the seedlings were irrigated with the distilled water.

Pepper seedlings plants with one well-developed crown of diameter 8 - 10 mm was planted in celled-trays containing peat (pH: 5.5, EC: 250 dS cm^{-1}, N: 300 mg/l, P_2O_5: 300 mg/l, K_2O: 400 mg/l, organic matter: 20%). Plants were transferred to free draining pots (25 and 18 cm top and bottom diameter respectively, and 20-cm height, with holes in the bottom) which filled an autoclaved mixture(1/1/2, v/v/v) of sand, manure and soil (pH 7.64, EC:1.12 dS cm^{-1}, total salt 0.035%, total nitrogen 3.05%, P: 18.5 mg kg^{-1}, exchangeable K 0.71 $cmol_{(+)}$ kg^{-1}soil) after 15 days of planting (DAP). All pots were randomized on the benches in the growth chamber.

Humic acid, phosphorus and salt (NaCl) treatments

Salinity treatments were established by adding 8 mM kg^{-1} of NaCl to the pot before the plants are transplanted. After adding 8 mM of NaCl kg^{-1}, the electrical conductivity of the soil solution was 2.12 dS m^{-1}. To support optimum pepper growth, no supplemental nutrients were applied to any of the treatments. Plots were treated with 0 (P_0), 50 (P_1), 100 (P_2) and 150 (P_3) mg kg^{-1} phosphorus, and 0 (HA_0), 750 (HA_1) and 1500 (HA_2) mg kg^{-1}humic acid (HA) solutions. The humic acid (HA) used contained polymeric polyhydroxy acid (85%, w/w); organic matter 86%, pH 3.5, 1% N, 0.9% K, 0.57% Mg, 2.3% S, 0.88% Fe and 0.02% Mn.

The experiments were conducted for 10 weeks. At the end of the study, some plant growth parameters such as seed germination, shoot length, root length, shoot width, cotyledon length, cotyledon width, hypocotyl length, shoot and root fresh weight, and shoot and root dry weight of the seedlings were determined. Plants were harvested by cutting the shoots from the soil surface and washed with de-ionized water. Plant roots were separated from the soil and washed with water until it is free of soil and then washed three times with de-ionized water.

Soil and plant analysis

Soil samples were passed through a 2 mm sieve prior to physical and chemical analysis before the study. The Kjeldahl method (Bremner, 1996) and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N while plant-available P was determined using the sodium bicarbonate method of Olsen et al. (1954). Electrical conductivity (EC) was measured in saturation extracts according to Rhoades (1996). Soil pH determinations were done in 1:2 extracts according to McLean (1982). Soil organic matter was determined using the Smith-Weldon method as described in Nelson and Sommers (1982). Ammonium acetate buffered at pH 7 (Rhoades, 1982) was used to determine exchangeable cations.

Following harvest of the experiments, plant shoots and roots were dried for 48 h at 68°C and ground to pass 1 mm. The Kjeldahl method (Bremner, 1996) and a Vapodest 10 Rapid Kjeldahl Distillation Unit were used to determine total N. Phosphorus and S contents were determined after wet digestion using a HNO_3-$HClO_4$ acid mixture (4:1 v/v) (AOAC, 2005). Phosphorus and S in the extraction solution was measured spectrophotometrically using the indophenol-blue and ascorbic acid method Aqumat UV/VIS spectrophotometer (Thermo Electron Spectroscopy LTD, Cambridge, UK) (AOAC, 2005). Potassium, Na, Ca, Mg, Fe, Mn, Zn, and Cu were determined after wet digestion using a HNO_3-$HClO_4$ acid mixture (4:1 v/v). In the diluted digests, K, Na, Ca, Mg, Fe, Mn, Zn, and Cu analysis were determined by atomic absorption spectrometry (Perkin-Elmer 360 Atomic Absorption Spectrophotometer, Waltham, Massachusetts, USA) (AOAC, 2005).

Statistical analyses

All data were subjected to analysis of variance (ANOVA) and significant means were compared with Duncan multiple range test method, performed using SPSS package.

RESULTS AND DISCUSSION

Seedling growth parameter

P applications with humic acid (HA) significantly affected the shoot and root fresh and dry weight of plant grown under moderately salt stress condition. The highest fresh and dry weights of shoot and root of plants resulted from HA_2 applications depend on P application doses. Humic acid (HA) and phosphorus applications increased the growth and growth parameter of plants. Shoot and root dry matter of plant was increased with increasing P appli-

Table 1. Influence of phosphorus and humic acid on shoot and root fresh and dry weight of plant grown under moderately saline condition (n = 20).

Treatment	Shoot fresh weight g plant^{-1}	Root fresh weight g plant^{-1}	Shoot dry weight g plant^{-1}	Root dry weight g plant^{-1}
HAoPo	4.14 a	1.22 ab	3.88b	0.83 b
HAoPI	3.85 a	1.51 a	4.50b	0.91 b
HAoP2	3.56 a	1.26 ab	5.26a	1.13 a
HAoP3	4.18 a	1.11 b	3.80ab	0.83 b
Mean	3.93 B	1.27 B	4.36B	0.93 B
HAIPo	4.01 a	1.47 a	3.92a	0.87 a
HAIPI	4.67 a	1.30 a	4.098a	0.96 a
HAIP2	4.31 ab	1.05 a	4.61a	1.05 a
HAIP3	3.85 a	1.54 a	5.02a	1.22a
Mean	4.21 AB	1.34 B	4.41B	1.02 B
HA$_2$Po	4.25 a	1.51 b	4.42a	1.16 a
HA$_2$PI	5.36 a	2.06 a	5.83a	1.66 a
HA$_2$P$_2$	5.28 a	1.74 ab	5.42a	1.32 a
HA$_2$P3	4.39 a	1.55 ab	4.75a	1.17 a
Mean	4.82 A	1.72 A	5.10A	1.33A

cation doses until P_2 (Table 1). Shoot and root dry weight of plants was 5.06 g pot^{-1}, 1.01 g pot^{-1} with 66 and 73 mg P kg^{-1} application without HA application according to regression analysis, respectively. But the maximum shoot (5.78 g pot^{-1}) and root (1.51 g pot^{-1}) yield was obtained from 63 and 80 mg P kg^{-1} application with 1500 mg HA kg^{-1} application according to regression analysis, respectively (Figure 1). This effect may be attributed to the efficient increase of the phosphorus used with in-creased HA application.

Dry and fresh weight of root was more affected than the shoot part of the plants by HA application (Table 1). The highest total dry matter obtained 69 mg kg^{-1} P application and 1500 mg HA kg^{-1} application according to regression analysis, respectively (Figure 1). P and HA applications did not statistically affect seed germination.

There were no statistically significant differences with P application levels on growth parameter except for root and cotyledon length, but P applications with HA significantly affect growth parameter except for shoot width. The highest value was obtained from HA_2P_I and HA_2P_2 treatments (Table 2).

Shoot and root nutrient contents of pepper seedling

N content of shoot were significantly influenced by level of HA and P applications. N content of root were significantly influenced by level of HA, but P applications was not effective. The highest content of N in shoot and root resulted from HA_2 and P_3 application levels with shoot, and HA_2 and P_1 application levels with root, respectively. However, HA application was more effective than P application (Table 3).

P contents of pepper shoot and root were significantly affected by all HA and P applications and the highest P contents of shoot and root resulted from the HA_2 and P_3 application levels (Table 3).

HA levels significantly affected the K contents of seedling shoot and root, whereas, no significant effect on P applications. The highest K contents in shoot and root occurred at the HA_2 application levels (Table 3).

HA and P applications significantly influenced the seedling Ca contents of shoot and root of pepper seedling, except for P applications which was not effective on Ca content of shoot (Table 3).

Mg content in shoot was significantly affected by HA levels, and the highest Mg content of shoot was measured in plants treated at the HA_2 levels (Table 3).

HA applications significantly influenced the S content of shoot and root of pepper seedling, and the highest S contents of both plant organs were determined at the HA_2 applications levels (Table 3).

The effect of HA rates on Na contents by shoot and root of pepper seedling in moderate salinity soil conditions is illustrated in Table 3. HA levels were significantly decreased. The decrease was similar to that of Na contents of shoot and root of pepper seedling. Na content of shoot and root decreased in line with P applications levels, but these decreased were not statistically significant (Table 3).

HA and P applications significantly affected Fe content in shoot and root of seedling. P applications decreased Fe content in shoot root of seedling. HA applications increased Fe content in root, whereas, IT decreased Fe content in shoot (Table 3).

HA and P applications significantly affected Mn content in shoot and root of seedling. HA applications increased

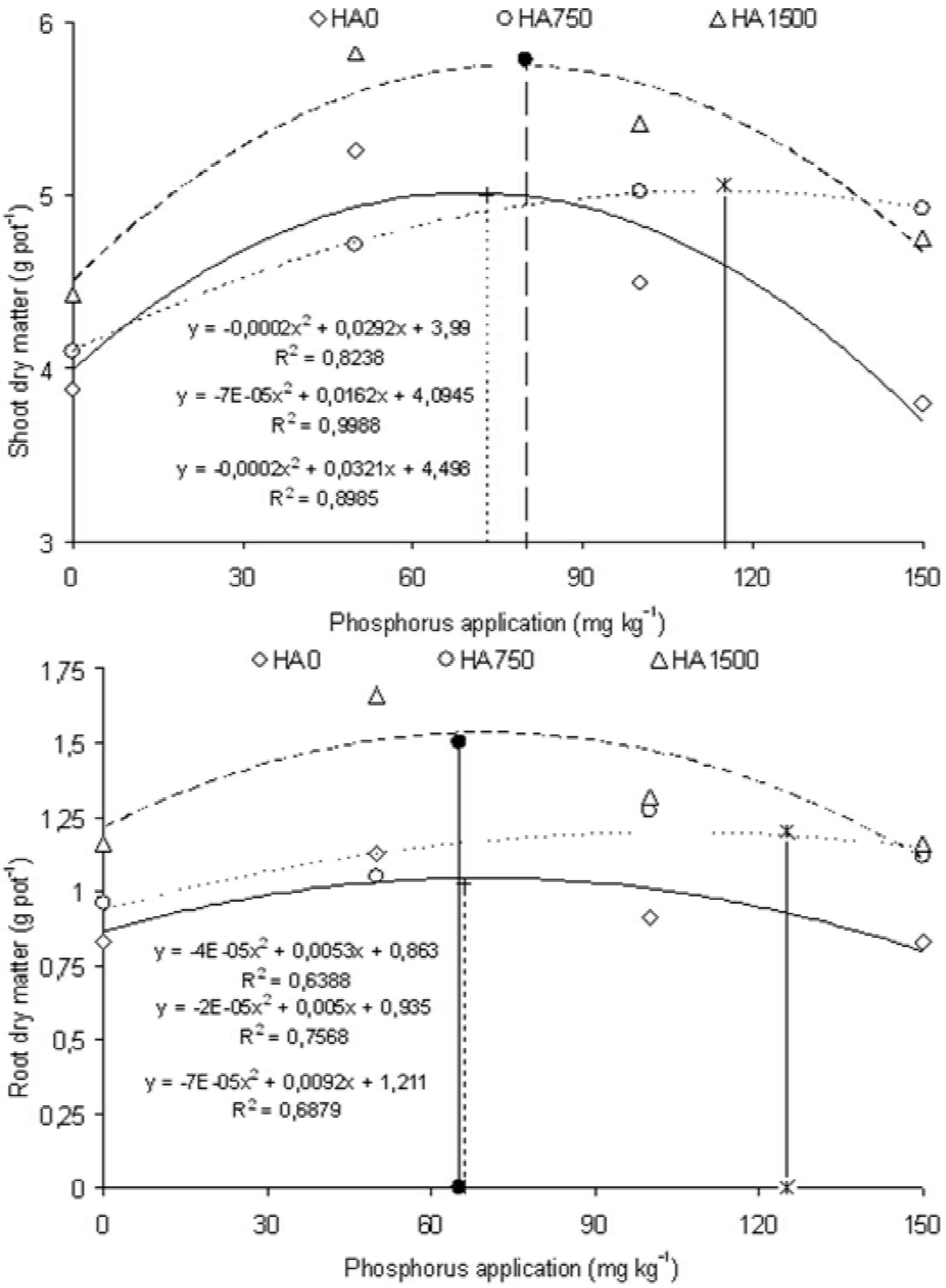

Figure 1. Effects of phosphorus and humic acid on shoot, root and total weight of pepper plants under grown in moderately saline condition.

Mn content in shoot root of seedling. P applications also increased Mn content in shoot, while it decreased Fe content in root (Table 3).

All HA and P applications significantly affected the Zn content of shoot and root of pepper. Zn content of shoot and root was decreased in line with P application levels. HA applications increased Zn content in root, while it decreased Zn content in shoot (Table 3).

All HA and P applications significantly affected the Cu content of shoot and root of pepper. Cu content of shoot and root was increased in line with HA application levels. P applications increased Cu content in shoot, while it decreased Zn content in root (Table 3).

Humic acid (HA) applications to plant growth in the

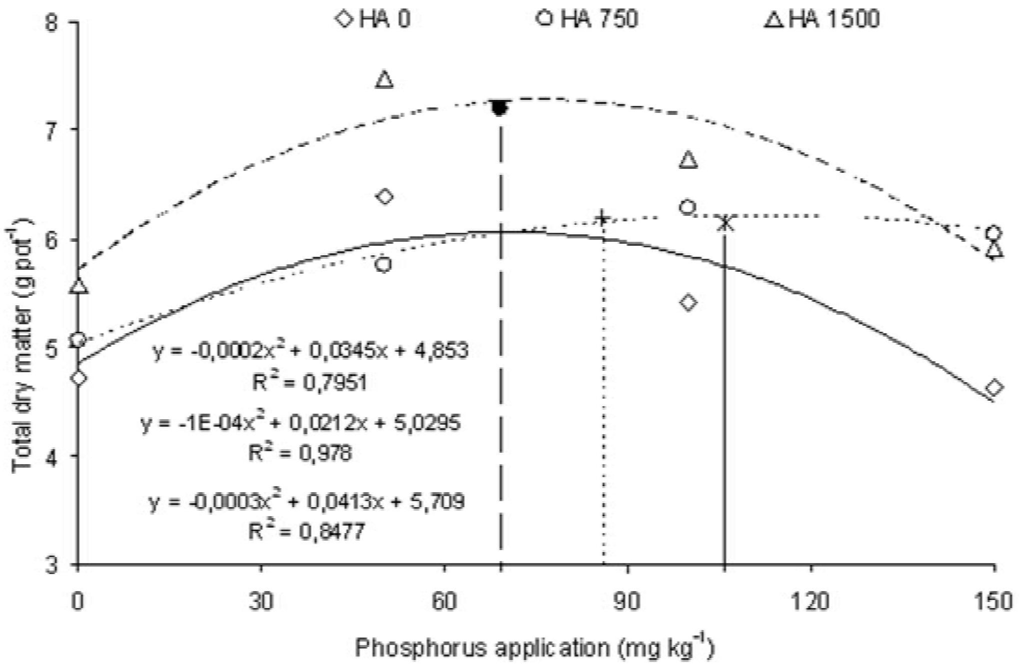

Figure 1. Continued.

Table 2. Influence of phosphorus and humic acid on some growth parameter of plant grown under moderately saline condition (n = 20).

Treatment	Shoot length (cm)	Shoot width (cm)	Root length (cm)	Cotyledon length (mm)	Cotyledon width (mm)	Hypocotyls length (cm)
HAoPo	12.85 a	2.96 a	12.62 ab	15.40 b	6.08a	20.38 a
HAoPı	13.78 a	3.03 a	14.08 a	16.58 a	6.40 a	19.70 a
HAoP2	13.23 a	2.63 a	13.33 a	16.58 a	6.58 a	20.83 a
HAoP3	12.28 a	2.96 a	11.43 b	15.83 b	6.13 a	20.63 a
Mean	13.03A	2.89 A	12.86 B	16.09 B	6.29 C	20.38 B
HAıPo	12.26 a	3.12 a	12.77 a	17.28 b	6.73 a	20.78 a
HAıPı	12.03 a	2.83 a	12.63 a	17.55 b	6.90 a	21.50 a
HAıP2	11.84 a	2.96 a	12.52 a	17.20 b	6.30 b	21.85 a
HAıP3	12.21 a	2.94 a	12.87 a	18.08 a	6. 65 a	21.00 a
Mean	12.08 B	2.96 A	12.70 B	17.53A	6.64 B	21.28 A
HA_2Po	13.05 b	3.28 a	14.35 a	16.60 b	6.30 b	20.20 b
HA_2Pı	14.64 a	3.18 a	15.16 a	16.95 b	6.58 b	19.88 b
HA_2P_2	14.58 a	3.20 a	14.44 a	18.75 a	7.50 a	23.63 a
HA_2P_3	12.69 b	2.81a	13.21 b	18.73 a	7.80 a	23.18 a
Mean	13.74 A	3.12 A	14.29 A	17.76 A	7.04 A	21.72 A

moderate saline conditions increased the growth of both shoots and roots fresh and dry weight, shoot and root lengths, shoot width, cotyledon length and width and hypocotyls length. The highest of all the parameters resulted from HA_3 applications (Tables 1 and 2). These results are in line with those reported by Chen and Aviad (1990), Çimrin et al. (2001), Türkmen et al. (2004a, b). It is possible that the positive effects of humic acids on plant growth could be mainly due to hormone like actives which were reported by Vaguhan et al. (1985), Chen and Aviad (1990) and (Piccolo et al., 1992).

Phosphorus application increased root length, cotyledon length and width and hypocotyls length, contents of N, P, Mn and Cu in shoot and P, Ca in root, and decreased Fe and Zn in shoot, Fe, Mn, Zn and Cu in root of pepper (Tables 2 and 3). The results were similar with the

Table 3. Influence of phosphorus and humic acid on shoot and root N, P, K, Ca, Mg, S, Na, Fe, Mn, Zn and Cu contents grown under moderately saline condition (n = 20).

Treatment	N	P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu
Shoot	%						ppm				
P_0	2.96 c	0.26 d	1.95 a	0.73 a	0.33 a	0.21 a	1030 a	176 a	42.2 b	74 a	19.4 c
P_1	3.11 b	0.39 c	1.95 a	0.74 a	0.35 a	0.22 a	1014 a	153 b	43.4 b	61 b	23.2 b
P_2	3.23 a	0.46 b	1.97 a	0.75 a	0.34 a	0.22 a	999 a	146 c	45.4 a	58 c	23.8 b
P_3	3.26 a	0.55 a	1.98 a	0.75 a	0.33 a	0.22 a	997 a	144 c	46.2 a	53 d	26.4 a
HA_0	2.83 c	0.36 c	1.83 c	0.59 c	0.27 c	0.17 c	1214 a	160 a	33.1 c	72 a	16.3 c
HA_1	3.15 b	0.40 b	1.86 b	0.68 b	0.31 b	0.19 b	969 b	150 c	43.4 b	60 b	21.5 b
HA_2	3.45 a	0.48 a	2.19 a	0.96 a	0.43 a	0.25 a	832 c	154 b	56.4 a	53 c	31.8 a
Root											
P_0	1.41 a	0.12 c	0.58 a	0.23 c	0.14 a	0.10 a	1355 a	70.3 a	13.4 a	38.1 a	8.4 a
P_1	1.43 a	0.13 c	0.57 a	0.25 b	0.12 b	0.10 a	1319 a	54.0 b	12.3 b	31.1 b	7.3 b
P_2	1.42 a	0.16 b	0.56 a	0.26ab	0.13ab	0.10 a	1315 a	48.3 c	10.7 c	30.1bc	6.6bc
P_3	1.40 a	0.19 a	0.58 a	0.27 a	0.13ab	0.10 a	1267 a	38.3 d	10.4 c	28.6 c	6.1 c
HA_0	1.13 c	0.13 c	0.50 c	0.19 c	0.13 a	0.08 b	1573 a	49.0 c	10.3 b	30.8 b	4.9 c
HA_1	1.41 b	0.14 b	0.56 b	0.23 b	0.13 a	0.10 a	1312 b	53.0 b	10.9 b	30.3 b	7.4 b
HA_2	1.69 a	0.18 a	0.65 a	0.34 a	0.13 a	0.11 a	1056 c	56.0 a	13.9 a	34.8 a	9.0 a

a, b, c, and d: Different letters indicate statistical significance ($p < 0.05$).

findings of Güneş et al. (1999) whom reported that salinity and increased doses of phosphorus reduced zinc content and uptake. This reduction can be explained by taking into account different mechanisms, but as Marschnner (1995) remarked, the main effect is considered to be related with the formation of insoluble phosphate compounds for zinc. Similar as both Fe absorption by roots and partitioning of Fe to shoots decreased with increasing concentration of P in the nutrient solution were declared by Elliott and Laeuchli (1985).

The beneficial effects of HA on plant growth may be related to their indirect (increase of fertilizer efficiency), or direct (improvement of the overall plant biomass) effects. Under moderate saline conditions, HA rates significantly increased N, P, K, Ca, Mg, S, Mn and Cu contents of shoot of pepper seedling, but the reverse was the case with Na, Fe and Zn of shoot of pepper. Humic acid have been reported to enhance mineral nutrient uptake by plants, because it affects the permeability of membranes of root (Valdrighi et al., 1996; Türkmen et al., 2004a,b). HA rates significantly increased N, P, K, Ca, S, Fe, Mn, Zn and Cu contents of root of pepper seedling, but the reverse was the case with Na of shoot of pepper. Na contents of both shoot and root of pepper decreased with increased HA doses. Also, root Na contents of plant was higher than the shoot Na contents. Similarly, the increase of root growth rate is higher than the shoot growth rate by HA application. Within this text, it was observed that the negative effects of excessive Na in saline soil conditions could partly be eliminated by HA applications. This may be attributed to the fact that HA application has improved root growth and increased salt tolerance of plant via Na accumulation in root not sent shoot part of the plants. These results are in line with the finding of Walker and Bernal (2008), they also reported that the application of poultry manure and compost to a saline soil can improve significantly the soil chemical environment by increasing the CEC and soluble and exchangeable-K, limiting the entry of Na into the exchange complex, and the K and P supplied by the amendments may have been responsible for the improvement of plant growth, leading to simultaneous decreases in Na and Cl in *Batis maritima*.

Salina soils and saline irrigations constitute a serious production problem for vegetable crops as saline conditions are known to suppress plant growth. The present study demonstrates salinity stress induced lower root and shoot biomass production, mineral element content of plant. HA added to moderate saline soil significantly improved the variables affected by salinity and also increased both root and shoot macro and micro element except for root Mg, enhanced plant growth by allowing nutrients, incorporated into the HA matrix, to release to plant as needed.

Conclusion

The results indicated that HA treatment can ameliorate the deleterious effects of salt stress by increasing root growth, altering mineral uptake, and decreasing membrane damage, thus inducing salt tolerance in pepper plants. The addition of HA could offer an economical and simple application to salt sensitive plant of pepper production problems in aridisol caused by moderate salinity but further studies are required in order to determine the efficiency of these materials under natural field condition.

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