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## Effects of Arbuscular Mycorrhizal Fungus and Humic Acid on the Seedling Development and Nutrient Content of Pepper Grown under Saline Soil Conditions

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**Abstract:** The present study tested the implications of dual application of two of the practices whose effects on salinity have already known: Humic Acid (HA) application and Arbuscular Mycorrhizal Fungus (AMF) (*Glomus intraradices* (Gi)) inoculation. Pepper cultivar Demre was used in the study carried out in a growth chamber. Four different levels HA and two levels of Gi were applied to growth media supplemented with 50 mg NaCl kg<sup>-1</sup> before seed sowing. Hypocotyl length, cotyledon width and length, stem-neck diameter, shoot length, leaf number, leaf area and shoot and root dry weights of the seedlings were determined. Mineral contents of plant shoots and roots were also determined. Almost all of the seedling growth parameters and plant nutrient contents were positively affected by both HA application and Gi inoculation. It was observed in the interaction of these two factors that HA application triggered and increased the positive effects of Gi inoculation. Therefore, it is advisable that the dual application of HA and Gi could promote much more growth in pepper seedlings grown in salty condition.

**Key words:** Arbuscular mycorrhizal fungus, humic acid, pepper, saline soil

### INTRODUCTION

Salinity problem is encountered in more than one third of the world's agricultural lands and causes yield decreases in many crops<sup>[1]</sup>. Turkey uses the 35.6% of its land (78 million ha) for agriculture and the 3.2% of the agricultural land has salinity problem. Pepper is not a salt tolerant vegetable. For pepper, the threshold salt limit causing yield loss is 1.5 dS m<sup>-1</sup> and about 14% of fruit yield loss is resulted by each additional salt level (1.0 dS m<sup>-1</sup>)<sup>[2]</sup>. Pepper is an important vegetable species for the protected cultivation. The salinity is one of the important problems of protected cultivation<sup>[3]</sup>.

Plants grown in salty conditions come across with two major drawbacks. The first is the increase in osmotic stress due to high salt concentration in soil solution and consequently the decrease in the soil-water potential. The later is the increase in concentrations of Na<sup>+</sup> and Cl<sup>-</sup> and the imbalance in overall concentrations of the ions. These mentioned factors cause abundances of the Na<sup>+</sup> and Cl<sup>-</sup> and deficiencies of K<sup>+</sup> and Ca<sup>++</sup> in plants<sup>[4]</sup>. The excessive accumulation of Na<sup>+</sup> in plants inhibits their K<sup>+</sup> uptake<sup>[5]</sup> and the excessive accumulation of Cl<sup>-</sup> in plants negatively affects their NO<sub>3</sub><sup>-</sup> uptake; therefore, the balance in ion concentrations is damaged<sup>[6-8]</sup>.

It is important that salinity tolerance level in seedling period of a plant should be taken into the consideration because if plants are sensitive to salinity in their late growing periods, they are most probably sensitive to salt in their seedling periods<sup>[1]</sup>. The natural and technological ways which might reduce the salinity damages in agricultural crops have been among the most studied subjects for the last decades. Humic substances and arbuscular mycorrhizae could be used in order to obtain some level salinity tolerance.

Humic substances (humic and fulvic acids), constituting 65-70% of the organic matter in soils, are the subjects of studies in various areas of agriculture, such as soil chemistry, fertility, plant physiology as well as environmental sciences, because the multiple role by these materials can greatly improve plant growth. The major functional groups of humic acid include carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone and quinoid<sup>[9]</sup>. The mechanism of humic acid that is active in promoting plant growth is not completely known. However, 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 it<sup>[9,10]</sup>. It was also reported that humic acid application increased the plant

growth and the plant nutrient uptake<sup>[11]</sup>. Böhme and Thi Lua<sup>[12]</sup> also reported that humic acid had beneficial effects on nutrient uptake by plants and was particularly important for the transport and availability of micro nutrient.

Arbuscular Mycorrhizae (AM) is one of the most widespread mycorrhizal associations between soil microorganisms and higher plants<sup>[13]</sup>. The function of all mycorrhizal systems depends on the ability of the fungal symbiont to absorb inorganic and/or organic nutrients available in soil<sup>[14]</sup>. AM has importance due to its great capability on the increase in plant growth and yield under certain conditions. Major reason for this increase in the growth and yield can be attributed to the ability of plants in associations with AM to uptake some nutrients efficiently, such as phosphorus<sup>[15-21]</sup>. AM also enables plants to cope with abiotic stress by means of alleviating nutrient deficiencies, improving drought tolerance, overcoming the detrimental effects of salinity, enhancing tolerance to pollution, adaptation of sterile micropropagated plants to non-sterile substrates and to field conditions<sup>[22,23]</sup>. AM widely exist in saline soil condition<sup>[24]</sup>. Many studies have demonstrated that inoculation with AM fungi improves growth of plants under a variety of salinity stress conditions<sup>[25-27]</sup>. To some extent, AM has been considered as bio-ameliorators of saline soils<sup>[23,28]</sup>.

The sole applications of either HA or AMF (Gi) could be relatively successful in attaining some level salinity tolerance. This study, therefore, aimed to investigate the effects of dual application HA and AMF(Gi) on the salinity tolerance of pepper, one of the salt sensitive vegetable and to see whether this dual application has an increased further amelioration on the emergence and seedling development in pepper.

## MATERIALS AND METHODS

The experiment was conducted at the Agriculture Faculty of Yuzuncu Yil University in Turkey from April to November in 2004. Four different levels of humic acid (HA) (HA<sub>0</sub>: 0, HA<sub>1</sub>: 500, HA<sub>2</sub>: 1000 and HA<sub>3</sub>: 2000 mg HA kg<sup>-1</sup>) and two levels of AMF, *Glomus intraradices* (Gi) (Gi<sub>a</sub>: absent, Gi<sub>p</sub>: present) were applied to growth media supplemented with NaCl (50 mg kg<sup>-1</sup>) before seed sowing. The humic acid used had pH 3.5 and contained polymeric poly-hydroxyl acid (85% w/w); organic matter 86% and N, K, Mg, S, Fe and Mn were 1, 0.9, 0.57, 2.3, 0.88 and 0.021%, respectively.

Inoculum of AMF, *Glomus intraradices* (Gi), consisted of spores, external mycelium and Gi colonized roots, was laid into around the seed with 5 g

(25 spores/g)<sup>[29]</sup>. The same amount of sterilized growth medium was laid into the control pots. Pepper roots were dyed in order to determine the existence of *G. intraradices* by the method modified by Phillips and Hayman<sup>[30]</sup> the percentage of mycorrhizal colonization was estimated by grid line intersect method<sup>[31]</sup>.

The average colonization rates of Gi inoculated as the above procedure were found for HA<sub>0</sub>, HA<sub>1</sub>, HA<sub>2</sub> and HA<sub>3</sub> as 44.73, 37.83, 26.36 and 25.08%, respectively.

The study using pepper cultivar Demre was designed as Completely Randomized Factorial Block Design with four replications each having twelve pots without any drainage. Three pepper seeds were sown to each pot having 250 cc volume and autoclaved mixture of soil, sand and manure and then the seedlings were thinned to one. The growth mixture used in the study had a loamy texture, pH 6.80, 0.035 and 3.05% of total NaCl and N, respectively and 18.53 and 275 mg kg<sup>-1</sup> of P and K, respectively. The pots were placed in a growth chamber at the temperatures of 22±1 °C, with 12 h fluorescent illumination with 8000 lux light intensity and the seedlings were irrigated with the distilled water. The experiment was ended 8 weeks after the seed sowing.

Hypocotyl length, cotyledon width and cotyledon length in the seedlings were determined in the first three weeks of the study, while stem-neck diameter, shoot length, leaf number, leaf area and shoot and root dry weights of the seedlings were determined at the end of the study. Leaf area was measured with a planimeter. Mineral contents of plant shoots and roots were also determined after these samples were oven-dried at 68°C for 48 h and were then ground. Nitrogen was determined by Kjeldahl method<sup>[32]</sup>. The amounts of K and Ca were determined after wet digestion of dried and ground sub-samples in a H<sub>2</sub>SO<sub>4</sub>-Se-salisilic acid mixture. In the diluted digests, P and S was measured spectrophotometrically<sup>[32]</sup>. Potassium and Ca were determined by flame photometry, Mg, Cu, Fe, Mn and Zn by atomic absorption spectrometry using the method of AOAC<sup>[33]</sup>.

All data were subjected to a one-way analysis of variance (ANOVA) and separated by Duncan's multiple range test performed using the COSTAT statistical software.

## RESULTS AND DISCUSSION

**Hypocotyl length:** The Gi inoculation and the interaction of Gi and HA had significant effects on the hypocotyl lengths of seedlings, while HA application did not affect on it alone (Table 1). The Gi inoculated seedlings had 1.73 mm (7.9%) longer hypocotyls than the

**Table 1: Effects of HA application and Gi inoculation on length of hypocotyl, length and width of cotyledon and stem-neck diameter in pepper grown in saline condition**

	Hypocotyl length (mm)			Cotyledon length (mm)			Cotyledon width (mm)			Stem-neck diameter (mm)		
	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave.	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave.	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave.	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave.
0	22.03bc*	23.06ab	22.54	18.75	20.59	19.67b**	6.90cd*	7.38ac	7.19b*	2.69	3.14	2.91c***
500	23.60ab	22.60ac	23.10	20.19	20.82	20.51ab	7.17bc	7.65ab	7.42ab	2.93	3.21	3.07c
1000	21.21bc	25.01a	23.11	20.52	20.63	20.58ab	7.65ab	7.90a	7.78a	3.28	3.93	3.61a
2000	20.22c	23.31ab	21.76	20.34	22.48	21.41a	6.52d	7.88a	7.20b	3.07	3.46	3.27b
Mean	21.76b**	23.49a		19.95b***	21.13a		7.09b***	7.71a		2.99b**	3.43a	

\*\*\*: There were significant differences among the different letter(s) at p<0.001, \*\*: There were significant differences among the different letter(s) at p<0.01  
\*: There were significant differences among the different letter(s) at p<0.05

**Table 2: Effects of HA application and Gi inoculation on shoot length, number and area of leaf, dry weight of shoot and root in pepper grown in saline condition**

	Shoot length (cm)			Leaf No. (number/seedling)			Leaf area (cm <sup>2</sup> /seedling)			Dry shoot weight (g) per seedling			Dry root weight (g) per seedling		
	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave.	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave.	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave.	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave.	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave.
0	8.65	10.82	9.74b**	10.17	10.74	10.46	147.82d*	198.31ab	173.06b***	0.411	0.583	0.490	0.089h***	0.140e	0.110c***
500	9.95	13.59	11.77a	10.02	11.90	10.96	179.30bc	212.95a	196.12a	0.486	0.723	0.600	0.107g	0.184b	0.140b
1000	9.17	13.61	11.39a	10.15	11.51	10.83	163.80cd	164.50cd	164.15b	0.485	0.784	0.630	0.143d	0.221a	0.180a
2000	10.72	13.64	12.19a	10.15	10.72	10.44	148.85d	174.95c	161.90b	0.514	0.664	0.580	0.126f	0.146c	0.130b
Ave.	9.62b***	12.92a		10.12b**	11.22a		159.94b***	187.67a		0.470b***	0.680a		0.110b***	0.170a	

\*\*\*: There were significant differences among the different letter(s) at p<0.001, \*\*: There were significant differences among the different letter(s) at p<0.01

non-inoculated ones. The longest hypocotyls length (25.01 mm) was obtained from the treatment inoculated with Gi and applied with 1000 mg HA kg<sup>-1</sup>.

**Cotyledon length and width:** Both traits were significantly affected by the Gi inoculation and the HA application (Table 1). The Gi inoculated seedlings had 1.18 (5.9%) and 0.62 mm (8.7%) longer and wider cotyledons, respectively than the non-inoculated ones. The applications of 2000 and 1000 mg HA kg<sup>-1</sup> gave the longest (21.41 mm) and the widest (7.78 mm) cotyledons than the other HA doses. The interaction of Gi and HA had only significant effects on the cotyledon widths of seedlings; the longest cotyledon width (7.90 mm) was obtained from the treatment inoculated with Gi and applied with 1000 mg HA kg<sup>-1</sup>.

**Stem-neck diameter:** The Gi inoculation and the HA application, but not their interaction had significant effects on the stem-neck diameters of seedlings (Table 1). The Gi inoculated seedlings had 0.44 mm (14.7%) thicker stem-necks than the non-inoculated ones. The applications of 1000 mg HA kg<sup>-1</sup> caused the thickest stem-neck (3.61 mm) and this value 0.70 mm (24.1%) higher than the control dose of HA.

**Shoot length:** It was significantly affected by the Gi inoculation and the HA application (Table 2). The Gi inoculated seedlings had 3.3 cm (34.3%) longer shoots than the non-inoculated ones. The HA applied seedlings had at least 1.65 cm longer shoots than the non-applied ones. The longest shoot length (13.64 cm) was obtained from the treatment inoculated with Gi and applied with 2000 mg HA kg<sup>-1</sup>.

**Leaf number:** The only Gi inoculation had significant effect on the leaf number of seedlings (Table 2). There were about 1.1 (10.9%) increases in the leaf number of Gi inoculated seedlings compared to the non-inoculated ones.

**Leaf area:** This trait was significantly affected by the Gi inoculation, the HA application and their interaction (Table 2). The Gi inoculated seedlings had 27.73 cm<sup>2</sup>/seedlings (17.3%) larger leaf areas than the non-inoculated ones. The applications of 500 mg HA kg<sup>-1</sup> produced the largest leaf area per seedling (196.12/cm<sup>2</sup>/seedlings) compared to the non-applied ones. The largest leaf area (212.95/cm<sup>2</sup>/seedlings) was obtained from the treatment inoculated with Gi and applied with 500 mg HA kg<sup>-1</sup>.

**Dry weights:** The dry root weight was significantly affected by the Gi inoculation, the HA application and their interaction, while the dry shoot weight was only significantly affected by the Gi inoculation (Table 2). The Gi inoculated seedlings had 0.21 g (44.7%) and 0.06 g (54.5%) heavier dry shoot weights and dry root weights, respectively than the non-inoculated ones. The applications of 1000 mg HA kg<sup>-1</sup> produced the heaviest dry root weight (0.180 g) among the HA applications. The applications of 1000 mg HA kg<sup>-1</sup> caused the heaviest dry root weight (0.221 g) among all treatments.

**Nitrogen contents:** The Gi inoculation and the HA application had significant effects on the nitrogen contents in roots and shoots and their interaction was significantly effective in the nitrogen contents of shoots (Table 3). The Gi inoculated seedlings had 0.46%

Table 3: Effects of HA application and Gi inoculation on macro nutrients in roots and shoots of pepper seedlings grown in saline condition

	N (%)			P (%)			K (%)			S (%)			Ca (%)		
	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave	Gi <sub>1</sub>	Gi <sub>2</sub>	Ave
Shoot															
0	2.86d**	3.13cd	2.99d***	0.36d***	0.38d	0.37c***	3.08c***	3.13c	3.11d***	0.21	0.21	0.21b***	1.25	1.90	1.23d***
500	3.36bc	3.73b	3.54c	0.36d	0.39d	0.38c	3.24c	3.68b	3.46c	0.22	0.23	0.23b	1.31	1.38	1.35c
1000	3.75b	4.57a	4.16b	0.40d	0.62b	0.51b	3.74b	3.83b	3.78b	0.25	0.28	0.27a	1.57	1.63	1.60b
2000	4.51a	4.91a	4.71a	0.55c	0.73a	0.64a	4.39a	4.39a	4.39a	0.28	0.25	0.27a	1.83	1.87	1.86a
Ave	3.62b***	4.08a		0.42b***	0.53a		3.61b**	3.76a		0.24	0.25		1.49	1.52	
Root															
0	0.75	0.74	0.75d***	0.12d***	0.13cd	0.12d***	1.21f	1.30c	1.26ab**	0.10	0.11	0.10b**	0.22	0.19	0.21c***
500	0.88	1.00	0.94c	0.13cd	0.15c	0.14c	1.16g	1.23e	1.20b	0.11	0.12	0.12a	0.20	0.21	0.21c
1000	1.13	1.29	1.21b	0.14cd	0.19b	0.17b	1.24d	1.15h	1.20b	0.13	0.12	0.13a	0.26	0.26	0.26b
2000	1.38	1.43	1.41a	0.20b	0.30a	0.25a	1.33a	1.31b	1.32a	0.13	0.13	0.13a	0.32	0.34	0.33a
Ave	1.04b**	1.12a		0.15b***	0.19a		1.23	1.25		0.11	0.12		0.25	0.25	

\*\*\*: There were significant differences among the different letter(s) at  $p < 0.001$ , \*\*: There were significant differences among the different letter(s) at  $p < 0.01$

(relatively 12.7%) and 0.08% (relatively 7.7%) more nitrogen contents in shoots and roots, respectively than the non-inoculated ones. Increasing doses of HA treatments significantly increased the nitrogen contents of both shoots and roots. The highest amounts of nitrogen in shoots and roots were obtained from the application of 2000 mg HA kg<sup>-1</sup> as 4.71 and 1.41%, respectively. The highest amounts of nitrogen in shoots (4.57 and 4.91%) were obtained from the Gi inoculated applications of 1000 or 2000 mg HA kg<sup>-1</sup>, respectively.

**Phosphorous contents:** The phosphorous contents of shoots and roots were significantly affected from the Gi inoculation, the HA application and their interaction (Table 3). The Gi inoculated seedlings had 0.09% (relatively 21.4%) and 0.04% (relatively 26.7%) more phosphorous contents in shoots and roots, respectively than the non-inoculated ones. Increasing doses of HA treatments significantly increased the phosphorous contents of both shoots and roots. The highest amounts of phosphorous in shoots and roots were obtained from the application of 2000 mg HA kg<sup>-1</sup> as 0.64 and 0.25%, respectively. The highest amounts of phosphorous in shoots and roots were obtained from the Gi inoculated applications of 2000 mg HA kg<sup>-1</sup>, as 0.73 and 0.30%, respectively.

**Potassium contents:** The HA application had significant effects on the potassium contents in roots and shoots; the Gi inoculation had significant effect on only the potassium contents in shoots; the interaction of Gi and HA was significantly effective in the potassium contents of both shoots and roots (Table 3). The Gi inoculated seedlings had 0.15% (relatively 4.2%) more potassium content in shoots than the non-inoculated ones. The highest amounts of potassium in shoots and roots were obtained from the application of 2000 mg HA kg<sup>-1</sup> as 4.39 and 1.32%, respectively. The highest amounts of potassium in shoots (4.39%) were obtained from the Gi inoculated or non-inoculated applications of 2000 mg HA kg<sup>-1</sup>. The highest amount of potassium in

shoots (4.39%) was obtained from the Gi non-inoculated application of 2000 mg HA kg<sup>-1</sup>.

**Sulphur contents:** The sulphur contents of shoots and roots were significantly affected from only the HA application (Table 3). Increasing doses of HA treatments significantly increased the sulphur contents of both shoots and roots. The highest amounts of sulphur in shoots were obtained from the application of either 1000 or 2000 mg HA kg<sup>-1</sup> as 0.27%. The HA applied seedlings had significantly more sulfur content in roots than the non-applied ones.

**Calcium contents:** Only the HA application significantly affected the calcium contents of shoots and roots (Table 3). Increasing doses of HA treatments significantly increased the calcium contents of both shoots and roots. The highest amount of calcium in shoots was obtained from the application of 2000 mg HA kg<sup>-1</sup> as 1.86%. The highest amount of calcium in roots was also obtained from the application of 2000 mg kg<sup>-1</sup> HA as 0.33%.

**Magnesium contents:** The HA application significantly affected the magnesium contents of shoots and root, while the Gi inoculation significantly affected just the magnesium contents of roots (Table 4). The Gi inoculated seedlings had 0.02% (relatively 7.7%) more magnesium content in roots than the non-inoculated ones. Increasing doses of HA treatments significantly increased the phosphorous contents of both shoots and roots. The highest amounts of magnesium in shoots and roots were obtained from the application of 2000 mg kg<sup>-1</sup> HA as 0.64 and 0.31%, respectively.

**Iron contents:** The Gi inoculation and the HA application had significant effects on the iron contents in roots and shoots and their interaction was significantly effective in the iron content of shoot (Table 4). The Gi inoculated seedlings had 17.0 (relatively 21.6%) and 14.0 mg kg<sup>-1</sup> (relatively 11.0%) more iron contents in shoots and roots, respectively than the non-inoculated ones. Increasing

Table 4: Effects of HA application and Gi inoculation on micro nutrients in roots and shoots of pepper seedlings grown in saline condition

	Mg (%)			Fe (mg kg <sup>-1</sup> )			Mn (mg kg <sup>-1</sup> )			Zn (mg kg <sup>-1</sup> )			Cu (mg kg <sup>-1</sup> )		
	Gi	HA	Ave	Gi	HA	Ave	Gi	HA	Ave	Gi	HA	Ave	Gi	HA	Ave
Shoot															
0	0.53	0.54	0.54c***	54.25e**	61.25de	57.75c***	38.75	37.75	38.25d***	67.25b***	70.00b	68.63c***	14.25e**	17.25de	15.75d***
500	0.56	0.56	0.56bc	63.50de	75.00cd	69.25b	42.50	41.50	42.00c	74.75b	72.25b	73.50b	20.25cd	18.75ce	19.50c
1000	0.58	0.60	0.59b	61.50de	86.50c	74.00b	44.75	51.75	47.50b	70.25b	75.75b	73.00b	23.00c	26.75b	24.88b
2000	0.66	0.61	0.64a	136.25b	160.75a	148.50a	54.00	67.00	60.50a	72.25b	93.75a	83.00a	26.50b	33.25a	29.88a
Ave.	0.59	0.58		78.87b***	95.87a		44.63b***	49.50a		71.13b***	77.94a		21.00b***	24.00a	
Root															
0	0.21	0.22	0.22c***	123.25	128.25	126.00c***	96.00ac	90.00c*	93.00c***	129.00d*	134.00cd	131.00c***	32.50	40.00	36.00d***
500	0.27	0.25	0.26b	111.25	131.00	121.00c	94.25bc	93.00bc	94.00c	141.25cd	140.50cd	141.00b	44.75	41.75	43.00c
1000	0.26	0.30	0.28ab	120.50	139.50	130.00b	92.00c	108.75ac	100.00b	139.75cd	145.50c	143.00b	51.00	54.25	53.00b
2000	0.28	0.33	0.31a	154.00	167.50	160.00a	116.50a	114.25ab	115.00a	166.25b	183.75a	175.00a	57.00	64.25	61.00a
Ave.	0.26b*	0.28a		127.00b***	141.00a		99.00	101.00		144.00b**	151.00a		46.00b*	50.00a	

\*\*\*: There were significant differences among the different letter(s) at p<0.001, \*\*: There were significant differences among the different letter(s) at p<0.01

\*: There were significant differences among the different letter(s) at p<0.05

doses of HA treatments significantly increased the iron contents of both shoots and roots. The highest amounts of iron in shoots and roots were obtained from the application of 2000 mg kg<sup>-1</sup> HA as 148.5 and 160.0 mg kg<sup>-1</sup>, respectively. The highest amount of iron in shoots (160.75 mg kg<sup>-1</sup>) was obtained from the Gi inoculated applications of 2000 mg kg<sup>-1</sup> HA.

**Manganese contents:** The HA application significantly affected the manganese contents of shoots and roots, while the Gi inoculation significantly affected just the manganese contents of roots (Table 4). The Gi inoculated seedlings had 4.87 mg kg<sup>-1</sup> (relatively 10.91%) more magnesium content in roots than the non-inoculated ones.

**Zinc contents:** The zinc contents of shoots and roots were significantly affected from the Gi inoculation, the HA application and their interaction (Table 4). The Gi inoculated seedlings had 6.81 (relatively 9.6%) and 7.0 mg kg<sup>-1</sup> (relatively 4.9%) more zinc contents in shoots and roots, respectively than the non-inoculated ones. Increasing doses of HA treatments significantly increased the zinc contents of both shoots and roots. The highest amounts of zinc in shoots and roots were obtained from the application of 2000 mg kg<sup>-1</sup> HA as 83.0 and 175.0 mg kg<sup>-1</sup>, respectively. The highest amounts of zinc in shoots and roots were obtained from the Gi inoculated applications of 2000 mg kg<sup>-1</sup> HA, 93.75 and 183.75 mg kg<sup>-1</sup>, respectively.

**Copper contents:** The Gi inoculation and the HA application had significant effects on the copper contents in roots and shoots and their interaction was significantly effective in the copper contents of shoots (Table 4). The Gi inoculated seedlings had 3 (relatively 14.3%) and 4 mg kg<sup>-1</sup> (relatively 8.7%) more copper contents in shoots and roots, respectively than the non-inoculated ones. Increasing doses of HA treatments

significantly increased the copper contents of both shoots and roots. The highest amounts of copper in shoots and roots were obtained from the application of 2000 mg kg<sup>-1</sup> HA as 29.88 and 61.00 mg kg<sup>-1</sup>, respectively. The highest amount of copper in shoot (33.25 mg kg<sup>-1</sup>) was obtained from the Gi inoculated application of 2000 mg kg<sup>-1</sup> HA.

It has been long known that salinity is a limiting or even extremely troublesome factor to deal with in agriculture<sup>[1,4]</sup>. Various methods have been employed in order to overcome the salinity. Therefore, the present study tested the implications of dual application of two of them whose effects on salinity have already known: HA application<sup>[9-12]</sup> and Gi inoculation<sup>[25-27]</sup>.

In general, HA application positively affected the plant growth parameters of pepper grown in salinity condition. Most researchers have also reported the ameliorative effects of HA on plant growth<sup>[9-12,34,35]</sup>. The present study determined that the application of 1000 mg kg<sup>-1</sup> HA found to be more suitable dose for most of the growth parameters except for cotyledon and shoot lengths. In general, Gi inoculation also positively affected the plant growth parameters of pepper grown in salinity condition. There have been several other studies in agreement<sup>[25-28]</sup>. When we consider the interaction of HA application and Gi inoculation, it has been noticed that HA application triggered and increased the positive effects of Gi inoculation. Based on the morphologic observation, it was observed that the dual application of HA and Gi promoted much more growth in pepper seedlings grown in salty condition. Plant nutrient contents analyses gave harmonious results with the seedling growth parameters and were in agreement with the several other studies; increasing doses of HA application increased the plant nutrient contents<sup>[9-11,34,35]</sup>. The application of 2000 mg kg<sup>-1</sup> HA found to be more superior dose for most of the plant nutrient contents. The Gi inoculation also increased the plant nutrient contents as seen in several other studies<sup>[16,17,19,36]</sup>.

In conclusion, the present study revealed in controlled conditions that dual application of HA and Gi whose sole applications have already known to be useful in the plant growth could be much more efficiently employed in pepper, one of the salinity sensitive vegetable species, grown in salty condition.

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