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The effects of humic acid on growth and ion uptake of mung bean (*Vigna radiata* (L.) Wilczek) grown under salt stress

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The study aimed to determine the effects of humic acid (HA) on seedlings of a salt-sensitive plant, mung bean (*Vigna radiata* (L.) Wilczek), grown on 50 mM (S1) and 100 mM (S2) NaCl concentrations. In controlled room conditions, mung bean seedlings were planted in pots containing torf and perlite mixture and salt effects were observed. The growth parameters were plant height, number of leaves, leaf area, leaf and stem fresh and dry weights in which all parameters significantly decreased at both salt levels. Nutrient analyses of Na, K and Ca were conducted by flame photometry (FP), and Mg, Mn, Zn were tested by inductively coupled plasma atomic emission spectrometry (ICP-AES), for the above and below ground parts of mung bean seedlings. Both salt treatments increased Na content significantly, however 10 ml addition of HA to those samples (S1HA and S2HA; 50 mM NaCl and 100 mM NaCl, respectively) caused reductions in Na contents of the above-ground parts of mung bean compared to plants watered with only Hoagland-Arnon solutions (HO). On the other hand, in the roots of mung bean seedlings, Na content rose significantly in S2 compared with control, but the amount of Na in S2HA significantly increased compared with S2 treated plants. K content was significantly decreased in both salt concentrations, while SHA1 and SHA2 caused slight increases in both above and underground parts of seedlings. In the experiment, SHA2 increased the content of K, Mg, Ca, Mn and Zn in the root of mung bean seedlings compared with both S1 and S2 treatments.

Key words: NaCl, mung bean, growth, nutrient *Vigna radiata*, humic acid.

INTRODUCTION

On a global basis, soil salinity is one of the stress factors prevalent in arid and semi-arid zones. It inhibits plant growth and development, resulting in serious reduction in yield, particularly on salt-sensitive plants (Example glycophytes) (Grattan and Grieve, 1998; Allbed and Kumar, 2003; Pessarakli and Szabolcs, 1999; Pitman and Lauchli, 2002; Asik et al., 2009; Amirjani, 2010). Salt-affected lands were first recorded in human agricultural

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history in aluvial soils of the Tigris (Mesopotamia), in present day it is Iraq (Russel et al., 1965, Pitman and Lauchli, 2000).

According to the Food and Agricultural Organization (FAO, 2005) more than 800 million ha is affected by salinity worldwide, with saline and sodic pressure affecting 23 and 37% of cultivated lands, respectively. Salt affected areas extend widely over more than 100 countries across all continents, equating to 10% of arable areas (Szabolcs, 1989). In Turkey, 1,513,645 ha of agricultural lands face salinity and alkalinity pressure due to mismanagement of irrigation and inefficient drainage systems, as well as features of water quality (Dinc at al., 1993).

Salinity plays a major role in plant growth in many ways, including physiological and biochemical mechanisms that affect cell level processes. These include inhibiting osmotic regulation, reducing stability of nutritional balance and specific ion toxicity (Neumann,1997; Yao, 1998; Alam, 1999; Jacoby, 1999; Hasegewa et al., 2000; Munns, 2002), and in the growing tissue salt impairs the supply of assimilates of photosynthesis as well as hormones (Munns, 1993).

The many salt-related studies have ranged from seed germination effects to whole plant growth, physiological to anatomical changes, biochemical composition differences to biomass and yield reduction and covered a very wide range of plant species (Niazi et al., 1987; Katerji et al., 1994; Poljakoff-Mayber et al., 1994; Villiers et al., 1994; Rogers et al., 1995; Reinhardt and Rost, 1995 a, b; Zidan and Elewa, 1995; Huang and Redmann, 1995; Wahid et al., 1998; Croser et al., 2001; Al-Mutawa, 2003; Turhan and Ayaz, 2004; Song et al., 2008; Maial et al., 2010; Ruffino et al., 2010).

Although Tavakkoli (2010) stated that most plants are thought to accumulate both Na^+ and Cl^-, Neumann et al. (1988) concluded that salt (particularly Na^+) toxicity in various plants has visible symptoms such as leaf burn, necrotic spots on leaves and limited expansion of leaf cells, particularly in salt sensitive plants. Studies have covered a very wide range of salt applications, such as NaCl, Na_2SO_4, $CaCl_2$, $MgCl_2$, individually or in various mixture compositions, looking at effects on seeds and propagule germination, commonly focusing on seed plant growth-development (Example, biomass) and physiological changes, such as nutrient composition in varied plant tissue and organs in different plant species (Jamil et al., 2005; Sagib, 2006; Turan et al., 2007a, b; Saffan, 2008; Rui, 2009; Turan et al., 2010; Memon et al., 2010).

Mung bean is a very sensitive plant to salt (Maas and Hoffman, 1977; Ashraf and Rasul, 1988) and an important crop widely grown in the Indian subcontinent, South East of Asia, Africa, South America and Australia. In South Asia, annual mung bean production is 3.1 million tonnes and it is grown on an area of 3 million ha under rainfed and irrigated conditions. It is consumed cooked, fermented, roasted etc. and has a high protein content (26.4 g/100 g dry weight) from the Fabaceae family (Ashraf and Rasul, 1988; Oplinger et al., 1990; Malik, 1994; Shangyandram, 2009; Mondal et al., 2012; Waqas et al., 2014) and 4.5 g ash, 1.75 g fat, 6.15 g crude fiber and 61.2 g carbohydrates per 100 g dry weight (El-Adawy et al., 2003).

In Turkey, mung bean is grown in South East Anatolia, particularly in Gaziantep and its environs, although there are no statistical figures on yield and cropping area (Akdag, 1995). There is some research on mung bean yield in relation to agronomical, morphological, and phenological yield components (Toker et al., 2002; Canci and Toker, 2005;2014) as well as genotypic study of its varieties (Peksen et al., 2015).

Humic substances have been widely used for agricultural research, affecting the quality of soil as well as yield quantity. Humic acids are responsible for pH adjustment, enhancing soil cation exchange capacity and extending the survival mechanism of plants grown under stress conditions such as salinity, drought and harmful effects of toxic and heavy metal elements in the soil (Fagbenro and Agboda, 1983; David et al., 1994; Stevenson, 1994; Dursun et al., 1998; Pilanali and Kaplan, 2003; Sharrif, 2002; Kolsarici et al., 2005; Fong et al., 2007; Buyukkeskin and Akinci, 2011; Khaled and Fawy, 2011)

In the present study, the salt-sensitive plant mung bean was subjected to the following treatments: a set of replicates watered only with Hoagland and Arnon (1950) solution (HO); two experimental sets exposed to 50 and 100 mM salt (NaCl) concentrations; and the remaining two sets of replicates exposed to the same two salt solutions with added 10 ml humic. The five different growth media replicates were tested for growth and physiological changes in mung bean seedlings, to determine the remediative role of humic acid on growth parameters and the mineral composition of both under and above ground parts of seedlings in all treatments.

MATERIALS AND METHODS

The study species

Superdivision: Spermatophyta; Division: Magnoliophyta; Order: Fabales; Family: Fabaceae (Leguminosae) Pea family; Genus: *Vigna* Savi (cowpea); Species:*Vigna radiata* (L.) R. Wilczek (mung bean)

Growing method under growth room conditions

The experiments were carried out in controlled growth room conditions in the department of Biology, Faculty of Arts and Sciences, Marmara University. Mung bean (*Vigna radiata* (L). Wilczek) seeds were obtained from Nickys Nursery Ltd. (SPR006) Kent, UK. The used liquid humic acid (Premium is a trade product of Turpex LTD, Turkey) contained organic, humic and fulvic acids and water soluble K_2O in ratios given in Table 1. For germination,

Table 1. Components of HA.

Components	Volume (%w/w)
Total organic substances	12
Total HA+ fulvic acid	16
Water-soluble K_2O	3

Table 2. Experimental salt and Humic acid preparations.

Experimental Groups	Contents and preparation
Control (C)	Full strength Hoagland solution (Hoagland-Arnon, 1950)
50 mM NaCl (S1)	50 mM NaCl is dissolved in HO solution and volume made up to1 L
100 mM NaCl (S2)	100 mM NaCl is dissolved in HO solution and volume made up to 1 L
50 mM NaCl + Humic acid (S1HA)	50 mM NaCl is dissolved in HO contained 10 ml humic acid and volume made up to 1 L
100 mM NaCl + Humic acid (S2 HA)	100 mM NaCl is dissolved in HO contained 10 ml humic acid and volume made up 1 L

mung bean seeds were kept overnight in a beaker containing distilled water and then transferred onto filter paper in the base of Petri dishes and covered for a week. Each germinated seed was planted in a plastic pot containing 280 g torf (Gardol) and perlite mixture in a 3:1 ratio, respectively. The five treatments were arranged in a completely randomized block design with eight replicates as follows: the control (C) contained only Hoagland and Arnon (1950); two salt concentrations (50 and 100 mM NaCl as S1 and S2, respectively); and humic treatments with 10 ml humic acid in addition to the same salt doses (S1HA and S2HA) (Table 2). The pots, with a seedling in each, were set up as blocks using a completely randomized method (Mead and Curnow, 1983) at 23±2ºC. The moisture level of the mixture was maintained at 55%±5 and sets were exposed to 4000 to 4200 lux plant fluorescence intensity for 14/10 day and night periods, respectively (Akinci et al., 2009). Growth parameters were determined at the time of harvesting, 55 days after planting, by measuring seedling heights (PH), number of leaves (NL), leaf area (LA), and fresh weights of leaves (FLW), stems (FSW) and roots (FRW). Dry weights of these parts (DLW, DSW, DRW) were evaluated after 24 h in a drying oven to fully remove tissue water in the parts.

Nutrient analyses

The dried samples of leaves and roots were prepared by the wet-ashing method after Kacar (1972). Dried parts of plants were crushed using a mortar and pestle until powdered. The powder was put in an Erlenmeyer flask and a mixture of 6 ml nitric acid + perchloric acid was added. The mixtures were digested for 30 min at 40ºC in a water-bath and the supernatant solution was removed by heating at 150 to 180ºC until 1 ml extract remained. The residue was treated with distilled water to dissolve and made up to 100 ml in coloured bottles. The elements Na, K, and Ca were determined by flame photometry, and Mn, Zn and Mg by ICP-AES.

Statistical analysis

All data from the eight randomized replicates of each treatment, including controls, were analyzed with SPSS (13.0 for Windows) for paired-sample T test. Growth and nutrient data were considered to be significant at the level of $P < 0.05$ (5% significance level for differences between means). Means are indicated with standard error (± S.E.) given in tables and shown as error bars in graphs.

RESULTS AND DISCUSSION

Effect of NaCl and HA on growth parameters and of mung bean seedlings

The results of tested growth parameters for the two levels of NaCl, controls (Hoagland) and humic acid treatments are presented in Table 3. Both salt concentrations (50 and 100 mM NaCl) significantly decreased plant height 39.9 cm and 41.7 cm, respectively compared to HO. Many studies had reported and agreed that salt stress caused similar plant height reduction in watermelon (Yetisir and Uygur, 2009); in pepper (Aktas et al., 2006); in maize (Irshad et al., 2002); in muskmelon (Carvajal et al., 1998); in tomato (Casierra-Posada et al., 2009); in common bean (Beltagi et al., 2006); in red-osier dogwood (*Cornus sericea*) (Mustard and Renault, 2006); in common bean (Gama et al., 2007).

In the study, HA caused an increase in plant heights in both salt treated plants, which were 17% and 9.8% taller in S1HA1 and S2HA2, respectively compared to S1 and S2, but these differences were not significant at the level of α=0.05 (Table 3). Numerous reports are available on the positive effect of HA on the growth of a wide variety of plants such as corn (*Zea mays* L.) (Tan and Noparmornbodi, 1979; Eyheraguibel et al., 2008), sunflower (*Helianthus annuus* L.) (Kolsarici et al., 2005), marigold (*Tagetes patula* v. Antigua Gold F1), pepper (*Capsicum annuum* grossum v. King Arthur), strawberry (*Fragaria ananassa* v. Tribute) and tomato (*Lycopersicon esculentum* v. Rutgers) (Arancon et al., 2003).

However, reports of HA addition to plants grown under salinity are limited, with positive effects on growth and

Table 3. Growth parameters of mung bean seedlings under salt stress and humic acid.

Growth parameters	Treatments				
	Control	S1	S2	S1HA	S2HA
Shoot heights (PH) cm	37.36±4.320	22.45±2.422*	21.79±1.377*	26.28±2.843	23.93±1.665
Root lenghts (RL) cm	22.10±2.096	16.34±1.255	16.33±0.943*	24.83±1.255**	23.33±1.632***
No of leaves (NL)	4.0±0.327	2.7±0.313*	2.6±0.183*	3.2±0.164	2.9±0.227
Leaf area (LA) cm^2	140.85±0.227	67.96±7.66*	61.55±12.23*	84.90±7.42	78.73±17.12
Leaves fresh weight (LFW) (g)	2.03±0.317	0.77±0.121*	0.78±0.121*	1.52±0.281**	1.16±0.227
Leaves dry weight (LDW) (g)	0.21±0.034	0.10±0.015*	0.10±0.033*	0.15±0.030	0.11±0.026
Stem fresh weight (SFW) (g)	1.52±0.289	0.70±0.098*	0.58±0.126*	0.85±0.115*	0.79±0.178
Stem dry weight (SDW) (g)	0.13±0.015	0.06±0.005*	0.05±0.008*	0.08±0.008**	0.07±0.011
Root fresh weight (RFW) (g)	0.80±0.146	0.41±0.072	0.35±0.060*	0.57±0.100	0.50±0.095
Root dry weights(RDW) (g)	0.04±0.004	0.03±0.006*	0.02±0.005*	0.04±0.008	0.03±0.006

±: Standard errors *: Significantly different from controls **: Significantly different from S1
***: Significantly different from S2

plant height reported by Unsal (2007) and Daur and Bakhashwain (2013) in chick pea; Sani (2014) in Canola; David et al. (1994) in tomato; Refaiy et al. (2016) in banana plantlets. Delfine et al. (2005) stated that in durum wheat, HA content caused increase in plant heights. Gulser et al. (2010) also stated that lower doses (1000 and 2000 mg kg^{-1}) of HA had a positive effect on pepper seedling growth under saline conditions, although higher HA application rate (4000 mg kg^{-1}) had a negative effect.

Root length of *Vigna* seedlings decreased in both salt treatments by 35.3 and 35.4% for S1 and S2 respectively, whereas the latter was significant at α=0.05 compared to controls (HO). HA treatment caused a significant increase in root length compared with S1 and S2, by 51.9 and 42.9% in S1HA and S2HA, respectively. Available reports stated that various salts caused a decrease in germination rate, root and shoot length in *Pisum sativum var., abyssinicum and Lathyrus sativus* (Tsegay and Gebreslassie, 2014), reduction of root length in tomato plants (Turkmen et al., 2002), root development inhibition in *Arabidopsis* and tobacco (Jang et al., 2007), and decrease in root length of pumpkin (Kurum et al., 2013); *Raphanus sativus* L. (Jamil et al., 2007).

However, humic acid addition caused an increase in length and dry weight of maize plant roots (Eyheraguibel et al., 2008) and root length in *H. annuus* L. (Kolsarici et al., 2005), stimulated root development in ryegrass (Bidegain et al., 2000), increased root biomass in broad bean (Buyukkeskin et al., 2015), increased root growth in pepper (Cimrin et al., 2010). These studies all agree with the present findings of an ameliorative effect of humic acid on root growth and development of mung bean.

According to David et al. (1994) humic substances promote plant growth through better-developed root systems that provide more minerals to the whole plant. Leaf number (NL) decreased in both salt treatments in the present experiment, with significant reductions compared to control plants leaf number (P= 0.002 and P= 0.014, < α= 0.05, in S1 and S2, respectively). Our results showing that NaCl has an inhibiting effect on plant growth parameters and reduction in developing parts of seedlings such as decreasing of leaves are similar to related works on corn (Irshad et al., 2002); *Pennisetum clandestinum Hochst* (Muscolo et al., 2003), tomato (Romero-Aranda et al., 2001; Lopez and Satti, 1996; Caines and Shennan, 1999), melon (Morabito et al., 1996).

On the other hand, with 50 and 100 mM humic acid addition to the salts, NL increased by 18.2 and 9.5% in S1HA and S2HA, respectively, although these did not differ statistically from the salt treatments. Similar effects of humic acid had been documented by various reseachers in agreement with the present study in plants such as tomato and eggplant (Dursun et al*.,* 1998), pepper (Cimrin et al., 2010), tomato (Turkmen et al*.,* 2004), and common bean (*Phaseolus vulgaris* L.) (Meganid et al., 2015).

Mean leaf area (LA) significantly decreased in both 50 and 100 mM NaCl treatments, with reductions of 51.8 and 56.3% (p=0.010; p=0.001 <α=0.05), respectively compared to HO. Some reports state that a reduction in LA is a result of harmful effects of salt in various plants such as eggplants (Yasar, 2003); pepper hybrids (Chartzoulakis and Klapaki, 2000); tomato (Casierra-Posada et al., 2009); *Phaseolus* (Bayuelo-Jimenez et al., 2012; Aydin et al., 2012); *R. sativus* L. (Jamil et al., 2007); *Vicia faba* L. (Qadas, 2011); and *Solanum lycopersicum* L. (Casierra-Posada et al., 2009).

The obtained data from the present experiment showed that with HA addition to the two different salt concentrations, LA of those seedlings increased 24.9 and 27.9% but neither of them was significant. A positive effect of humic acid was obtained by Aydin et al. (2012) in *Phaseolus*; Dursun et al. (1998) in tomato and eggplant; and Buyukkeskin and Akinci (2011) in broad bean grown under saline conditions.

The leaf fresh weights in the present experiment were significantly less in S1 and S2 treatments, at 0.772 g (P=0.006 <α=0.05) and 0.784 g (P= 0.009 <α=0.05) respectively, compared with weights in control plants of 2.031 g. For plants treated with humic acid, the fresh weights increased significantly by 98.1% in S1HA (P=0.044 <α=0.05), while the fresh weight increase in S2HA was 48.5%, although this did not differ significantly at the level of α=0.05. The obtained results from the present study is similar to studies on different plants such as potato (Heuer and Nadler, 1995); tomato (Lopez and Satti, 1996); pumpkin (Kurum et al., 2013); *R. sativus* L. (Jamil et al., 2007).

The mean leaf dry weights of mung bean seedlings decreased significantly in both S1 and S2, by 52.8% (P=0.006 <α=0.05) and 51.4% (P=0.049 <α=0.05), respectively compared to controls. This result agreed with various studies such as Dolatabadian et al. (2011) for soybean; Taban et al. (1999) for *Z. mays* L. cvs.; Rodriguez et al. (2005) for *Asteriscus maritimus*; Ramoliya and Pandey (2002) for *Salvadora oleoides*; Tuncturk et al. (2008) for *Glycine max* L. Merrill; and Reina-Sanchez et al. (2005) for tomato.

In our results, with HA addition to salt treatments LDW increased in S1HA (48%) and S2HA (10.8 %), however both increase were less than control plants without any significance at the level of <α=0.05. Stem fresh (SFW) and dry weights (SDW) significantly decreased in both salt treatments. The decrease in fresh stem weights was 53.7% (P= 0.025) and 61.2% (P=0.013), while dry weights of mung bean leaves were 53.4% (P= 0.004) and 60.2% (P=0.003) in S1 and S2, respectively compared to HO.

Reduction in shoot or stem fresh and dry weights as a result of salinity had been reported for different plant species (Adams, 1988; Ashraf and McNeilly, 1990; Mishra et al., 1991; Zaidi and Singh, 1993; Satti and Al-Yahyai, 1995; Ashraf and O'leary, 1997; Leidi and Saiz, 1997; Caines and Shennan 1999; Ashraf et al., 2001; Kaya et al., 2001; Cakir, 2004; Jamil et al., 2007; Turkmen et al., 2008; Tuncturk et al., 2008; Qados, 2011; Kurum et al., 2013).

For plants treated with HA, both fresh and dry weights of shoots were higher in HA1 and HA2 than plants grown only in NaCl (S1, S2), and the increase in fresh weights were 20.6% in S1HA and 35.8% in S2HA. On the other hand, the dry weights of S1HA significantly differed from S1 with a 41.9% (P=-0.017) increase, and S2HA increased by 30.2% compared to S2. Regarding positive effects of HA on salt treated plants, some reports state that stem/shoot weight increase occured in eggplant (Dursun et al., 1998); in *Chrysanthemum indicum* L. (Mazhar et al., 2012); in *Linum usitatissimum* L. (Bakry et al., 2014); in banana plantlets (Refaiy et al., 2016); and in pepper (Gulser et al., 2010).

Fresh weights of roots decreased by 48.4% in S1 but did not differ significantly. However, the root fresh weight decreased (57%) significantly (P=0.018) in S2 compared to controls. A decrease in root fresh weights have been recorded in various plants grown under salinity such as wheat and barley (Adiyaman, 2005); tomato (Lopez and Satti, 1996); Pumpkin (Kurum et al., 2013). The present study revealed that for mung bean seedlings grown with salt and HA together (S1HA and S2HA), RFW increased in both HA treatments, with a 40% and 43.4% increase in S1HA and S2HA, respectively, however, these differences were not significant.

On the other hand, dry weight of root decreased similarly in both salt treatments (S1, S2) and increased with HA treatment of mung bean seedlings (S1HA, S2HA). The decrease in S1 and S2 was significant, at 40 and 55.6% (P= 0.001; P= 0.008), respectively. The findings of the present study is supported by various reports that salinity causes root dry weight reductions in different plants such as soybean (Tuncturk et al., 2008); maize (Ashraf and McNeilly, 1990); spring wheat (Ashraf and O'leary, 1997); pepper (Turkmen et al., 2008).

Regarding the remediative role of humic acids on mung bean root dry weights, although weights increased in S1HA by 33.3% and in S2HA by 70%, neither differed significantly at the level of α=0.05. These results agree with the research articles on pepper (Cimrin et al., 2010; Gulser et al., 2010); and common bean (Aydin et al., 2012; Meganid et al., 2015).

Effects of HA on nutrient content of the mung bean seedlings

The measured nutrients, namely Na, K, Mg, Ca, Mn and Zn, were determined separately as root and above (shoot) parts of mung bean seedlings grown in C, S1, S2 and S1HA and S2HA. All results from the present study were evaluated and presented in Figures 1 and 2 as mg/g (Mn and Zn µg/g).

Sodium

Na concentration was the highest of the analyzed elements in both above and underground parts of mung bean, with higher concentrations in above ground parts than the roots.

1. **Roots:** The concentration of Na in roots increased 30.4% in S1 plants; however, this was not significant at the level of α=0.05. The increase in S2 roots was significant (p=0.047 < α=0.05), although the rise was slightly lower than S1 (Figure 1). Na accumulation in roots of S1HA plants was slightly increased compared to HO plants. Na content was highest in S2HA with the increase significant compared to S2.

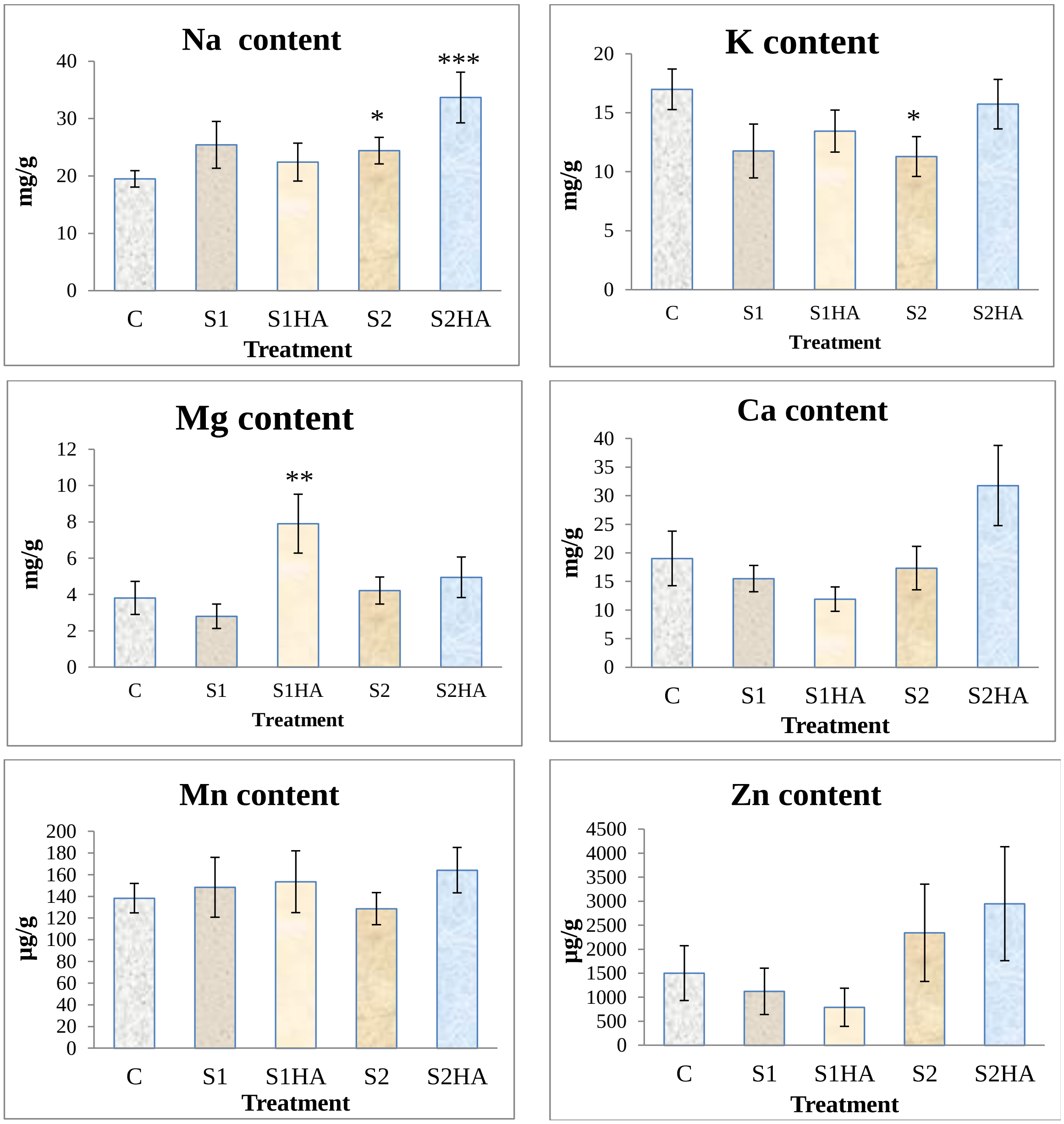

Figure 1. Mineral contents in mung bean root,.*: Significantly different from controls, **: Significantly different from S1, ***: Significantly different from S2.

2. **Shoots:** The tested Na amount in leaves and shoots together increased in all salt and humic-salt treatments compared to HO. Na content increased up to 40 and 60.7% in S1 and S2, respectively, and both were significant (p=0.01 < α=0.05). Humic addition in S1HA and S2HA caused a decrease of Na by 17.4 and 13%, however the decline was not significant in either case (Figure 2).

Potassium

K content was less than the amount of Na and was higher in upper parts of plants than in roots.

1. **Roots:** K content decreased significantly in both salt treated experimental groups by 30.8% (p=0.045 < α=0.05) and 43.6% (p=0.03< α=0.05) compared to controls (Figure1). Salt with humic treatments caused a slight increase in K in S1HA and S2HA, with rises of 14.3 and 39.4%, respectively, but neither was significant.

2. **Shoots:** The amount of K decreased with increasing salt concentration. The decreases were 28.8 and 38.9% in S1 and S2, respectively (Figure 2). K reduction in both salt treatments was significant at the levels of p=0.009 <

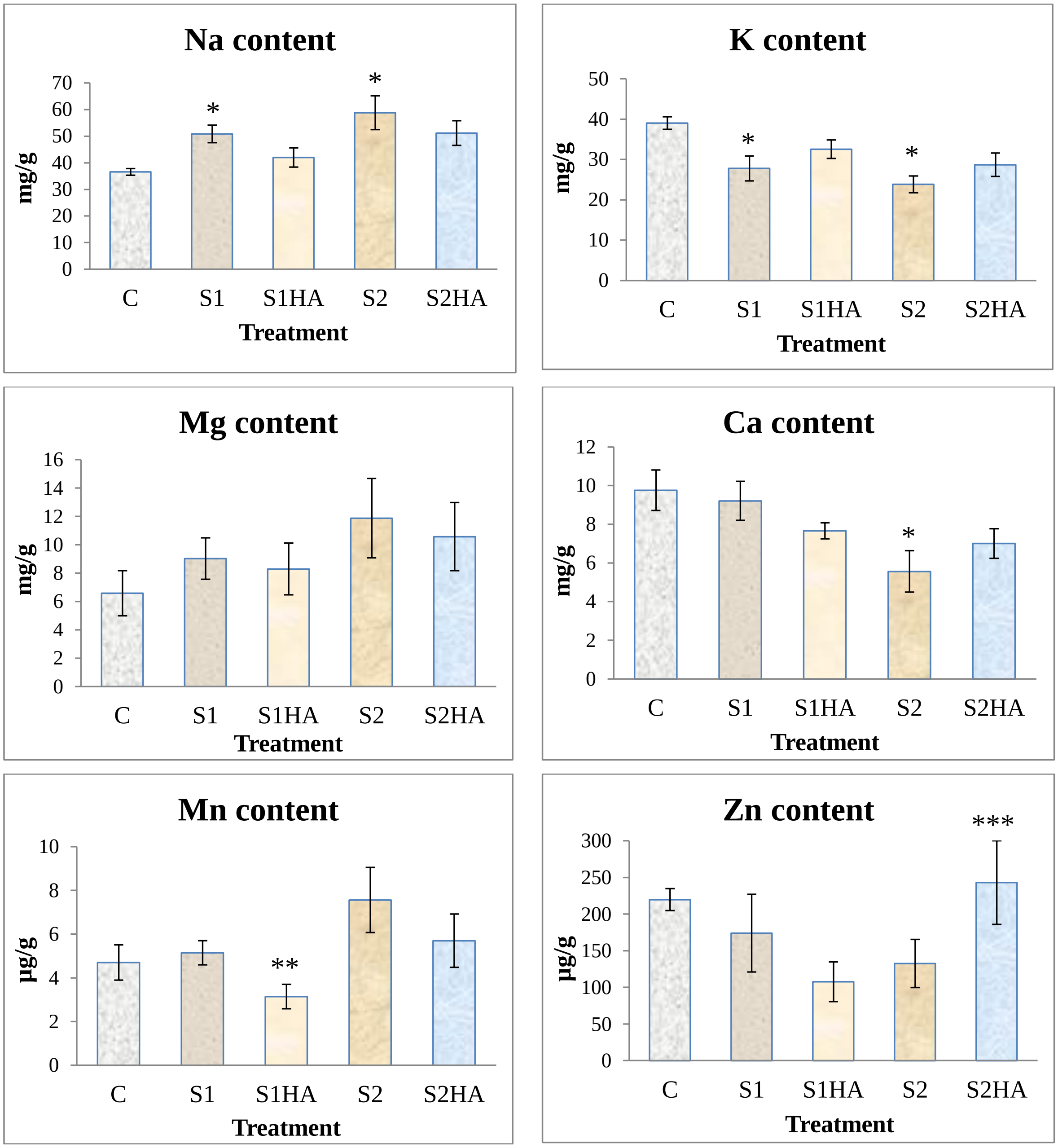

Figure 2. Mineral contents of above part of mung bean, *: Significantly different from controls, **: Significantly different from S1, ***: Significantly different from S2

α=0.05 for S1 and p=0.001< α=0.05 for S2. Humic acid addition to both salt treatments caused the K content to increase in S1HA and S2HA compared to S1 and S2 but both humic plus salt treatments decreased K compared to control values without any statistical significance.

Magnesium

Mg content was the lowest of the elements tested, after Zn and Mn, in both root and above parts of *Vigna* seedlings.

1. **Roots:** The Mg content varied in both salt and salt with humic treatments compared to controls. It decreased by 36.5% in S1 while it was increased in S2 (10.7%) without any significance. However, adding Humic acid increased the Mg content significantly in S1HA by 182.2% (p=0.008< α=0.05) compared to S1. In S2HA, the increase in Mg was only 17.3% above S2 root (Figure 1).

2. **Shoots:** Unlike roots, Mg content increased in all

treatments without any of these being significant. Increase in Mg was 37.1% and 80.3% in S1 and S2 respectively (Figure 2). HA treatment caused a slight decrease in Mg of 8.2% in S1HA and 11% in S2HA compared to both salt treated plants with 50 and 100 M NaCl.

Calcium

Ca as a proportion from all treatments of *Vigna* seedlings was quite close to K content present in root parts, however its ratio was lower than the content of K in above ground parts for all salt and salt-humic treated seedlings.

1. **Roots:** Ca content in both salt treatments and in S1HA was decreased but the latter differed significantly compared to 50 mM salt treated *Vigna* seedling root ($p=0.03 < \alpha=0.05$). Although, the treatment of S2HA increased the amount of Ca by 83.2%, this increase was not significant at the level of 5% (Figure 1).

2. **Shoots:** The proportion of Ca in all treatments including S2HA -contrary to root- decreased compared to HO values. The decrease of 43% in S2 was significant ($p=0.036 < \alpha=0.05$). The humic acid treatment with 100 mM salt caused a slight increase in Ca compared to plants treated with only 100 mM NaCl (S2) (Figure 2).

Manganese

Mn content showed fluctuations in both roots and shoot samples compared to controls. The ratio was found to be higher in roots than in shoots.

1. **Roots:** The changes in Mn concentrations in the root were higher in S1, S1HA and S2HA compared to controls (Figure 1). Both humic acid addition to salts increased Mn level by 3.4% in S1HA and 27.6% in S2HA compared to S1 and S2, respectively, but neither differed significantly.

2. **Shoots:** Mn content increased in both salt treatments by 9.4 and 60.7% compared to controls (HO), but without significance, while humic addition caused reductions in S1HA and S2HA when compared to salt-only treated samples (S1 and S2, respectively). The decrease in Mn concentration was 38.9% in S1HA (Figure 2) which was significant compared to 50 mM salt treatment ($p=0.034 < \alpha=0.05$).

Zinc

Zn is an essential micronutrient for crop production. It appeared as a high proportion of mung bean seedlings compared to Mn which is also a micronutrient measured in micrograms. Zn content in all treatments showed similar behaviour in both root and shoot samples.

1. **Root:** Zn content decreased in S1 by 25.2% compared to controls and in S1HA declined by 29.6% compared to controls. Zn concentration increased in S2 by 55.8% over HO and in S2HA also rose by 25.8% compared to S2 values. However, none of these changes showed any statistical difference at the level of $\alpha=0.05$ (Figure 1).

2. **Shoot:** As in roots, the Zn concentration decreased in S1 and S1HA by 20.8 and 38.1% compared to controls and S1 samples, respectively (Figure 2). The Zn content also decreased in S2 by 39.7%; however, humic addition to 100 mM NaCl caused a significant increase of 83.4% compared to S2 samples ($p=0.046 < \alpha=0.05$). The investigation above mainly focused on better understanding of physiological responses of six main crop nutrients, namely Ca, K, Na, Mg, Mn and Zn, to understand their uptake mechanism under salt stress. It is clear that salinity is one of the major stress factors that drastically affect many areas in the world, causing serious crop production impacts for salt sensitive plants particularly.

The study revealed the impact on growth of two NaCl levels on a very salt sensitive cultivar of mung bean (Ashraf and Ras, 1988) and the effective remediative role of humic acid when added to both salt treatments. It is well known that salinity has a drastic effect on general plant growth through osmotic stress and ion toxicity (Grattan and Grieve, 1998; Munns, 2002; Hanumantha et al., 2016), and causes a decline in the rates of net photosynthesis and decrease in nutrient uptake (Parida and Das, 2005; Cha-Um and Kirdmanee, 2009).

According to Dash and Panda (2001) and Delgado and Sanchez-Raya (2007) the decrease in root and shoot development was brought about by the inhibition of cytokinesis and cell expansion as a result of salt stress. Salinity may also cause a reduction in rhizobial nodulation directly or indirectly in legumes (Hanumantha et al., 2016) resulting in reduced N accumulation (Alam, 1994; Turan et al., 2007 a,b).

The present study revealed that the lower S1 salt concentration significantly affected the measured growth parameters except root lengths and fresh weights compared to control plants. However, the increased salt concentration of 100 mM in the S2 treatment caused a significant reductions in all parameters compared to control values. The findings for mung bean agree with reports for other Fabaceae species, including plant growth retardation in soybean (Grattan and Maas, 1988; Elsheikh and Wood, 1995), chickpea (Elsheikh and Wood, 1990), pea and faba bean (Delgado et al., 1994), shoot and root weight reduction in broad bean (Yousef and Sprent, 1983; Zahran and Sprent, 1986) and relative growth rate in bean (Gama et al., 2007).

Under salinity, many plant species have developed various mechanisms to cope with the osmotic and ionic effects and their inbition of plant growth (Munns and Tester, 2008), such as a reduction in osmotic potential by accumulation of ions (Na^+ Cl^- and K^+) or organic solutes (Example. soluble carbohydrates, glycinebetaine, proline) (Rodriguez et al., 1997; Azevedo Neto et al., 2004; Hasegawa et al., 2000; Lacerda et al., 2001, Bhaskaran et al., 2013), or increasing total soluble proteins (Ashraf and O'Leary, 1999) and free amino acids, particularly in salt sensitive plants (Azevedo Neto et al., 2009).

Humic substances have been known to have an enhancing effect on plant shoot and root growth, through effects on cell membranes by solubilization of macro and micro mineral nutrients, promoting photosynthesis, regulating enzyme activity, behaving like plant hormones, reducing toxic elements and increasing microbial populations (Chen and Aviad, (1990); Fagbenro and Agboda, (1993); David et al., (1994); Seyedbagheri, (2010); Gholami et al., (2013). Although enhanced stress resistance is demonstrated, its physiological mechanism has not been well established (Delfine et al., 2005).

Humic acids have been used to counter abiotic stress factors such as drought, cold, high-salinity and heat. It is believed that their application alleviates stress effects; in other words, it has positive effects on stress tolerance by stimulating nutrient uptake and improving the antioxidant system (Fernandez-Escobar et al., 1996; Buyukkeskin et al., 2011; Aydin et al., 2012). The humic acid preparation used was the same as from previous studies (Buyukkeskin et al., 2011), namely 10 ml/1L Hoagland – Arnon solution. The new mixture of solutions with humic acids and 50 and 100 mM NaCl concentrations was applied to the seedlings to assess the alleviative effects on a salt sensitive plant *V. radiata* (L.) Wilczek compared to salt treated control plants.

Nearly all growth parameters declined under salt treatments as expected, with salt causing reductions in plant growth and development, with the effect increasing with salt concentration (Table 3). Growth and development effects are mostly related to the excessive uptake of potentially toxic ions (Grattan and Grieve, 1998). Salt may cause restriction of water absorption and impact on the biochemical processes employed in plant growth, particularly a decline in the rate of net photosynthesis due to negative effects on the CO_2 influx into leaves, as well as excessive decrease in mineral nutrients in the roots (Parida and Das, 2005; Cha-Um and Kirdmanee, 2009).

Reduced dry weight on treatment with a low level of NaCl was reported by Al-Karaki (1997) and Taban et al. (1999). The results obtained was that the current study were similar to these findings for 50mM salt; however, 100 mM NaCl continued to inhibit growth parameters of mung bean seedlings except leaf fresh and dry weights (Khan et al., 2000; Asik et al., 2009). Application of humic acid to each salt level (50 and 100 mM) clearly revealed that the humic treatment mitigated the detrimental effect of salts on salt sensitive plants such as mung bean tested in this study.

The humic acid treatment affected all growth parameters compared to salt-only treatments (that is, S1 to S1HA and S2 to S2HA), with significant increases in S1HA and S2HA root length, but leaf fresh and weight increased in S1HA only, compared to S1 (Table 3). These findings supports reports by Turkmen et al., (2004) in tomato; Masciandaro et al., (2002) in maize; Pilanali and Kaplan (2002) in strawberry; Turkmen et al. (2005) and Gulser et al. (2010) in pepper. Aydin et al. (2012) reported that HA ameliorated soil and mitigated unfavorable effects on growth and development of beans grown in salty conditions. Meganid et al. (2015) found significant increases in plant height, number of leaves, root length, shoot and root fresh weights of common bean grown under salt stress following application of HA.

Francois and Maas (1999) reported that solubility of micronutrients is particularly low in plants exposed to salt stress which show mineral deficiencies. The same report stated that the detrimental effects of salt are observed at the whole plant level. Studies of mineral uptake by plants under saline conditions by various researchers report reduction of some nutrients such as N (Alam, 1994; Turan et al., 2007a,b), P (Navarro et al., 2001) and K (Lopez and Satti, 1996). Yermiyahu et al. (1997) stated that Na had an antagonistic effect on the uptake of Ca and Mg by reason of displacement of those elements by Na in roots. On the other hand, according to Kurum et al. (2013) NaCl caused Na, K and Ca increases in pumpkin varieties exposed to salt stress.

The mitigatitive role of humic acids on detrimental effects of salt on plants may be evaluated by growth parameters and mineral nutrients uptake together. HA effects on nutrient uptake in unstressed plants have been much studied, with varied HA quantites, growth conditions and species of plant. For instance, HA prolonged uptake of P, K, Na, Mg, Ca, Mn, Fe, Zn and Cu in tomato and eggplant (*Solanum melongena* L.) plants (Dursun et al.,1999); in maize N, P, K, Ca, Cu, Mn, Zn and Fe (Eyheraguibel et al., 2008; Sharif et al., 2002); N, P, K in maize and tomato stem (Abdel-Mawgoud et al., 2007) and in melon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai leaves (Salman et al., 2005) and P, K, Ca, Mn, Fe, Mg and Zn in tomato stem (David et al., 1994); P, K, Mg, Na, Cu and Zn in foliar application of humic in corn (Khaled and Fawy, 2011).

Foliar HA and soil application of humus increased the uptake of P, K, Mg, Na, Cu and Zn in wheat (Asik et al., 2009). Bakry et al. (2014) stated that using humic acids on previously salt stressed flax varieties increased the absorption of Fe and P as well as other nutrients to improve nutritional status of plants. In terms of nutrient availability, the current experiment evaluated mung bean root and shoot content of Na, K, Mg, Ca, Mn and Zn for controls (C), two levels of salt treatment (S1, S2), as well as HA treatments to previously salt stressed plants (S1HA, S2HA). Both HA treatments appeared to mitigate the effect of salt, enhancing growth parameters previously reduced by Na in shoots.

However, whilst S1HA decreased Na uptake in roots, S2HA increased uptake of Na when compared to S1 and S2 salt levels, respectively. The detrimental effect of Na was seen in shoots, where HA decreased the Na content in both salt treated plants. The findings agree with those of Cimrin et al. (2010) in which increasing HA doses decreased Na content in both root and shoot of pepper seedlings. Jarošová et al. (2016) stated that HA application to salt exposed cultured barley plants reduced Na accumulation in both above-and below-ground tissues.

In the present study, in mung bean root, HA continued to decrease Na in S1HA treatment, however, S2HA Na content increased significantly compared to samples grown in 100 mM salt stress (Figure 1). This increase may be related to excessive flow of Na cations accumulated in roots penetrating cell membranes, since humic acid stimulates root growth and increases membrane permability (Valdrigh et al., 1996). According to Khaled and Fawy (2011), application of humic acid 0.1% by foliar caused an increase in Na content, as well as N, P, K, Ca, Mg, Fe, Zn, and Mn, in corn. Asik et al. (2009) also stated that soil humus and foliar HA application increased Na uptake in wheat and significant Na increase was also reported by Liu and Cooper (2002) in creeping bentgrass.

K content in both root and shoot of mung bean, showed the opposite of Na, decreasing significantly in all salt treatments. Nevertheless, humic acid treated S1 and S2 (that is, S1HA and S2HA), increased K concentration in both root and shoot samples. These results agree with the studies carried out by Khaled and Fawy (2011) using foliar application of humic acid on corn, and Jarošová et al. (2014) in spring barley, who stated that K accumulation was found under saline and HA treatment. Ashraf et al. (1994), Turkmen et al. (2000) and Sensoy et al., (2007) found that increasing K has an important role in making plants more tolerant to salt stress.

In terms of magnesium content in mung bean root, the increase compared to HO on addition of HA to previously salinity-treated samples agree with reported Mg increase by Khaled and Fawy (2011) in corn, Liu and Cooper (2012) in creeping bentgrass, and Cimrin et al. (2010) in pepper shoot.

Shoot Mg content was slightly higher than it was in root, and while Mg accumulated in both salt treatments compared with controls, HA decreased the Mg content compared to previously salt exposed samples (Figure 2). HA seemed to regulate the Mg in the shoot, thereby influencing growth parameters and decreasing stress under salinity. Mg may bind to HA, which is a polydentate ligand with high chelating capacity but this binding mostly depends on the number of available dentates and pH of the medium.

Although Ca content in root was relatively higher than in shoot, both root and shoot Ca concentrations behaved similarly. Ca was slightly decreased by 50 mM NaCl (S1) in root and shoot, while HA treatment caused a reduction in this decrease in both root and shoot, with the former significant compared with S1. The Na:Ca ratio in mung bean shoots was 5.5 in S1 and 10.5 in S2 plants. Simiarly, after HA treatment, the ratio increased to 5.4 and 7.3 in S1HA and S2HA, respectively. These Figures agree with previous studies showing that plant Ca level is usually between 5 and 10 mM under salt stress, depending on the salinity level and genotype (Cramer, 2002).

In root, Ca content in S2 was 8.9% less than HO values; however, in S2HA Ca increased by 83.2% compared with S2, although this was not significant. Similarly, shoot Ca content of S2 decreased significantly by 5.6% compared to controls (HO), while HA treatment resulted in an increase of 28.2% compared with S2. Since HA has carboxy and phenol groups, HA has the ability to form a calcium complex. Since Ca atomic diameter is larger than that of Na, with higher valence, Ca may bind to HA and be absorbed more easily by the roots. The obtained results from the present study of Ca increase in S2HA are supported by other studies stating that Ca and other nutrients increase in pepper (Cimrin et al., 2010) and where HA and $CaSO_4$ were used together in maize (Mohamed, 2012).

In the present study, Mn concentration in roots was nearly 20 times higher than in shoots. The finding of a higher amount of HA in previously salt treated root may relate to the chelating capacity of HA, which binds Mn tightly. The rapid decrease of Mn content in shoots supports previous Mn labelling experiments on young wheat (*Triticum aestivum* cv. Arina) plants which found fast Mn transport via xylem from root to shoot, but particularly that it is immobile in phloem (Page and Feller, 2005).

The same method on white lupin plants (*Lupinus albus*) gave similar results, with the presence of a large amount of labelled Mn in the root system, hypocotyls and stem, but release of Mn into xylem after a certain time (7 days) and a large amount reaching photosynthetically active young expanded leaves (Page et al., 2006). The accumulation of Mn in rhizosphere and subsequent uptake depends on pH changes, but HA may stabilize the soil pH near root area thus, facilitating absorption of available Mn and other nutrients.

The Zn content of root was 6 to 17-fold than in shoot. These findings agree with the report by Gupta et al. (2016), who stated that Zn levels are higher in roots than aerial parts because the photosynthetic shoots are protected against toxic effects of Zn. The finding from the present study can be explained by HA binding Zn similarly to Mn, with the high chelation capacity of humic acids holding Zn with the COOH unit. However, Zn was gradually released from Zn-HA complexes for plant consumption, which in cases of high concentration of Zn causes toxic effect (Bednerak et al., 2011).

Conclusions

The present study revealed that HA application caused bio-mass increase of mung bean root, which may lead to greater uptake of minerals such as K. The obtained results from the study also showed that humic acid mitigated the effects of moderate salt stress (50 mM NaCl) on mung bean growth, in other words it increased the resistance to salinity by decreasing the negative effect of Na. However, HA application in severe salt stress (100 mM NaCl) showed mixed results in terms of uptake of the nutrients tested, seemingly having no clear role in decreasing the detrimental effect of salt and uptake of those nutrients. Whilst HA increased K uptake Ca, Mg, Mn and Zn showed no significant changes, indicating that more detailed investigations are required to fully elucidate the role of HA on membrane permeability.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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