Medicinal and Aromatic Plant Science and Biotechnology ©2012 Global Science Books

Humic Acid Affects the Germination of Basil and Cumin and Alleviates the Negative Impacts of Salinity and Drought Stress

Maryam Haghighi[1*] • Jaime A. Teixeira da Silva[2] • Maryam Mozafariyan[3] • Fatemeh Roustaii[4]

[1] Horticulture Department, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
[2] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Ikenobe, 761-0795, Japan
[3] Horticulture Department, College of Agriculture, Shiraz University, Shiraz, Iran
[4] College of Natural Resources, Higher Education Complex Ardakan, Yazd, Iran

Corresponding author: * **haghighi@cc.iut.ac.ir, haghighi@shirazu.ac.ir**

ABSTRACT

An experiment was conducted to investigate the effect of humic acid (HA; 0, 1000, 2000 mg/L) on germination and on the length of stems and roots of cumin (*Cuminum cyminum*) and basil (*Ocimum basilicum*) under salinity (0, 3, 6, 9 mmhos/cm with NaCl) and drought (0, -2, -4, -8 Pa by PEG-6000) stress. HA – primarily at 1000 mg/L – had a significant effect on the germination of both medicinal plants. The effect of the interaction of HA with salinity and drought was significant, proving that HA could improve germination and stem and root length in the presence of salinity stress and drought, particularly at 1000 mg/L.

Keywords: *Cuminum cyminum*, humic substrate, *Ocimum basilicum*, organic
Abbreviations: FA, fulvic acid, **HA**, humic acid, **PEG**, polyethylene glycol

INTRODUCTION

The salinity of agricultural soil and irrigation water can be perceived as one of the most frequent causes reducing crop growth all over the world, especially in dry and semi-dry regions, such as Iran. More than half of Iran's area is considered to be semi-dry in which the amount of rainfall is ≤ 250 mm. Consequently, drought and the loss of crops it causes is an issue of concern in this area. An estimated 23% of the world's agricultural land is saline soil (i.e., 0.137 billion ha) (Jamil *et al*. 2005). Dryness and salinity affect different aspects of crop growth and tend to decreased germination and growth, delay development and reduce dry mass production, i.e., productivity (Jamil *et al*. 2005). Germination of *Lepidium latifolium* (Cao 2010) or cotton (Sun *et al*. 2002) seed was significantly inhibited under salt stress, more so as NaCl concentration increased.

Decreasing water potential caused by salinity and dryness negatively affected the germination and shoot/root growth of several pastural plants (Koochaki and Khetabi 1996). Drought stress reduced the germination rate, root and stem length of 6 *Salvia* spp. (Khoshkhoui *et al*. 2007) and the germination and growth of *Medicago sativa* (Tehranifar *et al*. 1997).

Humic substances consist of humic acid (HA) and fulvic acid (FA) (Tan 1998). Humic substances positively affected the germination of Chenopodium album (Šerá and Novák 2011). Nitrogen content of tomato (*Solanum lycopersicon*) increased by 4.1% and membrane permeability increased when 250 mg/L HA was used as the source of minerals (Valdrighi *et al*. 1996). Root length increased more than stem length in tomato when HA was added to the nutrient solution in the range of 0, 640, 1280, or 2560 mg/L (David *et al*. 1994), and similar root growth that exceeded shoot growth was observed in tobacco (*Nicotiana tabacum*) (Mylonas and McCants 1980), olive (*Olea europea*) with range of 30-240 mg/pot (Tattini *et al*. 1991) and other crops (Chen and Aviad 1990). Hartwigsen and Evans (2000) revealed that HA at 5000, 10,000, or 15,000 mg/L increased growth of *Pelargonium* × *hortorum* L.H. Bailey Freckles' (geranium) and *Tagetes patula* L. 'Bonanza' (marigold).

The effectiveness of HA on growth is due to the ready availability of HA to the plasmalemma of plant cells, as well as to the positive effect of HA on respiration and photosynthesis and enhancement of nutrient uptake like nitrogen, phosphorus and sulfur (Chen and Aviad 1990), and micronutrients, (Fe, Zn, Cu and Mn) (Chen and Aviad 1990).

Seed germination is a three-stage process: imbibition, lag phase, and protrusion of the radicle through the testa (Simon 1984). Improving germination in the first few days can enhance tomato production in saline conditions (Simon 1984). Basil and cumin are sensitive toward salinity and dryness at the germination stage. Some research about germination of cumin and basil in saline conditions has been conducted. Aliabadi Farahani and Maroufi (2011) showed that basil germination decreased with saline water used for irrigation although hydropriming (keep the seed in water before planting) could alleviate the stress. Basil germination in saline conditions was related to temperature; more seeds could germinate at 25°C than at 15 or 35°C with 0-15 ds/m salinity by mixing NaCl, Na_2SO_4, and $CaCl_2$ in a ratio of 2:1:1 (w/w/w) and then diluting the salt mixture in water to give solutions with electrical conductivities of 1, 3, 6, 9, 12, and 15 dS/m (Ramin 2006). Black cumin could tolerate salinity well up to 150 mM NaCl (Hajar *et al*. 1996).

However, the effect of organic matter on germination of basil (*Ocimum basilicum*) and cumin (*Cuminum cyminum*) in drought and saline stress has not yet been investigated and is the focus of this study.

MATERIALS AND METHODS

The experiment was conducted in a laboratory and in a factorial design arranged based on a completely randomized design (CRD) with 4 replications. Basil and *cumin s*eeds were surface sterilized in a 50% (v/v) solution of sodium hypochlorite solution for 10 min, rinsed 3 times with distilled water before transferring them to Petri

Received: 27 November, 2011. Accepted: 17 March, 2012.

Original Research Paper

Table 1 The effect of salinity and humic acid on germination and seedling characteristics of basil and cumin.

Osmium basilicum			*Cuminum cyminum*			S.O.V.
Germination (%)	**Root length**	**Shoot length**	**Germination (%)**	**Root length**	**Shoot length**	
52.629*	0.004ns	0.136ns	627.583**	0.142ns	0.022ns	Humic acid
490.104**	0.029ns	0.026ns	461.209**	0.178*	0.012ns	Salinity
89.236ns	0.051**	0.080ns	62.258ns	0.003ns	0.003ns	Humic acid× salinity
0.061ns	0.005ns	0.001ns	0.021ns	0.002ns	0.001ns	Drought
0.111ns	0.001ns	0.001ns	0.011ns	0.006ns	0.004ns	Humic acid×drought
58.488	0.013	0.008	34.152	0.001	0.005	Error
9.55	26.37	35.36	31.57	33.36	15.96	CV%

**Significant at 0.001 (LSD), * significant at 0.05 (LSD), ns not significant; SOV = source of variation.

Table 2 The effect of humic acid on the germination% and vigor index of basil and cumin.

Humic acid (mg/L)	Vigor index of basil	Germination% of basil	Vigor index of cumin	Germination of cumin (%)
H0	17.208 A	86.042 A	2.2262 C	11.13 C
H1	17.458 A	90.625 A	3.9405 B	25.506 A
H2	18.125 A	87.29 A	5.1012 A	19.702 B

Means within a column followed by the same letter are not significantly different at $P < 0.05$ (LSD test)

Table 3 The effect of salinity on the shoot and root length of basil and cumin.

Salinity mmoh/cm	Root length of basil (mm)	Shoot length of basil (mm)	Root length of cumin (mm)	Shoot length of cumin (mm)
S0	1.0307 A	0.6925 A	0.9588 A	0.8707 A
S1	1.0631 A	0.6551 AB	0.7927 AB	0.8239 A
S2	1.0035 A	0.6353 AB	0.6740 B	0.8138 A
S3	1.0412 A	0.5845 B	0.6508 B	0.7825 A

Means within a column followed by the same letter are not significantly different at $P < 0.05$ (LSD test)

Table 4 The effect of humic acid on the shoot and root length of basil and cumin.

Humic acid mg/L	Root length of basil (mm)	Shoot length of basil (mm)	Root length of cumin (mm)	Shoot length of cumin (mm)
H0	1.0784 A	0.6522 A	0.8569 A	0.8570 A
H1	1.0517 A	0.6521 A	0.8033 A	0.8375 A
H2	0.9738 A	0.6212 A	0.6470 A	0.7737 A

Means within a column followed by the same letter are not significantly different at $P < 0.05$ (LSD test)

Table 5 The effect of salinity on the germination% and vigor index of basil and cumin.

Salinity mmoh/cm	Vigor index of basil	Germination% of basil	Vigor index of cumin	Germination of cumin (%)
S0	17.944 A	89.722 A	5.3016 A	26.508 A
S1	17.889 A	89.444 A	4.4048 AB	22.024 AB
S2	15.528 B	95.139 A	3.3413 B	16.706 B
S3	19.028 A	77.639 B	1.9762 C	9.88 C

Means within a column followed by the same letter are not significantly different at $P < 0.05$ (LSD test)

dishes. 100 seeds were placed over Whatman No. 1 filter paper inside 6-cm Petri dishes (25 seed/Petri dish and 4 Petri dishes/ replicate). NaCl at 4 concentrations (S0 = 0, S1 = 3, S2 = 6, S3 = 9 mmoh/cm), drought stress at 4 levels (D0 = 0, D1 = -2, D2 = -4, D3 = -8 Pa with polyethylene glycol (PEG-6000) (Merck, Whitehouse station, NJ, USA) and HA at three concentrations (H0 = 0, H1 = 1000 and H2 = 2000 mg/L) were added to Petri dishes. Petri dishes were placed at 25 ± 2°C in a 16-h photoperiod under undefined light intensity.

The HA was humified forest soil sampled from Mount Jinyun (Chongqing, China) that contained 30% N, and 12% each of P_2O_5 and K_2O (all on a dry matter basis). Seeds that germinated were counted daily for 10 days and germination percentage (G%) was calculated on the last day. Seeds with a radical 2 mm long were considered to be germinated seeds.

After 10 days the length of root and stem was calculated with a ruler in mm.

Vigor index (VI) was calculated with a following formula according Moradi Dezfuli *et al.* (2008):

VI = G1+G2+…..GL/D1+D2+….DL

where G = number of germinated seeds; D = days after the last count; L = day.

Data was analyzed done with Statistix 8 (Tallahassee FL, USA). All data was subjected to a one-way ANOVA and the means were compared for significance by the least significant difference (LSD) test at $P < 0.05$.

RESULTS

The germination of cumin and basil was significantly affected by HA and salinity (**Table 1**). HA and salinity changed the G% significantly in basil (**Table 1**). G% and VI of basil increased when HA was applied and highest germination was observed with 1000 mg/L HA (**Table 2**). Salinity decreased G% and VI of basil and cumin. The G% of cumin was affected by salinity more than basil. This decrease was 30 and 13% in cumin and basil, respectively in S3. Germination of basil increased as salinity increased (Y = -0.255X + 112.95; $R^2 = 0.86$). Salinity decreased the root length of basil, and when HA was applied, root length increased under salinity stress but shoot length did not change (**Fig. 1; Table 4**). The effect of salinity and HA on root length of basil was significant (**Tables 1, 3**): when HA was applied, root length increased and shortest roots were observed in the control while the longest roots were in the presence of 2500 mg/L HA (**Fig. 1**). When HA1 was applied, G% was the same as the control under drought stress although HA2 improved G% of basil significantly (**Fig. 2**). HA1 and HA2 increased VI of basil seeds and HA1 alleviated the deleterious effect of drought stress and the results were similar to the control; on the other hand, HA2 decreased the VI of basil (**Fig. 3**). The interaction effect of drought and HA showed that a higher level of drought stress (-8 Pa) decreased G% of cumin while HA at both concentrations improved G% at all levels of drought stress (**Fig. 4**). Cumin root length decreased with drought stress and 1000 mg/L HA improved it significantly. HA2 reduced root length of cumin (**Fig. 5**) but HA1 increased cumin shoot length sig-

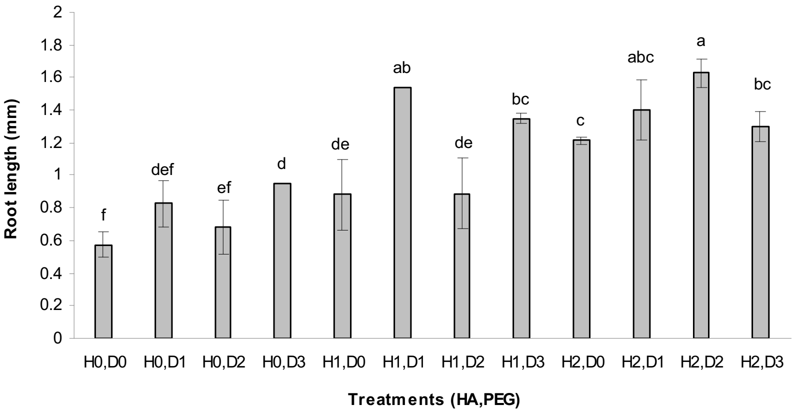

Fig. 1 The effect of salinity and humic acid on root length of basil. Means with the same letter are not significantly different at $P < 0.05$ (LSD test).

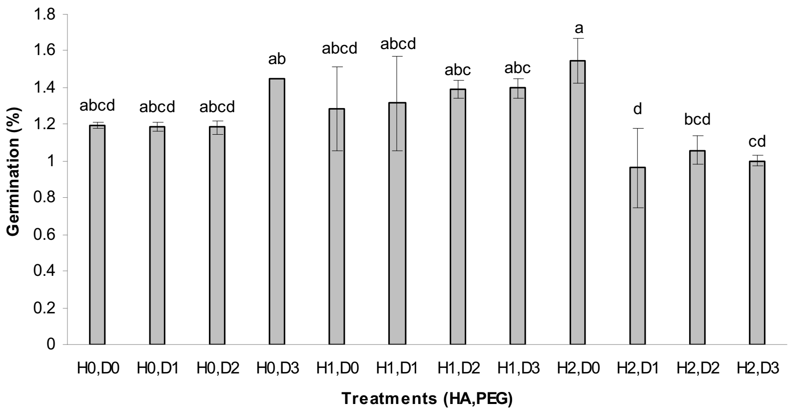

Fig. 2 The alleviation effect of humic acid on germination% of basil under drought stress. Means with the same letter are not significantly different at $P < 0.05$ (LSD test).

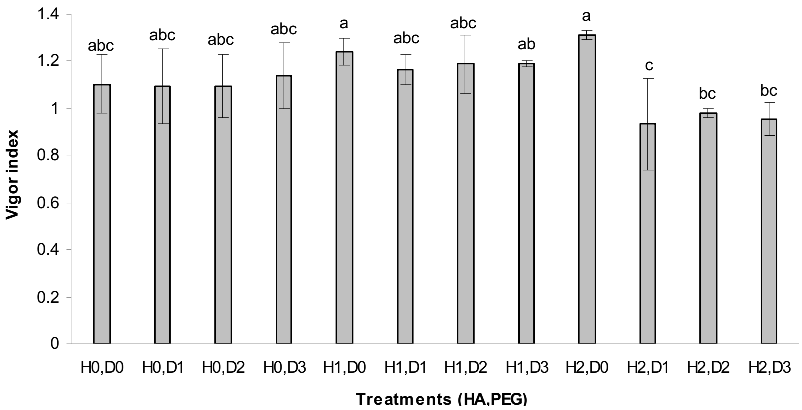

Fig. 3 The alleviation effect of humic acid on seed vigor index of basil under drought stress. Means with the same letter are not significantly different at $P < 0.05$ (LSD test).

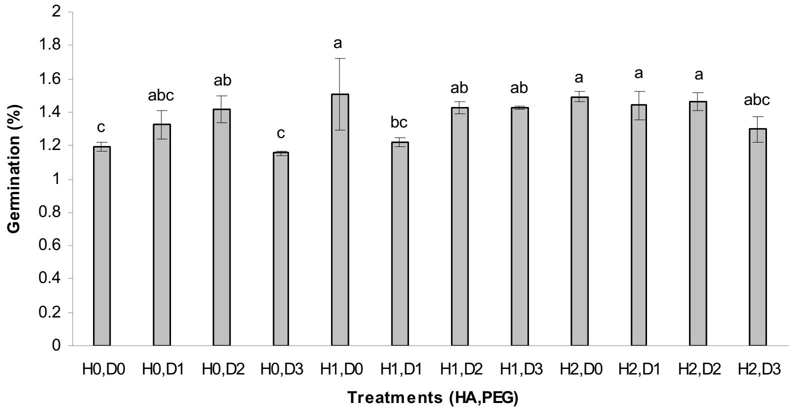

Fig. 4 The alleviation effect of humic acid on cumin germination% under drought stress. Means with the same letter are not significantly different at $P < 0.05$ (LSD test).

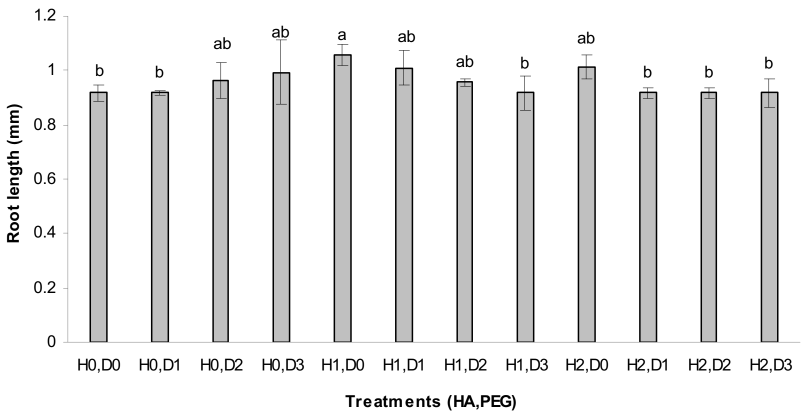

Fig. 5 The alleviation effect of humic acid on cumin root length under drought stress. Means with the same letter are not significantly different at $P < 0.05$ (LSD test).

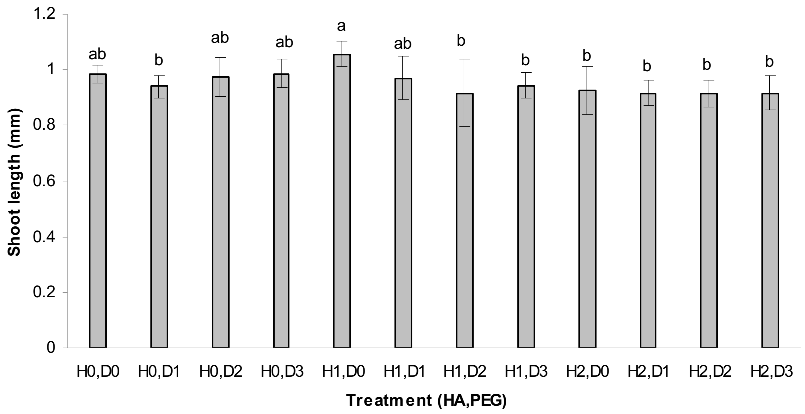

Fig. 6 The alleviation effects of humic acid on cumin shoot length under drought stress. Means with the same letter are not significantly different at $P < 0.05$ (LSD test).

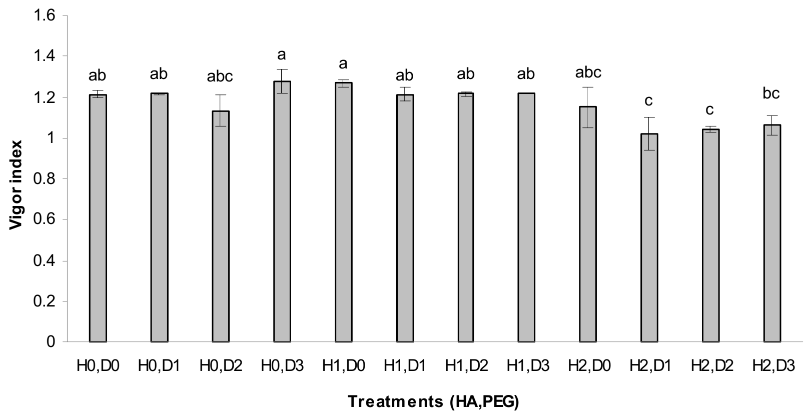

Fig. 7 The alleviation effect of humic acid on seed vigor index of cumin under drought stress. Means with the same letter are not significantly different at $P < 0.05$ (LSD test).

nificantly and alleviated drought stress; HA2 decreased shoot length (**Fig. 6**). Root length of cumin and shoot length of basil decreased with increasing NaCl concentration (**Table 3**). HA decreased the VI of cumin (**Fig. 7; Table 5**). Root length at 3, 6, 9 mmhos/cm in cumin was reduced by 16, 29 and 32% but no trend was observed for basil root length. Salinity reduced basil shoot length by 5, 7 and 15% at 3, 6, 9 mmhos/cm NaCl.

DISCUSSION

The loss in germination ability and speed is presumably because of the accumulation of toxic ions and a reduction of water uptake by seeds (Uhrits 1974; Koochaki *et al.* 1996). Salinity decreased alfalfa and lentil shoot length (Bagheri *et al*. 1988; Shariat Jafari 1997), possibly due to inadequate nutrition transfer from cotyledon to embryo (Shariat Jafari 1997). In this study, HA assisted basil (more than cumin) to better endure salinity. Other studies that used HA showed improved growth of crops, namely an increase in the length of the stem and roots (Vaughan and Linehan 1976 for wheat; Tan and Nopamornbodi 1979 for corn; Malik and Azam 1985 for *Triticum aestivum* L.; Sanders *et al.* 1990 for carrot; David *et al*. 1994 for tomato; Hartwigsen and Evan 2000 for *Pelargonium* × *hortorum* L.H.) but very few studies have shown the impact of supplemented HA on alleviating stress. Seong *et al*. (1990) amended soil with PEG resulted that drought stress descended the germination percentage and seedling growth. In our study, HA did not increase root growth. David *et al*. (1994) proposed that HA affected seedling growth through mechanisms other than nutrient supply. HA affected pepper seedling growth in saline conditions: 1000 and 2000 mg kg^{-1} HA increased fresh and dry leaf weight, fresh and dry root weight, root length and shoot length. Türkmen *et al*. (2004) similarly reported that 1000 g kg^{-1} of HA positively affected plant growth under saline soil conditions.

REFERENCES

Aliabadi Farahani H, Maroufi K (2011) Effect of hydropriming on seedling vigour in basil (*Ocimum basilicum* L.) under salinity conditions. *Advances in Environmental Biology* **5**, 828-833

Bagheri Kazem A, Sarmadnia GH, Hajrasooliha SH (1997) The response of alfalfa to salt and drought stress in germination stage. *Agricultural and Science Technology Journal* **2**, 41-55 (in Farsi)

Cao Y (2010) Effects of NaCl stress on seed germination of *Lepidium latifolium. Modern Agricultural Sciences and Technology* **1**, 71

Chen Y, Aviad T (1990) Effects of humic substances on plant growth. In: MacCarthy P, Clapp CE, Malcolm RL, Bloom PR (Eds) *Humic Substances in Soil and Crop Sciences: Selected Readings*, ASA and SSSA, Madison, WI, pp 161-186

David PP, Nelson PV, Sanders DC (1994) Humic acid improves growth of tomato seedling in solution culture. *Journal of Plant Nutrition* **17**, 173-184

Gulser F, Sonmez F, Boysan S (2010) Effects of calcium nitrate and humic acid on pepper seedling growth under saline condition. *Journal of Environmental Biology* **31 (5)**, 873-876

Hajar AS, Zidan MA, Al-Zahrani HS (1996) Effect of salinity stress on the germination, growth and some physiological activities of black cumin (*Nigella sativa* L.). *Arab Gulf Journal of Scientific Research* **14**, 445-454

Hartwigsen JA, Evans MR (2000) Humic acid seed and substrate treatment promote seedling root development. *HortScience* **35**, 1231-1233

Jamil A, Naim S, Ahmed S, Ashraf M (2005) Production of Industrially important enzymes using molecular approaches. Cellulases and xylanases. In: Thangadurai DT, Pullaiah P, Balatti A (Eds) *Genetic Resources and Biotechnology II*, Regency Publications, New Delhi, pp 143-183

Koochaki F, Ketabi H (1996) the effect of optimum temperature on germination of some forage plant under salinity and drought stress. *Desert* **1 (33)**, 75-77 (in Farsi)

Malik KA, Azam F (1985) Effect of humic acid on wheat (*Triticum aestivum* L.) seedling growth. *Environmental and Experimental Botany* **25**, 245-252

Moradi Dezfuli P, Sharif-Zadeh F, Janmohammadi M (2008) Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.). *ARPN Journal of Agricultural and Biological Science* **3 (3)**, 22-25

Mylonas VA, McCants CB (1980) Effects of humic and fulvic acids on growth of tobacco root initiation and elongation. *Plant and Soil* **54**, 485-490

Ramin AA (2006) Effects of salinity and temperature on germination and seedling establishment of sweet basil (*Ocimum basilicum* L.). *Journal of Herbs, Spices and Medicinal Plants* **11 (4)**, 81-90

Sanders DC, Ricotta JA, Hodges L (1990) Improvement of carrot stands with plant biostimulants and fluid drilling. *HortScience* **25**, 181-183

Seong RC, Park Y, Cho JY (1990) Effects of temperature on germination of Chinese milkvetch. *Korean Journal of Crop Science* **35**, 248-253

Šerá B, Novák F (2011) The effect of humic substances on germination and early growth of Lamb's Quarters (*Chenopodium album* Agg.). *Biologia* **66**, 470-476

Shariat Jafari MH (1997) The effect of salinity on lentil plants. Agronomy thesis, Ferdowsi University of Mashhad, Iran, 124 pp (in Farsi)

Simon EW (1984) Early events in germination. In: Murray DR (Ed) *Seed Physiology*, Australian Academic Press, Sydney, pp 77-115

Sun X-F, Zheng Q-S, Liu Y-L (2000) Salinity injury to germination and growth of cotton (*Gossypium hirsutum* L.) at emergence and seedling stages. *Journal of Plant Resources and Environment* **9**, 22-25

Tan KH (Ed) (1998) Colloidal chemistry of organic soil constituents. In: *Principles of Soil Chemistry*, Marcel Dekker, New York, pp 177-258

Tan KH, Nopamornbodi V (1979) Effect of different humic acids on nutrient content and growth of corn. *Plant and Soil* **51**, 283-287

Tattini M, Bertoni P, Landi A, Traversim ML (1991) Effect of humic acids on growth and biomass partitioning of container grown olive plants. *Acta Horticulturae* **294**, 75-80

Tehranifar A, Akramian M, Joharchi M (1997) The effect of salinity and drought stress on alfalfa as a ornamental plants. In: *Proceedings of the 5th Congress of Horticulture*, August 1997, Mashhad, Iran, pp 75-76 (in Farsi)

Türkmen O, Dursun A, Turan M, Erdinc C (2004) Calcium and humic acid affect seed germination, growth and nutrient content of tomato (*Lycopersicon esculentum* L.) seedlings under saline soil conditions. *Acta Horticulturae Scandinavica B* **54**, 168-174

Uhrits R (1974) Effect of the osmotic pressure on water absorption germination of alfalfa seeds. *American Journal of Botany* **33**, 278-285

Valdrighi MM, Pera A, Agnolucci M, Frassinetti S, Lunardi D, Vallini G (1996) Effects of compost-derived humic acids on vegetable biomass production and microbial growth within a plant (*Cichorium intybus*) soil system: A comparative study. *Agriculture, Ecosystems and Environment* **58**, 133-144

Vaughan D, Linehan DJ (1976) The growth of wheat plants in humic acid solutions under axenic conditions. *Plant and Soil* **44**, 445-449