Journal of Plant Nutrition, 38:224–236, 2015

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1080/01904167.2014.939759

EFFECT OF FOLIAR APPLICATIONS OF HUMIC ACID ON GROWTH, VISUAL QUALITY, NUTRIENTS CONTENT AND ROOT PARAMETERS OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.)

Naghmeh Daneshvar Hakimi Maibodi,[1] Mohsen Kafi,[1] Ali Nikbakht,[2] and Farhad Rejali[3]

[1]*Deptartment of Horticulture, Faculty of Agricultural Science and Engineering, Campus of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*
[2]*Deptartment of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan, Iran*
[3]*Institute of Soil and Water Research, Tehran, Iran*

□ *In recent years, applying humic acid (HA) has been common in turfgrass management. A series of experiments were conducted to evaluate the effect of HA on qualitative and quantitative characteristics of "Speedygreen" perennial ryegrass (*Lolium perenne *L.). Different concentrations of HA (0, 100, 400, and 1000 mg L^{-1}) were applied monthly as foliar application. Results showed that leaf phosphorus (P), potassium (K), and zinc (Zn) content, fresh and dry weight, chlorophyll content, and root fresh weights were not affected by HA. Meanwhile, HA improved the root and shoot development, except for root fresh weight. While just 100 mg L^{-1} improved height, visual quality, nitrogen (N) content, roots length, and surface of roots, all of HA concentrations were effective on iron content. These results suggest that HA foliar application might be of benefit to enhance some nutrients uptake and root development of ryegrass possibly leading to improved drought resistance.*

Keywords: leonardite, humic substances, foliar application, turfgrass, nutrition

INTRODUCTION

In recent years, many commercial products containing humic substances (HS) have been promoted for using on turfgrasses (Liu and Cooper, 2000). Humic acid (HA) is the main segment of HS and the most active component of soil and compost organic matter (Ferrara et al., 2007). These compounds are the products of decomposition of plant tissues, and are predominantly derived from lignified cell walls (Turkmen et al., 2004). These materials have

Received 3 April 2011; accepted 12 December 2012.
Address correspondence to Ali Nikbakht, Department of Horticulture, College of Agriculture, Isfahan University of Technology, 8415683111 Isfahan, Iran. E-mail: anikbakht@cc.iut.ac.ir

many benefits such as phyto-hormone-like activity (Pizzeghello et al., 2001; Fike et al., 2001) that directly and indirectly have stimulating effects on the physiological processes of plant growth (Yang et al., 2004). However, these materials have become the most commonly used organic materials in golf course management (Huang, 2007). Several researchers have noted that foliar application of HA positively affects plant growth. In this respect, there are effects on nutrient uptake (Adani et al., 1998; Tejada and Gonzalez, 2003), improving photosynthesis (Liu et al., 1998), increasing root growth (David et al., 1994; Nikbakht et al., 2008) and enhancing seed germination and seedling growth (Dorer and Peacock, 1997). It seems that foliar fertilization may reduce the need for soil applied fertilizers, thereby reducing leaching and run off of fertilizers nutrients and reducing environmental impacts (Tejada and Gonzalez, 2003). Liu et al. (1998) reported that 400 mg L^{-1} commercial preparation of HA could enhance net photosynthesis, root dehydrogenase activity and root mass re-growth in creeping bentgrass (*Agrostis stolonifera*). Zhang et al. (2003c) showed that plant metabolic enhancers (PMEs) such as seaweed extract and HA may reduce shipment heat injury and improve post-transplant rooting and quality of tall fescue (*Festuca arundinacea*) sod.

The use of HS has often proposed as a method to improve crop production (Nikbakht et al., 2008) and many researchers claim that proper use of these products could improves turfgrass quality and physiological growth with reduced input of mineral fertilizers (Zhang et al., 2003a). Sharif et al. (2002) showed that using HA in low concentrations caused significant increases in shoot and root dry weight of maize plants as compared to control, however it could not impact micronutrient concentrations of plants.

The main objective of this work was to investigate the efficiency of foliar application of HA on growth, chlorophyll and nutrient content, visual quality and root parameters of “Speedygreen” perennial ryegrass as there is little information on the effect of HA on this species.

MATERIALS AND METHODS

Site and Culture Condition

The experiment was conducted in pots and in the natural condition (open air) at the University of Tehran, Karaj, Iran from May to September 2009 after establishing plants (40 days after sowing). The seeds of “Speedygreen” perennial ryegrass were sown at the rate of 25 g m^{-1} on a sandy loam soil (79% sand, 7% clay, 14% silt), pH 8.3, electrical conductivity (EC) 0.55 dS m^{-1}, total nitrogen (N), 200 mg kg^{-1}, phosphorus (P) 15.9 mg kg^{-1}, and potassium (K) 120 mg kg^{-1}. The pots were polyethylene tubes (60 cm in length and 15 cm in diameter with holes pierced at the bottom for drainage; Parsian Pipe, Tehran, Iran) filled with the soil mixture. Plants were irrigated daily until establishment. Treatments were initiated with the establishment of plants. The experimental layout was a randomized complete block design (RCBD) with four replications and each replication contained 3 pots. Humic acid was used in this work prepared from leonardite [containing: carbon (C), 61.2%; N, 3.13 g kg^{-1} dry matter; and P, 2.89 g kg^{-1} dry matter] and was purchased from a Chinese company (Dalian Yano Agriculture Co., Dalian, China).

The experiment included four HA concentrations: 0 (control), 100, 400, and 1000 mg L^{-1}, by adding the commercial HA to deionized water (DI) and treatments sprayed every month during 6-month experimental period.

Measurements

Various morphological parameters were recorded including plant height; fresh and dry weight of plants, chlorophyll content (Chl a, Chl b, and total Chl), visual quality, nutrients content, and root factors (fresh weight, diameter, length, and root surface).

Fresh and Dry Weight

Fresh weight was determined for shoots (leaves) every 2 weeks after clipping. Mowing was done at a height of 3 cm. Then, shoot samples were bagged, oven-dried at 70°C for 48 h and dry weight was recorded and then averaged.

Height Measurement

Plant height was measured every 2 weeks, after treatment initiation and 2 days before turfgrass mowing. It was measured from the base to the tip of leaves by a ruler at three points of pots randomly and then averaged.

Turf Visual Quality

Turf visual quality was evaluated as the integration of shoot density, uniformity and color and ranked from 1 to 9 scale, where 1 was the worst quality and completely brown, 6 = acceptable and 9 = best quality. It was taken every 2 weeks after starting the treatments according to National Turfgrass Evaluation Program (NTEP) procedure (Zhang et al., 2003a, 2003b; Huang and Gao, 2000) and then averaged for the period of experiment.

Leaf Chlorophyll Content

Leaf chlorophyll content was determined every 2 weeks following mowing sample from plants and the data were averaged. Leaf material (0.1 g) was ground with a chilled pestle (3–4°C) and mortar in diffuse light using 5 ml of 80% acetone and the homogenate was centrifuged at 3000 x *g* for 2 min. Aliquots of 5 ml of 80% acetone were added to the pellet and centrifuged till it was non-green. The supernatants were pooled and protected from light prior to the estimation of chlorophyll pigments. Absorbance of extracts was measured at 663 nm and 645 nm with the Shimadzo UV2401 PC spectrophotometer (Shimadzu Corp., Kyoto, Japan) (AOAC, 2006). The chlorophyll (Chl) a, Chl b, and total Chl contents in leaves were determined by using the formula of Arnon (1949).

$$\text{Chl a} = 0.0127 \times A_{663} - 0.00269 \times A_{645}\ \text{mg mL}^{-1}$$

$$\text{Chl b} = 0.0229 \times A_{645} - 0.00488 \times A_{663}\ \text{mg mL}^{-1}$$

$$\text{Total Chl} = 0.0202 \times A_{645} + 0.00802 \times A_{663}\ \text{mg mL}^{-1}$$

Plant Analysis

Minerals content of plant shoots were determined after starting the treatments. Plant samples were oven-dried at 70°C for 48 h and were then ground to determine their mineral composition. The determination of total N in the leaf samples was based on the Kjeldahl method (Eaton et al., 1995). The extraction of K, P, iron (Fe), and zinc (Zn) from the plant tissue material was performed using 1 N hydrochloric acid (HCl) after dry ashing at 550°C for 5 h. The concentrations of Fe and Zn were determined by atomic absorption spectrometer (AAS) (Shimadzu, Kyoto, Japan) (AOAC, 2006) and K was analyzed by flame photometer (ELE, Model PFP7, Essex, UK), while that of phosphorus was estimated by the vanadomolybdophosphoric acid colorimetric method at 460 nm (Eaton et al., 1995). The colorimetrical determinations of P were performed using a ShimadzoUV2401 PC (Shimadzu, Torrance, CA, USA) spectrophotometer.

Rooting Analysis

At the end of the experiment, the roots of the plants were removed from the soil, washed carefully with tap water to remove the soil and separated from shoot and thatch, weighted and placed in plastic covers and stored at 4°C until analyzed (approximately 15 days after exhumation). The WinRHIZO system (Regent Instruments Inc., Quebec, Canada) was used to analyze root samples. Large root samples were cut into shorter samples to reduce root volume and overlap. Roots were scanned in gray scale color to determine root morphology including total root length (mm), diameter (mm), and root surface area (cm^2). Scanner resolution was set at 400 dots per inch.

Data Analysis

All obtained data were subjected to statistical analysis. This analysis was performed by using Statistical Analysis Systems software, version 9.1 (SAS Institute, Cary, NC, USA). Orthogonal contracts considered to determine the relationships between dependent variables and HA treatments. The variation due to treatments was split into linear, quadratic, and cubic orthogonal comparisons.

RESULTS

Effect of Humic Acid on Fresh and Dry Weight, Height, and Visual Quality

Table 1 shows the effect of HA on the growth parameters (height, fresh and dry weight) and visual quality. Humic acid application significantly influenced plants leaves by affecting their visual quality and height by 13% and 7.9% respectively at 1000 mg L^{-1}. The treatments had no significant effect on fresh and dry weight. Both visual quality and height show a linear relationship with HA concentrations ($P < 0.05$).

Effect of Humic Acid on Chlorophyll Content (Chl a, Chl b, and Total Chl)

HA had no significant effect on the chlorophyll content of the leaves (Chl a, Chl b, and total Chl) (Table 1).

Effect of Humic Acid on Nutrient Content

Table 2 shows the effect of HA on the nutrients accumulated in leaves. The effect of HA on P, K, and Zn content of leaves were not found to be affected by treatments. Increasing HA concentration had negative effect on N uptake and N concentration only increased significantly in response to 100 mg L^{-1} by 5%. However tissue N concentration decreased in higher doses of HA (400 and 1000 mg L^{-1}) and it showed a quadratic relationship with HA concentrations. All HA treatments significantly increased leaf Fe concentration compared to the control and it increased by 18% at 1000 mg L^{-1} (Table 2), however the treatments could not significantly influence the Zn content of the leaves in any concentration.

Effect of Humic Acid on Root Parameters

The effects of HA on root parameters (fresh weight, diameter, length, and surface) are presented in Table 3. Humic acid application did not significantly affect fresh weight, while 100 mg L^{-1} HA improved root diameter, length and surface in compression with other concentrations. In fact, a lower level (100 mg L^{-1}) increased the root development compared with the higher levels.

TABLE 1 Effect of spraying humic acid on visual quality, growth indices and chlorophyll content of perennial ryegrass (*Lolium perenne* L.) "Speedygreen" in natural conditions in Karaj, Iran

HA (mg/L)	Visual quality (out of max 9)	Fresh weight (g)	Dry weight (g)	Height (cm)	Chl a (mg/g fresh weight)	Chl b (mg/g fresh weight)	Total Chl (mg/g fresh weight)
0	6.70	1.15	0.67	5.22 b	2.89	0.64	3.54
100	6.65	1.15	0.74	5.54 b	2.71	0.65	3.37
400	6.75	1.13	0.75	5.58 b	2.74	0.63	3.38
1000	7.45	1.05	0.73	6.15 a	2.71	0.62	3.39
Linear	1.54*	0.013	0.0026	1.63**	0.0091	0.0012	0.017
Quadratic	0.164	0.007	0.0066	0.0004	0.0242	0.0000061	0.025
Cubic	0.0024	0.007	0.0055	0.14	0.0434	0.0003816	0.035
Error	0.269	0.014	0.0061	0.048	0.0159	0.0092	0.035

$^{**}P < 0.01$, and $^{*}P < 0.05$.

The spray treatments were applied monthly after establishment of plants (40 days after planting).

TABLE 2 Effect of spraying humic acid on nutrients uptake in leaf samples of perennial ryegrass (*Lolium perenne* L.) "Speedygreen" in natural conditions in Karaj, Iran

	Plant nutrient				
HA (mg/L)	N (%)	P (%)	K (%)	Fe (ppm)	Zn (ppm)
0	3.84	0.3	1.01	358.40	9.48
100	4.04	0.3	1.28	416.02	10.52
400	3.70	0.27	1.22	397.38	11.20
1000	3.74	0.26	1.22	421.79	11.00
Linear	0.074	0.002	0.0193	3024.97*	6.71
Quadratic	0.019*	0.00028	0.0333	351.09	1.33
Cubic	0.12	0.00004	0.0796	4000.16*	0.47
Error	0.0155	0.00073	0.023	373.98	1.41

** $P < 0.01$, and * $P < 0.05$.
The spray treatments were applied monthly after establishment of plants (40 days after planting).

DISCUSSION

Effect of Humic Acid on Plant Growth

The present study showed that foliar application of HA influenced growth of perennial ryegrass especially in low concentration for height. This is in agreement with the result of El-Ghamry et al. (2009) on *Vicia faba*. They showed that the application of HA and amino acids as foliar application can improve the plant growth. Katkat et al. (2009) showed that growth in wheat plant (*Triticum durum*) increased in response to treatments with low and medium concentration of HA. Hunter and Anders (2004) reported that HA did not stimulate growth of creeping bentgrass (*Agrostis stolonifera)* irrespective of the level of P applications but decreased significantly when N was applied at the experiment. It seems that species behavior is different in response

TABLE 3 Effect of spraying humic acid on root parameters of perennial ryegrass (*Lolium perenne* L.) "Speedygreen" in the natural condition in Karaj, Iran

HA (mg/L)	Fresh weight (g)	Diameter (mm)	Length (mm)	Root surface (cm^2)
0	61.62	1.43	1841.30	1687.23
100	65.05	1.56	2664.75	2208.55
400	66.49	1.31	1531.53	1232.87
1000	66.7	1.07	1390.33	897.60
Linear	36.41438	0.0193	1753483*	2766853**
Quadratic	21.016	0.0114	48049	53504
Cubic	8.59479	0.0047*	2104173**	1064334**
Error	91.58	0.001	4973.726	1139.716

**$P < 0.01$, and * $P < 0.05$.
The spray treatments were applied monthly after establishment of plants (40 days after planting).

to HA application. Also, Adani et al. (1998) found that the most favorite growth in tomato (*Lycopersicon esculentum*) was achieved with concentration of 20–50 mg L^{-1} HA and it was communicated with source of HA (peat or leonardite). This might be the reason that foliar application variously affects growth and development of different plants. Chen and Aviad (1990) documented that foliar spray of HS could influence plant growth and increased shoot and root growth, which were attributed to the enhanced uptake of micronutrients.

Pertuit et al. (2001) determined that tomato seedling growth increased linearly with leonardite concentrations compared with plants produced with fertilizer alone. In our research plant height increased consistently in plants treated with HA, but the treatments did not affect the other growth indices similarly. It is possible that the enhancement of turfgrass height following the HA foliar application could be attributed at least partially to increased iron content (Chen and Aviad, 1990). Bidegain et al. (2000) showed the HS extracted from poplar sawdust could improve yield and growth of container grown perennial ryegrass.

Effect of Humic Acid on Visual Quality

One of the major factors in turfgrass is visual quality. Some research has proven that any concentrations of HA could not affect visual quality (Liu and Cooper, 2000). But other researchers have shown that using biostimulants such as a mixture of bacteria and fulvic acid (FA) and HA enhanced visual quality and color in creeping bentgrass (*Agrostis stolonifera)* (Muller and Kussow, 2005). It seems that chlorophyll content contributes in this factor, too. In the current study, the highest concentration was more effective than other concentrations which is similar to the results obtained by Zhang et al. (2003a) that showed HA application improved turf quality and could enhance color and health. It seems HS have multiple effects that can benefit plant growth. One of them is hypothesizes as 'direct' action on the plants, which is hormonal in nature. It seems that these materials contain plant growth regulators such as indoleacetic acid (IAA), gibberellins and cytokinins and can significantly affect plant quality (Arancon et al., 2006). Some researchers believe root development probably provides the plant with more indigenous phytohormones production sites. These phytohormones such as cytokinins are transmitted into the leaves that consequently improve turfgrass quality (Huang, 2004; Zhang et al., 2003b), however this is not in agreement with our findings. As both visual quality and Fe content showed a linear relationship with HA concentrations (Tables 1 and 2), a linear increase in plant Fe content might lead to improved turfgrass quality in current research (Chen and Aviad, 1990).

Effect of Humic Acid on Chlorophyll Content

Chlorophyll is the molecule that performs photosynthesis and it is found in the chloroplasts of green plants. Although enhancement of chlorophyll content with application of HS in nutrient solutions or foliar spray has been reported (Vaughan and Malcolm, 1995; Ferrara et al., 2007), we observed no significant effect in this experiment. Our results contradict Chen et al. (1999) who reported improved chlorophyll content in creeping bentgrass (*Agrostis stolonifera)*. They explained HA-mediated maintenance of Fe and Zn at sufficient levels is the key factor in elevating chlorophyll content in the leaves; however we did not observe any significant correlation between Fe and Zn content of the leaf and chlorophyll content. Liu et al. (1998) reported that HA was not effective on chlorophyll content in creeping bentgrass (*Agrostis stolonifera)*, but could enhance net photosynthesis. Some researchers showed that no differences were observed in chlorophyll content of the turf with any HS treatment suggesting that turf color and visual quality are not enhanced by HS (Van Dyke et al., 2009). On the contrary, El-Ghamry et al. (2009) showed that chlorophyll content significantly increased by the application of HA interacted with amino acids. In the same way Tejada and Gonzalez (2003) observed that the highest chlorophyll a and b values were from plots receiving amino acids and HA treatments in asparagus (*Asparagus officinalis*) plants. Cheng et al. (2007) showed that chlorophyll content of perennial ryegrass grown in amended soil with 5–100% composted sewage sludge was greatly improved. Finally they concluded that the effect of HA on chlorophyll content and photosynthesis seems to be species and concentration dependent.

Effect of Humic Acid on Nutrient Content

Promotion in the nutrients uptake with the application of HA has been reported by various researchers (Chen and Aviad, 1990; David et al., 1994; Adani et al., 1998; Sharif et al., 2002). Obtained results being confirmed by these findings that HA can increase uptake of certain elements. The plants take more mineral elements due to the better-developed root systems. Besides, the stimulation of ion uptake by treatments with HS led many investigators to propose that these materials affect membrane permeability (Katkat et al., 2009). It seems Fe reduction in cell apoplast by HS might be the reason why Fe is accumulated in leaf tissues (Nikolic et al., 2003).

It is believed that besides the source of HA and the nature of container medium, efficiency of HA also differ according to the plant species. According to Katkat et al. (2009), foliar applications of HS had significant effect on elements uptake [N, P, K, calcium (Ca), Mg, sodium (Na), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn)] under calcareous soil conditions and raised the dry weight of wheat at non-limed pots. Liu et al. (1998) reported that HA had no influence on the concentrations of P, K, Fe, Mo, and Zn in creeping bentgrass (*Agrostis stolonifera)* although this increased tissue concentrations of Mg, Mn, and sulfur (S).

There was no increase in P, K, Fe, and Zn tissue concentration reported in creeping bentgrass (*Agrostis stolonifera)* when grown in hydroponic system containing HA (Liu et al., 1998). Cooper et al. (1998) raised creeping bentgrass (*Agrostis stolonifera)* in both sand and a hydroponic system. Spraying HA from different sources, they found that P content increased in sand culture by 3–5% but not in hydroponic system. They documented that HA might have limited promoting effects when plants receiving adequate nutrients. Our treatments could not elevate P content. It is in agreement with what Bidegain et al. (2000) report. They believe that HA can improve P uptake in ryegrass only in the case of very low P availability. Under field conditions, foliar application of leonardite extracts stimulated shoot growth and promoted the accumulation of K, boron (B), Mg, calcium (Ca), and Fe in leaves of olive (*Olea europea*) (Fernandez-Escobar et al., 1996). It seems HS spray can maximize the efficient use of some nutrients, reduce fertilizer costs and help release those plant nutrients presently bound is minerals and salts especially when incorporated in soil (El-Ghamry et al., 2009).

Effect of Humic Acid on Root Parameters

Increased rooting with HA application was found on creeping bentgrass (*Agrostis stolonifera)* (Zhang et al., 2003a) and other plants (Atiyeh et al., 2002, David et al., 1994). Humic acid affected root growth, especially in low concentration (100 mg L^{-1}), which is in agreement with Zhang et al. (2003a) and Van Dyke et al. (2009). Liu et al. (1998) reported that HA solution had no effect on root re-growth and actually reduced root length at low concentration, but 400 mg L^{-1} HA visually produced more developed root mass. The findings of Cooper et al. (1998) showed that incorporation of granular HA into the root zone produced significantly longer roots and greater root mass deeper in the root zone.

The roots of plants that received 100 mg L^{-1} HA in our experiment produced more developed roots and grew more than the roots of plants received higher concentrations. The optimal levels of humic materials to enhance root and shoot growth are 50 to 300 mg L^{-1} (Chen and Aviad, 1990), and is very species-dependent. Some researchers showed that spray application of HA on plants had no effect on root growth. Perhaps this problem related with no tangent HA with roots (Cooper et al., 1998; Liu et al., 1998).

Hunter and Anders (2004) found that HA were effective on root architecture and growth of creeping bentgrass (*Agrostis stolonifera)* and also increased the plant resistance to drought. In a study on gerbera (*Gerbera* *jamesonii*) Nikbakht et al. (2008) showed the addition of HS to nutrient solution had a greater effect on the growth of roots than shoots and expressed it could have facilitated more efficient absorption. Humic acid application in the present study had little effect on root parameters in higher rates and lower concentration was more effective. In consequence although HA foliar application could improve root architecture, but had little effect on root growth of the plants. This is in agreement with Kafi et al. results (2009) that demonstrated spraying HA is less effective on root development than incorporating HA in nutrient solution in hydroponically grown gerbera (*Gerbera jamesonii*). Origin of HA can be also of importance on root growth. Results of Ervin et al. (2008) experiments showed that application of peat-originated HA increased root mass 73% in Kentucky bluegrass (*Poa pratensis*), while HA with leonardite origin enhanced root growth just by 34%. According to Table 3, a cubic model in response of root parameters to HA concentrations is expected. This means in the lowest concentrations the response is linear but beyond this range the response is cubic. That might be at least partly a feature of N content in plants in the present research.

CONCLUSIONS

The results of this experiment showed that the lower concentration could be effective on N content. However, higher levels of HA had no effect or even adverse effect in many cases. By contrast high concentration had positive effect on Fe content. In conclusion, it appears that using low concentrations of HA could be more applicable, especially in improving *Lolium prenne* "Speedygreen" visual quality. Our findings suggest the importance of further investigation to understand the nature of humic substances and their effects on the plants in relation to the chlorophyll content and root growth. It is assumed that HA could play a major role in some growth indices, further study may be needed for more information about mechanism and effect of this material on this species and obtaining optimal concentration for growth improvement and turf quality in the field.

REFERENCES

Adani, F., P. Genevini, P. Zaccheo, and G. Zocchi. 1998. The effect of commercial humic acid on tomato plant growth and mineral nutrition. *Journal of Plant Nutrition* 21: 561–575.

AOAC. 2006. *Official Methods of Analysis*, 18th ed. Arlington, VA: Association of Official Analytical Chemists.

Arancon, N. Q., C. A. Edwards, S. Lee, and R. Byrne. 2006. Effects of humic acids from vermicomposts on plant growth. *European Journal of Soil Biology* 42: 565–569.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplast. I. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology* 24: 1–15.

Atiyeh, R. M., S. Lee, C. A. Edwards, N. Q. Arancon, and J. D. Metzger. 2002. The influence of humic acids derived from earthworm-processed organic wastes on plant growth. *Bioresearch Technology* 84: 7–14.

Bidegain, R. A., M. Kaemmerer, M. Guiresse, M. Hafidi, F. Rey, P. Morard, and J. C. Revel. 2000. Effects of humic substances from composted or chemically decomposed poplar sawdust on mineral nutrition of ryegrass. *Journal of Agricultural Science* 134: 259–267.

Chen, Y., and T. Aviad. 1990. Effects of humic substances on plant growth. In: *Humic Substances in Soil and Crop Science: Selected Readings*, eds. P. McCarthy, C. E. Clapp, R. L. Malcom, and P. R. Bloom, pp. 161–186. Madison, WI: SSSA and ASA.

Chen, Y., C. E. Clapp, H. Magen, and V. W. Cline. 1999. Stimulation of plant growth by humic substances: Effects on iron availability. In: *Understanding Humic Substances: Advanced Methods, Properties and Applications*, eds. E. A. Ghabbour and G. Davies, pp. 255–263. Cambridge: Royal Society of Chemistry.

Cheng, H., W. Xu, J. Liu, Q. Zhao, Y. He, and G. Chen. 2007. Application of composted sewage sludge (CSS) as a soil amendment for turfgrass growth. *Ecological Engineering* 29: 96–104.

Cooper, R. J., C. Liu, and D. S. Fisher. 1998. Influence of humic substances on rooting and nutrient content of creeping bentgrass. *Crop Science* 38: 1639–1644.

David, P. P., P. V. Nelson, and D. C. Sanders. 1994. A humic acid improves growth of tomato seedling in solution culture. *Journal of Plant Nutrition* 17: 173–184.

Dorer, S. P., and C. H. Peacock. 1997. The effect of humate and organic fertilizer on establishment and nutrient of creeping bent putting greens. *International Turfgrass Society* 8: 437–443.

Eaton, A. D., L. S. Clesceri, and A. E. Greenberg. 1995. *Standard Methods for the Examination of Water and Wastewater*. Washington, DC: American Public Health Association.

El-Ghamry, A. M., K. Abdel-hai, and K. M. Ghoneem. 2009. Amino and humic acids promote growth, yield and disease resistance of faba bean cultivated in clay soil. *Australian Journal of Basic and Applied Sciences* 3: 731–739.

Ervin, E. H., X. Zhang, and J. C. Roberts. 2008. Improving root development with foliar humic acid applications during Kentucky bluegrass sod establishment on sand. *Acta Horticulturae* 783: 317–322.

Fernandez-Escobar, R., M., Benlloch, D. Barranco, A. Duenas, and J. A. Guterrez Ganan. 1996. Response of olive trees to foliar application of humic substances extracted from leonardite. *Scientia Horticulturea* 66: 191–200.

Ferrara, G., A. Pacifico, P. Simeone, and E. Ferrara. 2007. Preliminary study on the effects of foliar applications of humic acids on 'Italia' table grape. In: *Proceedings of the XXXth World Congress*. Paris: Organisation Internationale de la Vigne et du Vin (OIV).

Fike, J. H., V. G. Allen, R. E. Schmidt, X. Zhang, J. P. Fontenot, C. P. Bagley, R. L. Ivy, R. R. Evans, R. W. Coelho, and D. B. Wester. 2001. Tasco-Forage: I. Influence of a seaweed extract on antioxidant activity in tall fescue and in ruminants. *Journal Animal Science* 79: 1011–1021.

Huang, B. 2004. Recent advances in drought and heat stress physiology of turfgrass: A review. *Acta Horticulturea* 661: 185–192.

Huang, B. 2007. Plant growth regulators: what and why. *Golf Course Management* 75: 157–160.

Huang, B., and H. Gao. 2000. Growth and carbohydrate metabolism of creeping bentgrass cultivars in response to increasing temperatures. *Crop Science* 40: 1115–1120.

Hunter, A., and A. Anders. 2004. The influence of humic acid on turfgrass growth and development of creeping bentgrass. *Acta Horticulturea* 661: 257–264.

Kafi, M., M. Babalar, A. Nikbakht, H. Ebrahimzade, N. Etemadi, and S. Samavat. 2009. Effect of humic acid spray on nutrients uptake, protein content and postharvest life of *Gerbera jamesonii* cv. Malibu. *Iranian Journal of Horticultural Sciences* 40: 69–75 (in Persian with English abstract).

Katkat, A. V., H. Celik, M. A. Turan, and B. B. Asik. 2009. Effects of soil and foliar applications of humic substances on dry weight and mineral nutrients uptake of wheat under calcareous soil conditions. *Australian Journal of Basic and Applied Sciences* 3: 1266–1273.

Liu, C., and R. J. Cooper. 2000. Humic substances influence creeping bentgrass growth. *Golf Course Management* 68: 49–53.

Liu, C., R. J. Cooper, and D. C. Bowman. 1998. Humic acid application affects photosynthesis, root development, and nutrient content of creeping bentgrass. *HortScience* 33: 1023–1025.

Muller, S. R., and W. R. Kussow. 2005. Biostimulant influences on turfgrass microbial communities and creeping bentgrass putting green quality. *HortScience* 40: 1904–1910.

Nikbakht, A., M. Kafi, M. Babalar, Y. P. Xia, A. Luo, and N. Etemadi. 2008. Effect of humic acid on plant growth, nutrient uptake and postharvest life of gerbera. *Journal of Plant Nutrition* 31: 2155–2167.

Nikolic, M., S. Cesco, V. Romheld, Z. Varanini, and R. Pinton. 2003. Uptake of iron (Fe-59) complexed to water-extractable humic substances by sunflower leaves. *Journal of Plant Nutrition* 26: 2243–2252.

Pertuit, A. J. Jr., J. B. Dudley, and J. E. Toler. 2001. Leonardite and fertilizer levels influence tomato seedling growth. *HortScience* 36: 913–915.

Pizzeghello, D., G. Nicolini, and S. Nardi. 2001. Hormone-like activity of humic substances in *Fagus sylvaticae* forests. *New Phytologist* 151: 647–657.

Sharif, M., R. A. Khattak, and M. S. Sarir. 2002. Effect of different levels of lignitic coal derived humic acid on growth of maize plants. *Communication in Soil Science and Plant Analysis* 33: 3567–3580.

Tejada, M., and J. L. Gonzalez. 2003. Influence of foliar fertilization with amino acids and humic acids on productivity and quality of asparagus. *Biological Agriculture and Horticulture* 21: 277–291.

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

Van Dyke, A., P. G. Johnson, and P. Grossl. 2009. Influence of humic acid on water retention and nutrient acquisition in simulated golf putting greens. *Soil Use and Management* 25: 255–261.

Vaughan, D., and R. E. Malcolm. 1995. Influence of humic substances on growth and physiological processes. In: *Soil Organic Matter and Biological Activity*, eds. D. Vaughan and R. E. Malcolm, pp. 37–75. Dordrecht, the Netherlands: Martinus Nijhof Publishing.

Yang, C. M., M. C. Wang, Y. F. Lu, I. F. Chang, and C. H. Chou. 2004. Humic substances affect the activity of chlorophyllase. *Journal of Chemical Ecology* 30: 1057–1065.

Zhang, X., E. H. Ervin, and R. E. Schmidt. 2003a. Physiological effect of liquid applications of a seaweed extract and a humic acid on creeping bentgrass. *Journal of American Society for Horticultural Science* 128: 492–496.

Zhang, X., E. H. Ervin, and R. E. Schmidt. 2003b. Plant growth regulators can enhance the recovery of Kentucky bluegrass sod from heat injury. *Crop Science* 43: 952–956.

Zhang, X., E. H. Ervin, and R. E. Schmidt. 2003c. Seaweed extract, humic acid and propiconazole improve tall fescue sod heat tolerance and post-transplant quality. *HortScience* 38: 440–443.