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Can We Reduce Salinity Effects by the Application of Humic Acid on Native Turfgrasses in order to Attain Sustainable Landscape?

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Abstract

Soil salinity is one of the most important problems in arid and semi-arid climate areas of the world. So, we investigated the effects of humic acid on visual quality and quantity parameters of native turfgrasses in compared to commercial variety under salinity stress to explore can we reduce salinity effects by humic acid or not? So, a research was arranged in factorial experiment based on a randomized complete design with three replications. The first factor was three grasses consisted of two native accessions of *Lolium perenne* 'Chadegan' and 'Yarand' and its commercial variety, the second factor was four concentrations of humic acid (HA) (0, 5, 10, 15 g kg^{-1}) and the third factor consisted of three levels of salinity (0, 150, 300 mM). Results of analysis of variance indicated that the interaction effects of cultivar × HA was significant in all traits ($P<0.01$) except on uniformity and plant height. The treatment of cultivar × salinity significantly affected color, texture, smoothness, uniformity and RWC ($P<0.01$) and total quality ($P<0.05$). Treatment of HA × salinity had not significant influence on smoothness and uniformity but other traits were significantly influenced ($p< 0.05$) and RWC ($P<0.01$). Also, triple interaction effect was significant on texture, quality after clipping, uniformity and RWC ($P<0.01$) and plant height ($P<0.05$). Application of HA could decreased salinity effect on darkness, texture and increased quality after clipping and relative water content of leaves under salinity stress. Also, increasing of severity of salinity had reduction effect on plant height and total quality even with HA application. In other hands, plants grow on soils which contain adequate humic acids (HA) are less subject to stress condition, are healthier because of having higher relative water content in compared to salinity condition.

Keywords: Color, Quality, Texture, Turf, Uniformity.

INTRODUCTION

Many research agencies such as the Plant Materials Center are developing varieties with broader genetic bases which have two advantages. First, they have more adaptability to a wide range of climate. Secondly, these materials are native genetic bases for future generations (Pfaff *et al.*, 2002). Therefore, native plants provide excellent choices for landscapes (Pessarakli and Kopec, 2008). They are excellent candidate species to produce cultivars which have more attractiveness and require less maintenance cost to be used as a turfgrass in arid and semi-arid areas (Bormann *et al.*, 2001). Some researchers have studied native turfgrasses in different condition for example Saeedi Pooya *et al.* (2013), Simmons *et al.* (2011) Bunderson *et al.* (2009) and etc.

On the other hand, soil salinity is one of the main problems in arid and semi-arid regions of the world. Salts tend to accumulate at ground level due to intensive evaporation conditions and inadequate leaching process (de Pascale and Barbieri, 1997). Some soil physical and chemical properties are deteriorated by accumulated salts. Exchangeable Na and high soil pH can decrease hydraulic conductivity and infiltration rate of soil and deform soil structure (Lauchli and Epstein, 1990). Khalegi and Ramin (2005) reported that *Cynodon dactylon* had more resistant to salinity stress rather than *Lolium perenne* L. and *Festuca arundinacea*. Growth index of the grasses had not large variation at salinity of 3 dS/m but the level of 6 dS/m had significant reducer effect on growth.

As we know, unsuitable soil conditions to plant growth generally is due to the lack of organic materials in the soil (Khaled and Fawy, 2011). So, an adequate fertilizer program is one of the most important factors to approach healthy and wear-resistant turf cover (Aamlid and Hanslin, 2009). To solve this problem, humic substances can be used. Piccolo *et al.* (1992) represented that humic acid (HA) could improve plant growth and enhance stress tolerance as a plant growth regulator. Humic acid is a bio-fertilizer, which acts as a growth stimulant by positive effects on soil and plant properties. It is a complex mixture of aromatic organic acid, with diverse functional groups bearing carbon, hydrogen, sulphur, nitrogen, phosphorous and oxygen in different percentage and metal ions such as calcium, zinc, magnesium, copper, etc. It chelates metal ions under alkaline soil conditions and improves the availability of nutrient to plants (Zhang *et al.*, 2010). Piccolo *et al.* (1992) indicated that humic extract with the highest acidity and smallest molecular size is the only material showing auxin-like activity. Khaled and Fawy (2011) reported that salinity had negative effects on the growth of corn such as reducer effect on the dry weight and the uptake of nutrients except Na and Mn. Foliar application of humic acids increased the uptake of P, K, Mg, Na, Cu and Zn while humus soil application improved the N uptake of corn. Although, the interaction effect of salt and soil humus application was found statistically significant unlike foliar humic acid application. Generally, humic substances have both direct and indirect effects on plant growth. The some indirect effects of humic compounds on soil fertility consist of:

(i) Increasing of soil microbial population including beneficial microorganisms

(ii) Improvement of soil structure

(iii) Increasing of the cation exchange and the pH buffering capacity of the soil.

Directly, humic acid substances may have various biochemical effects at cell wall, membrane surface and in the cytoplasm. Humic enhanced respiration and photosynthesis rates and increased protein synthesis (Chen and Aviad, 1990).

Recent research found that HA, when applied to turfgrass, can motivate shoot and root growth, and increase resistance to stress condition in turfgrass (Schmidt, 1990). Liu *et al.* (1998) reported that the effects of humic acid on photosynthesis, chlorophyll content, rooting, and nutrient concentration of *Agrostis stolonifera* L. They applied Hoagland's nutrient solution and HA at 0, 100, 200 and 400 mg L^{-1} and found that 400 mg L^{-1} HA significantly improved net photosynthesis but chlorophyll concentration was unaffected by HA. Also, HA enhanced tissue content of Mg, Mn, and S and decreased those of Ca, Cu, and N, but had no influence on the content of P, K, Fe, Mo, and Zn.

Application of humic acid to bentgrass in hydroponic culture significantly increased photosynthesis, root enzyme activity and root mass, but not chlorophyll content or root length (Liu and Cooper, 2000).

Therefore, according to recent research on application of HA to turfgrass and having stimulant and regulated effects of HA on plants growth and improves resistance to stress condition, the objective of this study was to evaluate the effect of soil application of humic acid in mitigating the negative or harmful effects of salinity on turfgrasses.

MATERIALS AND METHODS

Experimental design and plant materials

This pot research was conducted at the greenhouse of Agricultural Faculty, Ferdowsi University of Mashhad (latitude 36º 16´ N, longitude 59º 36′ E and 985 m elevation), Mashhad, Iran in 2013. The experiment was arranged in a factorial experiment based on randomized complete design with three replications.

Treatments included: three grasses consisted of two native accessions of *Lolium perenne* 'Chadegan' and 'Yarand' and one commercial variety of *Lolium perenne*, soil application of humic acid (HA) in four levels (0, 5, 10, and 15 g kg^{-1} soil) and salinity stress at three levels (0, 150, 300 mM).

Turfgrass pots were established by directly sowing the seeds. The soil mixture had a sandy-loam texture (1:1 ratio of soil: sand mixture), pH 7.5 and electrical conductivity of 0.8 dS/m.

HA (humic acid 65% Australia) was used as soil mixture just before sowing. During the growing period, plants were sprinkler irrigated as needed and weeds were controlled by hand. The salinity stress was applied for 30 days after establishment and full tillering stage.

Data collection

Visual quality was assessed using a visual score based on a 1–9 scale, as used in the National Turfgrass Evaluation Program (NTEP) in the USA (Beard, 1973; Salehi and Khosh-Khui, 2004). The lowest level (1) defines very poor turf quality and highest level (9) defines very ideal visual quality. A rating of 6 or greater was considered to be acceptable. For example, color is based on a visual rating scale with 1 being light green and 9 being dark green or leaf texture (9=fine, 1=coarse) and other traits such as; quality after clipping, smoothness, uniformity, total quality have also evaluated using rated on 1 to 9 scale (1= poorest, 9= best). NTEP data was recorded before and during the salinity stress. Plant height was measured by ruler (cm) and the percentage of relative water content (RWC) was calculated according to Saini *et al.* (2001): The leaves were weighed (fresh weight: FW), and were immersed in distilled water for 5 hours and weighted again (turgid weight: TW) and then were dried at 70 ºC inside an oven for 24 hours (dry weight: DW). RWC was calculated using the equation 1:

$$RWC\ (\%) = (FW - DW) / (TW - DW) \times 100 \qquad \text{Eq. 1}$$

Where FW is fresh weight, TW is turgid weight and DW is dry weight.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using JMP8 software and LSD test at 5% levels was used for mean separation.

RESULTS

The results of analysis of variance (Table 1) indicated that the effect of cultivar treatment was significant on all traits ($P<0.01$). Humic acid treatment had significant effect on color ($P<0.05$), texture, quality after clipping, uniformity, plant height and RWC ($P<0.01$). Salinity treatment significantly affected color ($P<0.05$), texture, smoothness, uniformity, total quality, height and RWC ($P<0.01$) (Table 1).

Table 1. Results of analysis of variance for the effects of cultivars, HA and salt stress on visual quality and quantity parameters of turfgrasses.

S.O.V	df	G color	Texture	Quality after clipping	Smoothness	Uniformity	Total quality	Height	RWC
Cultivar	2	9.61**	48.76**	40.55**	24.31**	3.04**	6.43**	274.62**	430.33**
HA	3	0.31*	0.42**	5.02**	0.04 ns	0.37**	0.15 ns	15.33**	1092.81**
Salinity	2	0.37*	4.99**	0.21 ns	1.58**	0.70**	13.27**	82.93**	1034.11**
Cultivar× HA	6	0.52**	0.55**	3.41**	0.21**	0.10 ns	0.65**	1.69 ns	734.23**
Cultivar× Salinity	4	0.60**	0.19**	0.14 ns	0.27**	0.37**	0.35*	1.98 ns	260.54**
HA× Salinity	6	0.21*	0.08*	0.49*	0.02 ns	0.16 ns	0.25*	7.37*	358.20**
Cultivar×HA×Salinity	12	0.13 ns	0.22**	0.56**	0.03 ns	0.23 **	0.09 ns	4.98*	291.75**
Error	72	7.39	0.03	0.21	0.03	0.09	0.11	2.52	34.6
CV (%)		7.39	14.51	15.05	9.73	5.6	10.2	30	51

*, ** and ns: Significant at P<0.05, P<0.01 and no significant respectively.

Table 2. Mean comparison of interaction effect of cultivar and HA on visual quality and quantity parameters of turfgrasses.

Cultivar	HA (%)	G color (1-9)	Texture (1-9)	Quality after clipping (1-9)	Smoothness (1-9)	Total quality (1-9)	RWC (%)
Chadegan	0	7.30 gh	7.37 d	8.57 a	7.69 de	7.19 bc	17.28 ef
	5	7.40 g	7.09 e	8.44 ab	7.67 e	6.79 ef	23.23 cd
	10	7.54 fg	7.47 d	8.60 a	7.86 cd	7.01 cde	26.53 bcd
	15	7.83 de	7.69 c	8.82 a	7.84 cd	6.81 def	23.61 cd
Yarandi	0	7.11 h	5.58 h	5.51 f	6.47 g	6.57 f	25.21 cd
	5	7.40 g	6.24 f	5.68 f	6.56 g	6.70 f	24.33 cd
	10	7.37 gh	6.16 fg	7.07 e	6.49 g	6.60 f	27.92 bc
	15	7.68 ef	6.06 g	7.81 cd	6.82 f	6.87 def	32.00 b
Commercial variety	0	8.44 ab	8.23 b	7.86 cd	8.28 a	7.11 bcd	27.17 bcd
	5	8.57 a	8.51 a	7.99 c	8.23 a	7.82 a	22.20 de
	10	8.26 bc	8.30 b	7.54 d	8.13 ab	7.73 a	15.80 f
	15	8.10 cd	8.23 b	8.11 bc	8.01 bc	7.38 b	52.45 a

In each column, means with the similar letters are not significant different (P< 0.05) using LSD test.

The interaction effect of cultivar × HA was significant in all traits (P<0.01) except uniformity and height. The interaction effect of cultivar × salinity significantly affected color, texture, smoothness, uniformity and RWC (P<0.01) and total quality (P<0.05). Interaction effect of HA × salinity had not significant influence on smoothness and uniformity but other traits were significantly influenced at 5% probability level and RWC at 1% level (Table 1).

Triple interaction effect was significant on texture, quality after clipping, uniformity and RWC (P<0.01) and height (P<0.05) (Table 1). According to results, interaction effects of treatments can be described as follows:

Color

The interaction effect of cultivar × HA indicates that the best color (darkest color) and the lightest one were belonged to 5% HA in commercial variety and 0% HA (control) in Yarandi, respectively. Turf color increased with increasing HA concentration and this process was more regular in both native cultivars than commercial one (Table 2).

According to Table 3, in treatment of cultivar × salinity the darkest green color was belonged to commercial one in all salinity levels. Increasing of salinity stress resulted in a substantial increase in darkness of turf color in two native cultivars.

But, the interaction effects of salinity × HA demonstrated that application of HA at three concentrations of salinity was effective on extreme darkness (Table 4). In fact, both of HA and salinity could increase turf color.

Texture

According to Fig. 1, due to triple interaction effect, the regularity of slender texture among three grasses was recorded in commercial variety> Chadegan> Yarandi. In three grasses with increasing of salinity stress texture quality increased (leaf width decreased). HA had synergistic effect on turf texture in salinity condition. The smallest width was recorded in commercial variety at 300 mM salinity without HA application while, the biggest leaf width (least texture quality) was showed in Yarandi at control treatment (without salinity and HA).

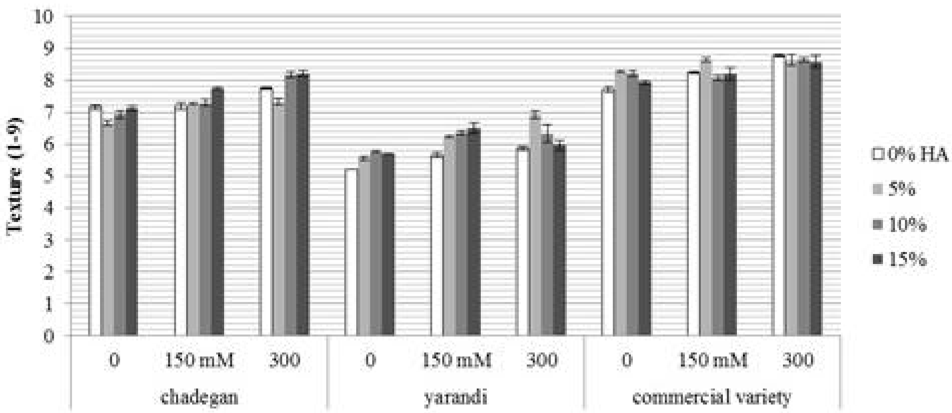

Fig. 1. Triple Interaction effect of turfgrass, humic acid and salinity stress on texture. Error bars represent standard error.

Table 3. Mean comparison of interaction effect of cultivar and salinity on visual quality and quantity parameters of turfgrasses.

Cultivar	Salinity(mM)	G color (1-9)	Texture (1-9)	Smoothness (1-9)	Uniformity (1-9)	Total quality (1-9)	RWC (%)
Chadegan	0	7.18 c	6.98 f	7.48 e	7.72 cd	7.62 b	25.58 cd
	150	7.74 b	7.38 e	7.83 d	7.74 c	6.95 cd	20.70 e
	300	7.63 b	7.86 d	7.98 c	7.78 c	6.28 e	21.72 de
Yarandi	0	7.29 c	5.56 h	6.22 g	7.93 bc	7.08 c	31.70 ab
	150	7.29 c	6.19 g	6.70 f	7.49 de	6.73 d	28.66 bc
	300	7.58 b	6.28 g	6.83 f	7.30 e	6.24 e	21.75 de
Commercial variety	0	8.43 a	8.03 c	8.13 ab	8.28 a	8.24 a	35.28 a
	150	8.40 a	8.28 b	8.10 bc	8.08 ab	7.53 b	34.90 a
	300	8.20 a	8.65 a	8.26 a	8.05 ab	6.77 d	18.04 e

In each column, means with the similar letters are not significant different (P< 0.05) using LSD test.

Table 4. Mean comparison of interaction effect of salinity and HA on visual quality and quantity parameters of turfgrasses.

Cultivar	Salinity(mM)	G color (1-9)	Texture (1-9)	Quality after clipping (1-9)	Total quality (1-9)	Height (1-9)	RWC (%)
0	0	7.30 c	6.69 h	7.14 f	7.34 bc	12.11 ab	26.30 b
	150	7.72 ab	7.03 f	7.19ef	7.01 d	12.22 a	23.96 bc
	300	7.83 a	7.46 cd	7.60 cde	6.51 e	9.89 cd	19.39 c
5	0	7.78 ab	6.83 gh	7.59 cde	7.82 a	12.89 a	28.11 b
	150	7.87 a	7.38 de	7.32 def	7.17 cd	9.89 cd	22.70 bc
	300	7.72 ab	7.63 ab	7.20 ef	6.32 e	7.56 e	18.95 c
10	0	7.54 bc	6.97 fg	7.79 c	7.63 ab	10.56 c	23.12 bc
	150	7.79 ab	7.24 e	7.71 cd	7.17 cd	10.11 cd	23.26 bc
	300	7.83 a	7.71 a	7.71 cd	6.54 e	8.67 de	23.86 bc
15	0	7.91 a	6.92 fg	8.49 a	7.78 a	10.67 bc	45.87 a
	150	7.87 a	7.48 bcd	8.32 ab	6.93 d	10.67 bc	42.40 a
	300	7.83 a	7.58 abc	7.93 bc	6.34 e	8.33 e	19.79 c

In each column, means with the similar letters are not significant different (P< 0.05) using LSD test.

Quality after clipping

Totally, between three grasses, quality after clipping of Chadegan was better in different treatments. It is clear that with increasing of HA concentration quality after clipping increased even in severe salinity condition. But, the highest variation of quality after clipping with increasing of HA concentration and salinity level was belonged to Yarandi (Fig. 2).

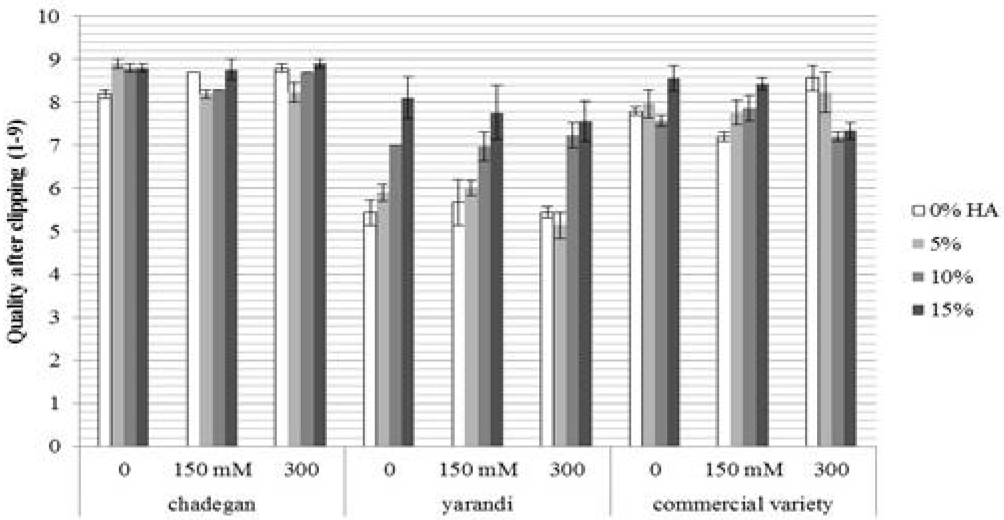

Fig. 2. Triple interaction effect of turfgrass, humic acid and salinity stress on quality after clipping. Error bars represent standard error.

Smoothness

The results of interaction effect of cultivar × HA showed that, different concentrations (5, 10 and 15 g) of humic acid increased turf smoothness in two native turfs unlike in commercial variety (Table 2). Also, smoothness was gradually increased by increasing salinity levels in three cultivars (Table 3).

Uniformity

According to Fig. 3, the highest and the lowest uniformity were belonged to commercial variety at 10% HA and salinity control treatment and Yarandi at 15% HA and 300 mM salinity, respectively.

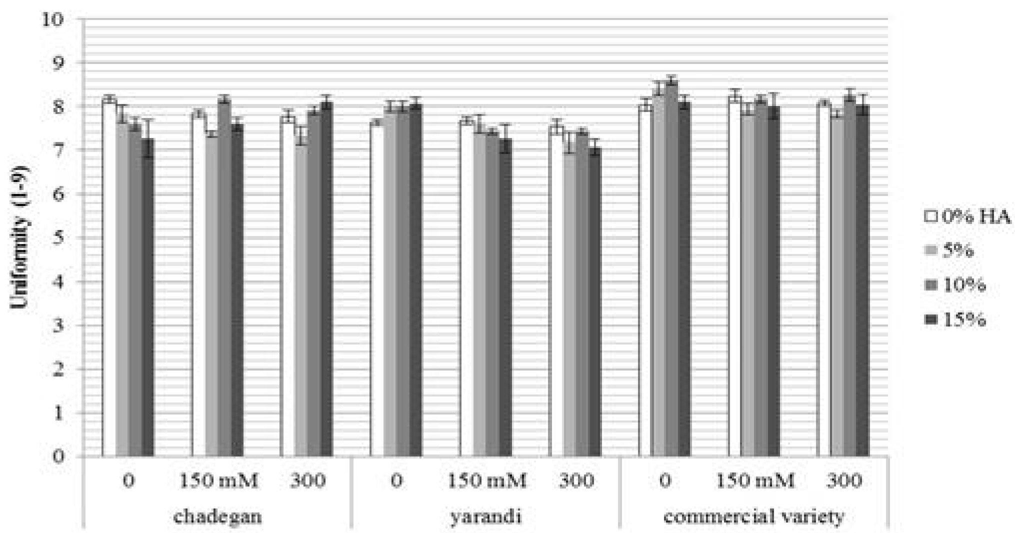

Fig. 3. Triple interaction effect of turfgrass, humic acid and salinity stress on uniformity. Error bars represent standard error.

Total quality

Results of interaction effect of cultivar× HA (Table 2) showed that, the highest total quality was observed in commercial turf at 5% HA and the lowest was belonged to Yarandi at control treatment. Increasing of salinity stress significantly decreased total quality of all tested turfgrasses (Table 3). The lowest value of quality was observed in all turfgrasses at 300 mM salinity. The application of HA could not improve total quality of turfgrasses under salinity condition (Table 4).

Height

Increasing of severity of salinity had reduction effect on plant height with or without HA application. The lowest growth was observed in commercial variety with 300 mM salinity and 10% HA and the highest was belonged to Chadegan without HA and salinity stress (control condition) (Fig. 4).

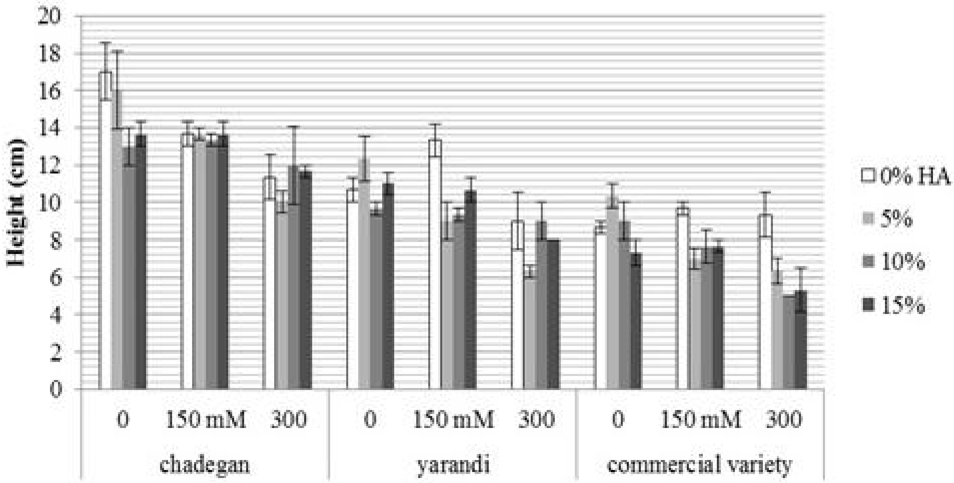

Fig. 4. Triple interaction effect of turfgrass, humic acid and salinity stress on plant height. Error bars represent standard error.

RWC

According to Fig. 5, the results showed a regular process in relative water content of two native cultivar than commercial variety. With increasing of HA concentration the RWC was increased than control. The highest and the lowest RWC were shown in Yarandi with 15% HA and without salinity stress and commercial variety at 15% HA with 300 mM salinity stress, respectively. Totally, application of HA could preserve relative water content of leaves in condition of severe salinity stress.

DISCUSSION

Piccolo *et al.* (1992) represented that humic acid (HA) could improve plant growth and enhance stress tolerance as a plant growth regulator and it is a bio-fertilizer, which acts as a growth stimulant by positive effects on soil and plant properties. However, there are not many researches into humic acids application and their effects on plant salinity resistance (Mahmoudi *et al.*, 2013).Some researchers used NTEP for visual quality rating such as; Garling and Boehm (2001), Salehi and Khosh-Khoi (2004) on some cool and warm season grass species, Bunderson *et al.* (2009) and Saeedi Pooya *et al.* (2013) on native turfgrasses. Turfgrass with high stress tolerance must demonstrate not only functional quality, but also visual quality. Mintenko and Smith (2001) suggested that visual quality was more important than other characteristics such as yield resulting from the aesthetical function of turfgrass.

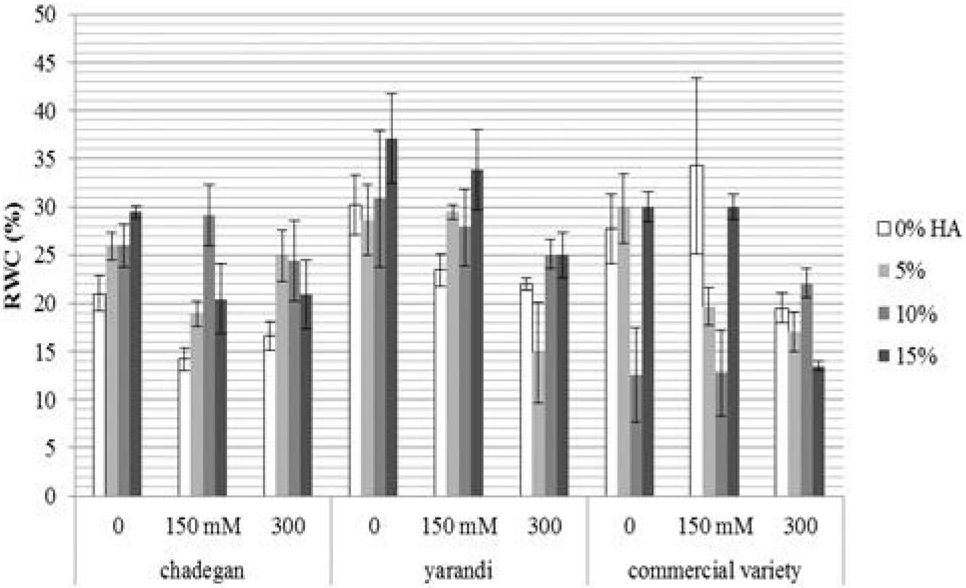

Fig. 5. Triple interaction effect of turfgrass, humic acid and salinity stress on relative water content. Error bars represent standard error.

According to results of this study, turf quality was declined linearly with increasing salinity stress. In summary, as salinity increased, turfs exhibited reduction in turf quality. This result was similar to Munshaw *et al.* (2004) on bermudagrass, Berndt (2007) on *Seashore paspalum*, Walworth *et al.* (2009) on some turfgrasses, Wang and Zhang (2011) on *Prairie junegrass* and Pessarakli (2011) on saltgrass.

In this experiment humic acid improved color, texture, quality after clipping and RWC in all tested turfgrasses. But humic acid had not similar effect on total quality of grasses. To similarity, the results of other researchers were different; Liu and Cooper (2000) reported that humic acid did not improve visual quality compared with untreated turf while, Zhang *et al.* (2003) declared that application of HA improved turf quality.

Beard (1973) stated that most individuals prefer a dark green turf and turf color improved with increasing of salinity stress and HA application.

The complex biological activity of humic matter depends on its concentration, chemical characteristics and molecular size and weight (Muscolo *et al.*, 2007).

Leaf width (or texture) is also one of the important growth characteristic contributing to overall plant growth under normal or saline conditions. However, decrease in leaf width is the main strategy which makes it possible to reduce the effects of water deficit under saline condition (Alem *et al.*, 2002). In other words, the reduction in the availability of water has a significant effect on leaf width and growth of leaves by reducing the rate of photosynthesis (Akhzari and Dehghani Bidgoli, 2013). The leaf area of tolerant genotypes is less affected by salinity stress, as was reported by Hameed *et al.* (2008) on *Cynodon dactylon*, Imperata *cylindrica* and *Sporobolus arabicus*. These reports are in accordance with the present findings where leaf width of tolerant genotype (Yarandi) was less affected by salinity than salt sensitive genotypes.

Between growth variables affected by salt stress, shoot growth is one of the important attribute in evaluating the salt tolerance of plants. However, it may change with species, cultivars, and even populations. According to results, Chadegan had the highest growth at all of salinity levels than other grasses. The regularity of growth in different salinity and HA was observed in Chadegan> Yarandi> commercial turf (Fig. 4).

Mechanism of adaptation to saline condition is very specific. Ecotypes grasses shows stunted growth, perhaps to save energy for normal developmental and other metabolic processes (Mladenova, 1990; Mansour and Salama, 1996). The higher salinity levels caused a significant reduction in plant growth over the lower levels (Fig. 4). Munshaw *et al.* (2004) found similar results on bermudagrass, Li *et al.* (2010) on *Spartina alterniflora* (Poaceae). Akhzari and Dehghani Bidgoli (2013) reported that combined treatment of salinity and drought stress significantly reduced plant height and it is due to limitation of available water or water uptake. Alshammary *et al.* (2004) reported 50% reduction in shoot growth in Kentucky bluegrass, tall fescue, alkaligrass, and saltgrass at 49, 100, 200, and 349 mM salinity, respectively.

In this study, in salinity stress condition, RWC was decreased in all tested turf. But, application of HA could preserve relative water content of leaves in severe salinity stress condition. Akhzari and Dehghani Bidgoli (2013) reported that water content decreased in salinity and aridity stress, too. Because of, humic acid application provides many benefits to agricultural soil, including increased ability to preserve moisture, a better nutrient-holding capacity, a better soil structure and a higher level of microbial activity. The humic acids can significantly decline water evaporation and improve water uptake by plants in non-clay, arid and sandy soils (Ouni *et al.*, 2014). Water status in terms of water stress level of kentucky bluegrass, tall fescue and creeping bentgrass was significantly improved with foliar application of HA (Zhang, 1997).

CONCLUSIONS

According to results of this study, turf quality and RWC were declined with increasing salinity stress and humic acid improved color, texture, quality after clipping and RWC in all tested turfgrasses. One of the important effect of HA application was preserve relative water content of leaves in severe salinity stress condition.

Among grasses, Chadegan and commercial turf had the highest and lowest growth at all of salinity levels, respectively. So, this study confirms the role of humic acid in increasing of growth and quality of turfgrass. Plants grow on soils which contain adequate humic acids (HA) are less subject to stress, are healthier because of having higher relative water content in contrast salinity condition. Generally, according to results we can use humic acid in salt area to have more sustainable and attractive landscape.

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