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ORIGINAL PAPER

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INFLUENCE OF NANO-SILICA AND HUMIC ACID ON PHYSIOLOGICAL CHARACTERISTICS OF BERMUDA GRASS (*Cynodon dactylon* L.) UNDER SALINITY STRESS

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ABSTRACT

This research was conducted to evaluate the effects of water salinity and nano-silica (NS) and humic acid (HA) on Bermuda grass. The study was carried out under greenhouse and exterior space conditions in a completely randomized design with factorial arrangements. Treatments included 4 water salinity levels (0, 5, 7 and 9 dS/m) and 4 level of NS (0, 1, 2 and 3 mm/l) as well as 4 level of HA (0, 50, 100 and 150 mg/l). Results indicated a decrease in chlorophyll content, and increase in proline, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and electrolyte leakage (EL) with increasing levels of salinity. The chlorophyll content in greenhouse and exterior space also increased with increasing levels of NS and HA, while proline in two environments, with increasing levels of NS and HA decreased. EL with increasing levels of NS fluctuated, but with increasing levels of HA, it decreased in two environments. With increasing levels of NS, CAT in both environments was eventually decreased after the oscillation, but there was no particular trend in HA levels. With increasing levels of NS and HA, MDA in the greenhouse decreased and in the exterior space – it increased. Finally, by increasing levels of NS and HA, SOD did not show any change in the greenhouse condition, but in the exterior space, the SOD was decreased.

Key words: nano-silica, humic acid, chlorophyll, proline

INTRODUCTION

Bermuda grass (*Cynodon dactylon* L.) is widely distributed throughout the world between 45°N and 45°S and one of the most widely used warm-season turf grass species in temperate and tropical regions, which has shown good tolerance to salinity and can survive in saline soil [Akram et al. 2012]. Soil salinity, a common problem in turf grass management, is caused by various activities such as deficient precipitation, water percolation from high water tables, low-quality irrigation water, and salts from fertilizers and deicing agents [Akram et al. 2012, Zhang et al. 2012]. In some of cities in Iran, increase use of saline water for irrigation is becoming a serious problem in providing either a playing surface or a green ground cover in sports enterprises and home lawns. This situation needs an investigation to observe the response of grasses to different salinity levels. Salinity adversely affects the plant growth and development, resulting in reduced aesthetical and playable functions of turf grass [Zhang et al. 2012]. Most studies in Bermuda grass examined under salinity conditions were at either morphological or physiological levels [Akram et al. 2012]. Little re-

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search has investigated antioxidant response to salinity at the level of enzyme activity.

Nano fertilizers in plant nutrition are one of the major roles of nanotechnology in agriculture, soil and water sciences. Cell wall of plants prevents the entrance of elements into cells, and nano particles that have less diameter than the pores of cell wall, therefore can easily cross through the pores after application of nano fertilizers, which is one of the promising methods for increasing the resources to use and reduce the environmental pollutions. The use of nano fertilizers encourages the plant growth, production and reduces toxicity of the soil. Also the use of nano fertilizers reduces negative effects caused by the excessive consumption of fertilizers and reduces the frequency of fertilizer application [Karimi and Mohsenzadeh 2016]. Some nanoparticles are reported to be useful in increasing plants productivity grown under adverse conditions as silica dioxide (SiO_2). Recently, silica has given a lot of attention by the agricultural researchers [Karimi and Mohsenzadeh 2016]. It had been reported that silica might play a critical role in increasing the plant's tolerance against environmental stresses [Biglari et al. 2012]. Our objectives of this study were to determine the effects of saline irrigation, NS and HA effects on some of the physiological traits and proline concentration of Bermuda grass.

MATERİAL AND METHODS

Experiments were conducted in the research greenhouse of the Department of Horticulture Science, College of Agriculture, Ilam University, Ilam, Iran. The soil used was a mixture of garden soil, vermin compost, and wind sand. The soil was crushed to pass through a 10-mm sieve and air-dried to disinfect the planting bed, a systemic Metalaxyl GS 5% fungicide at the concentration of 1.5/1000 was used. 192 plastic pots (60 cm in height and 15 cm in diameter) were filled with 5 kg of air-dried soil with a layer of gravel filter at the bottom. Seeds of Bermuda grass were weighed and sowed at the rate of 100 seeds in every pot. Irrigation was carried out daily before seed germination and after grass establishment using tap water. Until the full deployment and establishment of grass, which lasted about 2 months, irrigation was carried out regularly and to the extent that the water was removed slowly from the drainage end of the pots. During this period, the soil water content was kept at field capacity level by adding tap water. During germination and plant establishment, weed control was carried out manually and regularly. Fertilizer was added to the pots with full dose (N : P : K – 20 : 20 : 20) monthly and manually (15.75 g/100 m^2). The saline irrigation water treatments were tap water (control) and 5, 7 and 9 ds/m obtained by the addition of equal proportions of NaCl to the tap water. Salinity treatment was performed 40 days after planting, three times a week, to prevent shock to the plant. NS treatments (0, 1, 2 and 3 mm/l) and HA treatments (0, 50, 100 and 150 mg/l) were added to the soil in a water-soluble solution.

Leaf membrane damage was determined by recording of EL and MDA content [Stewart and Bewely 1980]. Chlorophyll content was calculated using method of Strain and Svec [1966]. Proline content was determined according to the method described by Bates et al. [1973] based on proline's reaction with ninhydrin using a spectrophotometer (PD-303 UV; APEL) at 520 nm wavelength and the appropriate proline standards. SOD was measured following Beauchamp and Fridovich [1971] and finally, CAT activity was recorded as described by Aebi [1984].

This study was carried out with factorial split-plot based on a complete randomized block design with 3 replications. Measurement of parameters was done in two steps. Greenhouse comparisons were performed in all treatments at 90 days and comparison of the treatments in the outer space was carried out 180 days after cultivation. Data were analyzed using SAS software and means were compared using the least significant difference (LSD) test at $p \leq 0.05$.

RESULTS

Chlorophyll Content. Environment, salinity, NS and HA had significant effects ($P < 0.01$) on chlorophyll a, b and t content (except chlorophyll a in HA) (Tab. 1). Exposure to exterior space and salinity reduced the leaf chlorophyll content. The highest and lowest chlorophyll content was observed in control and 9 ds/m level, respectively. The trend of decreasing the chlorophyll content (all three types) under saline treatment in both studied environments is somewhat the same (Fig. 1). The use of HA and NS had significant increase in chloro-

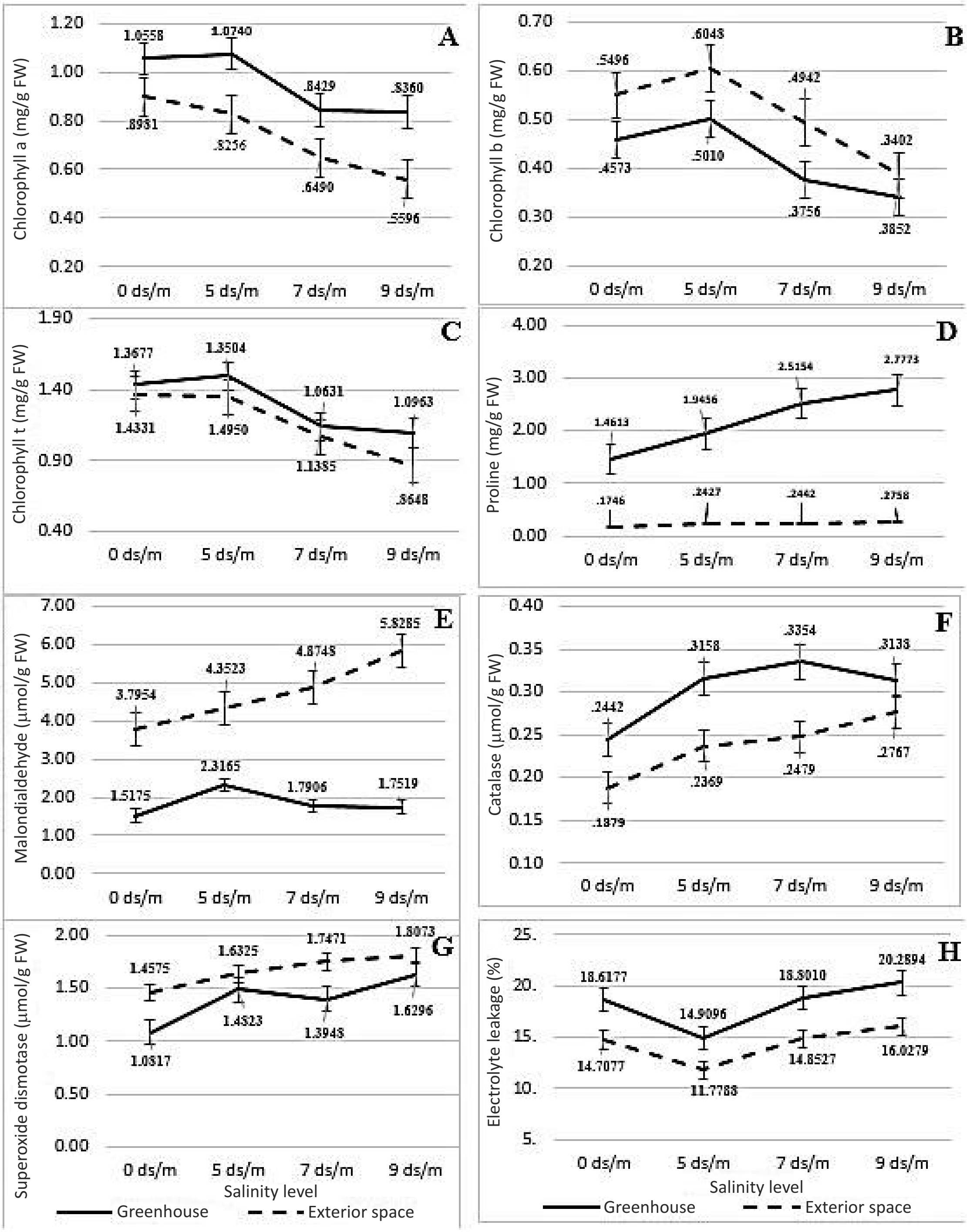

Fig. 1. Physiological characteristics in Bermuda grass cultivar subjected to salinity conditio in green house and exterior space

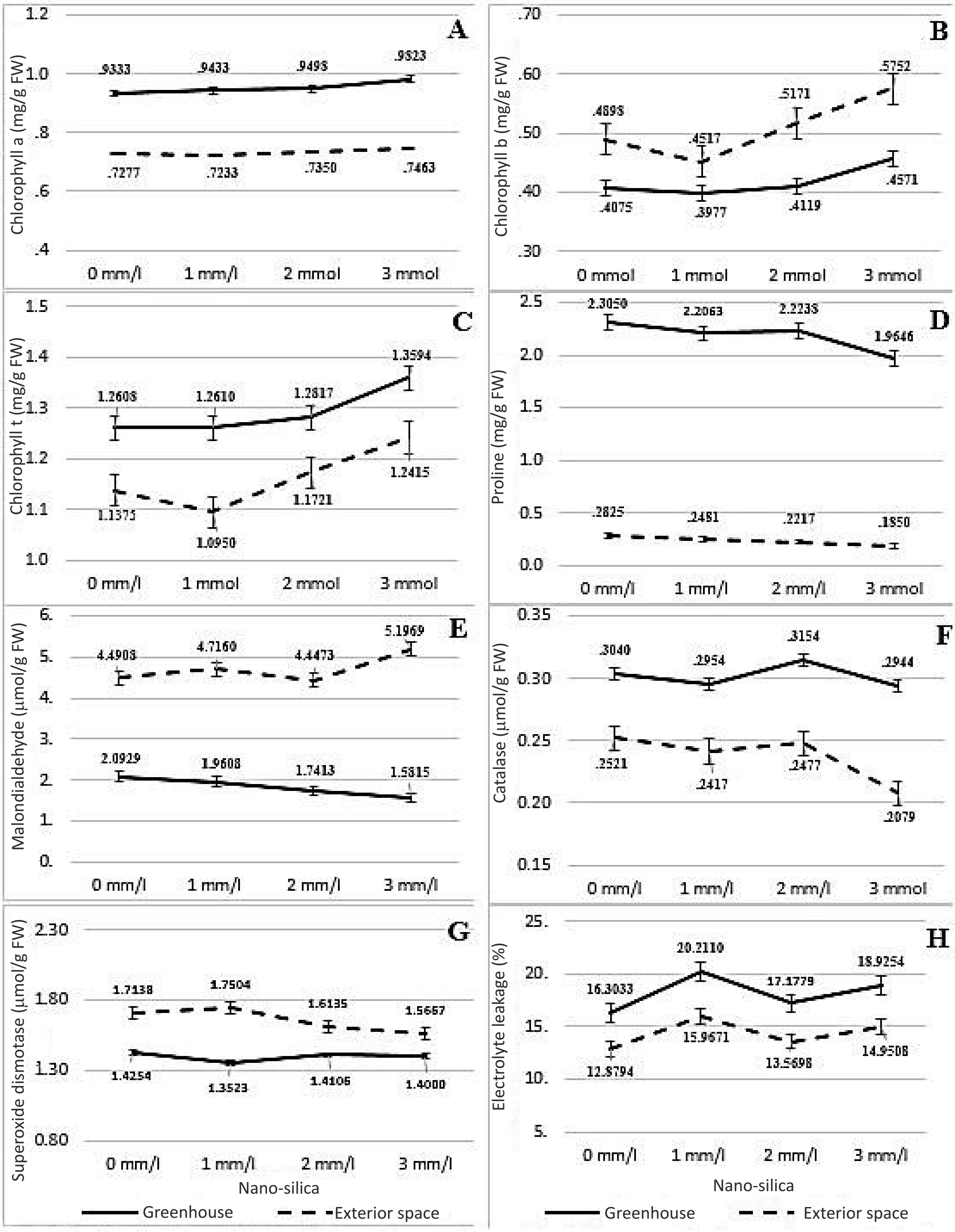

Fig. 2. Physiological characteristics in Bermuda grass cultivar subjected to nano-silica in green house and exterior space

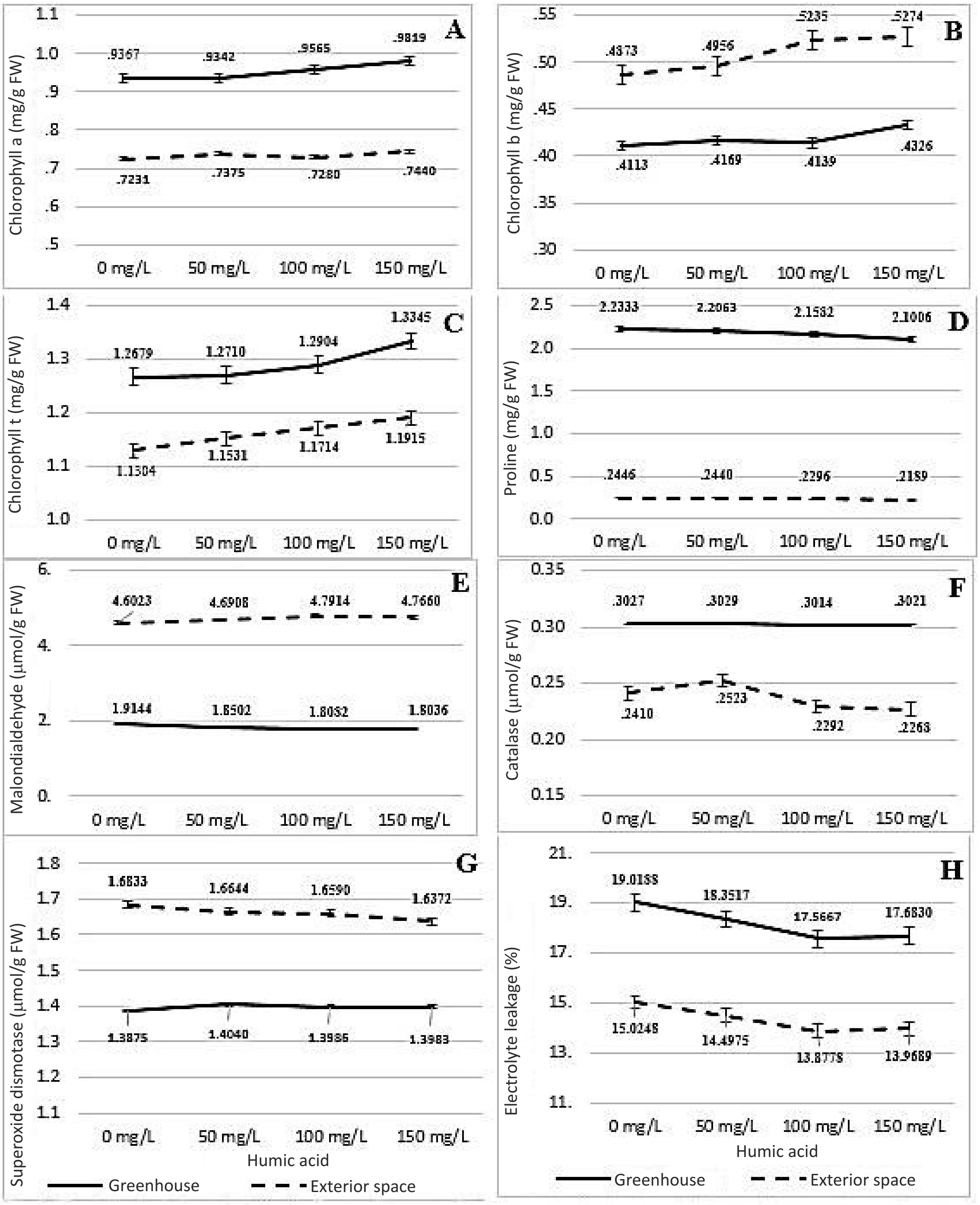

Fig. 3. Physiological characteristics in Bermuda grass cultivar subjected to humic acid in green house and exterior space

phyll a, b and t content. The lowest and highest levels of chlorophyll contents were from control and 2 mm/l for NS and 150 mg/l for HA, respectively (Tab. 2). In NS treatment, chlorophyll b and t in the exterior space was reduced to the 1 mm/l level and then increased (Fig. 2), but in HA treatment, the process of change was the same and incremental (Fig. 3).

Proline Content. All of the main effects of the treatments were significant ($P < 0.01$) for proline content (Tab. 1). Proline in greenhouse conditions was much higher than in exterior space. Proline accumulation increased with increasing salinity level and decreased with increasing of NS and HA levels (Tab. 2). The trend of proline increase in the greenhouse was much more severe than in the exterior space, so that an increase of 90% was observed from the control to 9 ds/m level (Fig. 1). In contrast, the trend of proline decreasing in the NS and HA, in both environmental conditions, is somewhat the same (Figs. 2 and 3).

Malondialdehyde. Environment, salinity, and NS treatments had significant effect ($P < 0.01$) on MDA (Tab. 1). Leaf MDA content as a marker of tissue lipid peroxidation in exterior space, was much higher than the greenhouse and this increase was very impressive. Salinity increased the MDA content, therefore the lowest and highest amount of it was observed in the control and 9 ds/m level, respectively. In NS treatment, the lowest and highest MDA was observed in 2 mm/l and 3 mm/l levels, respectively. In general, NS levels do not seem to cause significant differences in MDA content (Tab. 2).With increasing levels of NS, the MDA content decreased with a roughly steady gradient in greenhouse, but in exterior space, an irregular increase was observed (Fig. 2).

Superoxide dismutase. Environment, salinity, and NS treatments had significant effect ($P < 0.01$) on SOD (Tab. 1). Increasing the plant growth period and exposure to exterior space increased SOD. Increasing

Table 1. Analysis of variance of physiological traits under environmental conditions, salinity, NS and HA

Source	df	CA	CB	CT	Proline	MDA	CAT	SOD	EL
Environment (E)	1	4.599**	0.767**	1.609**	359.99**	786.55**	0.402**	6.636**	1379.06**
Error	2	0.001	0.004	0.006	0.061	0.372	0.008	0.135	70.551
Salinity (S)	3	1.905**	0.649**	4.603**	9.407**	20.81**	0.13**	3.38**	371.6**
NS (NS)	3	0.042**	0.145**	0.274**	0.818**	1.589**	0.017**	0.14**	226.1**
HA (HA)	3	0.016	0.017**	0.065**	0.11**	0.028	0.003	0.004	42.975
E*S	3	0.069**	0.025**	0.143**	7.214**	20.157**	0.013**	0.328**	5.123
E*NS	3	0.004	0.018**	0.015	0.294**	6.449**	0.006	0.257**	3.105
E*HA	3	0.008	0.006	0.005	0.045	0.448	0.003	0.015	0.590
S*NS	9	0.032**	0.038**	0.063**	0.181**	3.229**	0.008**	0.025	114.36*
S*HA	9	0.014*	0.006	0.029**	0.026	0.180	0.003	0.003	99.477
NS*HA	9	0.001	0.003	0.002	0.035	0.324	0.003	0.018	361.29**
E*S*NS	9	0.046**	0.02**	0.052**	0.163**	1.127**	0.004	0.108**	1.575
E*S*HA	9	0.006	0.003	0.013	0.026	0.095	0.002	0.001	1.368
E*NS*HA	9	0.006	0.01**	0.03**	0.048	0.333	0.002	0.015	4.968
S*NS*HA	27	0.008	0.003	0.013	0.041	0.431**	0.003	0.006	136.48**
E*NS*S*HA	27	0.010	0.013**	0.034**	0.040	0.129	0.001	0.015	1.879
Error	252	0.007	0.004	0.008	0.036	0.192	0.002	0.025	59.784
CV%	–	9.95	13.95	7.51	15.66	13.42	18.38	10.43	37.51

CA – chlorophyll a, Cb – chlorophyll b, CT – total chlorophyll, MDA – malondialdehyde, CAT – catalase, SOD – superoxid dismutase, EL – electrolyte leakage; * significant at 1%, ** significant at 5%

Table. 2. Physiological responses under environment condition, salinity, NS and HA

Treatments	CA		CB		CT		Proline		MDA		CAT		SOD		EL	
Greenhouse	0.9522	a	0.4185	b	1.29	a	2.1749	a	1.8441	b	0.3022	a	1.3971	b	18.154	a
Exterior space	0.7351	b	0.5099	a	1.1651	b	0.2334	b	4.6942	a	0.2372	b	1.6605	a	14.123	b
Control	0.9769	a	0.5034	b	1.4004	a	0.8179	d	2.6565	c	0.216	c	1.2969	c	16.663	a
5 dS/m	0.9498	b	0.5529	a	1.4227	a	1.0942	c	3.3344	b	0.2763	b	1.5574	b	13.344	b
7 dS/m	0.7459	c	0.4349	c	1.1008	b	1.3798	b	3.3327	b	0.2917	a	1.5709	b	16.827	a
9 dS/m	0.7013	d	0.3627	d	0.9839	c	1.5529	a	3.7331	a	0.2962	a	1.7184	a	17.798	a
control	0.8305	b	0.4486	b	1.1992	c	1.2938	a	3.2919	a	0.278	a	1.5696	a	14.591	b
1 mm/l	0.8398	ab	0.4259	c	1.1856	c	1.2471	ab	3.2717	a	0.2689	a	1.5478	ab	17.727	a
2 mm/l	0.8424	ab	0.4645	b	1.2269	b	1.2227	b	3.0943	b	0.2879	a	1.5121	bc	15.374	ab
3 mm/l	0.8643	a	0.5161	a	1.3004	a	1.0748	c	3.3892	a	0.2511	b	1.4833	c	16.938	ab
control	0.8299	d	0.4493	a	1.1991	c	1.2389	a	3.2583	a	0.2718	a	1.5554	a	17.022	a
50 mg/l	0.8389	c	0.4572	bc	1.2161	bc	1.2348	a	3.2368	a	0.278	a	1.5321	a	16.186	a
100 mg/l	0.8455	b	0.4697	ab	1.2353	b	1.2033	ab	3.267	a	0.2654	a	1.5269	a	15.563	a
150 mg/l	0.8629	a	0.48	a	1.2629	a	1.1598	b	3.2848	a	0.2645	a	1.5178	a	15.826	a

All the values followed by the same letter in each column for each treatment are not statistically different at the 0.05 probability level

CA – chlorophyll a, CB – chlorophyll b, CT – total chlorophyll, MDA – malondialdehyde, CAT – catalase, SOD – superoxid dismutase, EL – electrolyte leakage

salinity level has increased the amount of SOD, thus most of the SOD was observed at the 9 ds/m level of salinity. In contrast, by increasing of NS, the amount of SOD was reduced. Therefore, the lowest and highest amount of SOD was observed in the 3 mm/l and control level, respectively (Tab. 2). The trend of SOD changes in salinity treatment was incremental. However, in the greenhouse, this trend was irregular and in exterior space, it was regular with a steady gradient (Fig. 1). The effects of salt stress on SOD activity varied at interspecific and intraspecific levels and the age of tissue or organ.

Catalase. Environment, salinity, and NS treatments had significant effect ($P < 0.01$) on CAT (Tab. 1). CAT declined with increasing growth and exposure to exterior space. The highest amount of CAT was found in the 9 ds/m level of salinity, which indicates that increasing the salinity level increases of CAT. In contrast, there was the lowest amount of CAT at the 3 mm/l level of NS, which indicates that when the NS level increases, the CAT eventually decreased (Tab. 2). The process of CAT change in greenhouse and exterior space to the 7 ds/m level of salinity was incremental, but from the 7 to the 9 ds/m level, it decreased in greenhouse and increased in the exterior space (Fig. 1). In NS treatment, the process of CAT change was the same in both conditions, thus it was initially reduced (control to 1 mm/l), then increased (1 to 2 mm/l) and ultimately reduced in 3 mm/l (Fig. 2). Of course, falling from the 2 to 3 mm/l level was more severe in exterior space.

Electrolyte leakage. Environment, salinity, and NS treatments had a significant effect ($P < 0.01$) on EL (Tab. 1). Increasing the plant growth period and exposure to exterior space decreased the EL. Increasing the salinity level from control to 5 ds/m level reduced, but increasing salinity from 5 to 9 ds/m increased the EL. In contrast for NS treatment, by increasing the amount of NS to 3 mm/l, irregular oscillation was observed in EL, therefore the amount of EL initially increased (control to 1 mm/l), then decreased (1 to 2 mm/l) and at the end, also increased (2 to 3 mm/l). No considerable difference was observed between levels of HA (Tab. 2). The EL fluctuations in greenhouse and exterior space for different salinity levels were exactly the same as initially decreased and increased further (Fig. 1).

DİSCUSSİON

The decrease in Chl content under salt stress is a commonly reported phenomenon and it was used as a sensitive indicator of the cellular metabolic state

[Al-aghabary et al. 2005]. Leaf chlorophyll content is an indicator of general plant health. Amirjani [2011] found that Chl a and b contents of leaves were reduced by salinity stress. In this research, chlorophyll a, b and t decreased when the inappropriate effect of salinity was inflicted. Our finding is in agreement with previous studies [Mane et al. 2011]. Marcum [1999] stated that chlorophyll loss under saline conditions might be due to oxidative stress and mass production and aggregate of ROS. It might be related to Mg^{2+} shortage. Photosynthetic responses of plants to abiotic stress such as salinity are very intricate and the severity and period of stresses may change this response [Flower et al. 1977]. According to the results, photosynthetic rate diminished with intensity of salinity stress. One reason might be destruction of chlorophyll, which occurred under saline conditions. Another reason is that salinity may modify basic metabolic processes in a way that results in the decreased photosynthetic rate [Flower et al. 1997]. Salinity can cause synthesis and aggregation of abscisic acid, which leads to restriction of available CO_2 for photosynthesis and stomatal closure [Amirjani 2011]. Another reason is that the outcome of the electron transport chain and the activity of rubisco enzyme may decrease leading to a decrement in the photosynthetic rate [Flower et al. 1997].

NS increased the chlorophyll contents. Si enhances resistance of mature leaves, makes their texture rougher and to stand more horizontally; it has more chlorophyll content, carboxylase activity and delayed senescence [Zhu 2003]. The results revealed that in two conditions applying NS results in an obvious increase in chlorophyll content, which could eventually enhance the photosynthetic efficiency in salt stressed plants. These findings are supported by the results of Al-aghabary et al. [2005] in tomato showing an improvement in the photosynthetic efficiency after application of Si. The results confirmed that HA improved chlorophyll content. Strawberries treated with HA in various dates presented higher chlorophyll concentrations and a greater photosynthetic efficiency [Neri et al. 2002].

In this research, the amount of proline density and activity of free radical scavenging enzymes increased significantly under salinity stress conditions. Proline was synthesized in stress conditions in order to reduce the negative effect of stress on the osmotic balance of plants [Ashraf and Foolad 2007]. The response of plants to salinity includes synthesis of compatible solutes such as proline as is in the case of this investigation. Finally, these responses will cause the detoxification, recovery of cellular homeostasis, and therefore survival under saline stress [Mane et al. 2011]. Aggregation of proline is a general adaptation to reduce the intensity of most abiotic stresses in higher plants. Our results are in accordance with remarks of Akram et al. [2012] on responses of two populations of *Cynodon dactylon* Pers. to salt stress and of Marcum [1999], who studied the salinity tolerance mechanisms of grasses in the subfamily Chloridoideae.

Proline accumulation decreased with increasing of NS levels. It seems that the effect of NS on the amount of proline of different plants and different environmental conditions is different. Karimi and Mohsenzade [2016] reported that NS (50 and 100 mg/L) partly improves the plant resistance to stress and high concentrations of NS (200, 400, and 800 mg/L) can have adverse effects on a plant. Similar studies of NS effects on proline contents in plants showed similar results [Siddiqui and Al-Whaibi 2014]. Proline accumulation decreased with increasing of HS levels treatment. It seems that the effect of HA on the proline of different plants and environmental conditions is different.

The level of MDA produced during peroxidation of membrane lipids is often used as an indicator of oxidative damage. In this study, MDA levels increased significantly during the salt stress compared to controls. These results are in agreement with those of DaCosta and Huang [2007], who found that MDA levels increased under drought stress.

Because of the derangement of electron-transfer chains under salinity stress, elevated levels of ROS can be collected, which can lead to damages to bio-membranes and molecules such as proteins [Benavides et al. 2000]. One of the plant obstacles against ROS collections, is synthesis and accumulation of free radical scavenging enzymes such as SOD and CAT, as we observed in this research. Salt tolerance is related to a higher activity of antioxidant enzymes in plants [Shalata et al. 2001].

In Bermuda grass, the active interference of these enzymes is related to salt-induced oxidative stress tolerance [Mane et al. 2011]. The SOD as one of the metalloproteins, is considered a main enzyme for retaining the normal physiological processes and confronting with oxidative stress by converting O_2^- to O_2 and H_2O_2. In this research, salt stress increased SOD activity in leaves in two environments. Therefore, these results indicated that the SOD activities can be induced by ROS under salinity stress under greenhouse and exterior space. The responses of antioxidant enzymes to stresses depend on stress severity, period of time and plant species of perennial grasses [DaCosta and Huang 2007, Bian and Jiang 2009]. These results suggested that the SOD activities can be induced by ROS under salt stress and the salt-tolerant genotype has a better O^{2-} radical scavenging ability. CAT causes detoxification of H_2O_2, which is localized in peroxisomes [Amirjani 2011]. The results indicated that CAT activity changes may depend on the development and metabolic state of the grass, as well as on the duration and severity of the stress [Uddin et al. 2012]. In *Poa pratensis* L. under drought condition, Bian and Jiang [2009] indicated an increase of CAT. However, unchanged CAT activity under salt stress was also observed in potato [Benavides et al. 2000]. Intense levels of drought or salinity stress may decrease the activity of CAT as it was in the case of greenhouse condition at 9 ds/m level [DaCosta and Huang 2007]. It can be said that decline in antioxidant enzymes' production under saline stress would negatively affect other characteristics, such as photosynthetic rate, chlorophyll content and proline content, which results in reduction of the efficiency of cells under this condition. Contrary to the results of this study, in which catalase has been reduced due to the use of NS, other researchers have reported different results in different plants. Biglari et al. [2012] reported that treatment of rice with NS increased the activity of catalase.

Da Costa and Huang [2007] reported that EL was a symptom of cell membrane deterioration in response to stress. Bermuda grass was treated with short photoperiod duration, Esmaili and Salehi [2012] noted that electrolyte leakage was increased. Agarie et al. [1999] reported that silicon decreased electrolyte leakage and increased cell walls polysaccharides in rice. They suggested that silicon could be involved in thermal stability of lipids in cell membranes and prevent structural and functional deterioration of cell membranes, when rice plants are exposed to abiotic stresses. HA increases the cell and decreases the EL. Increasing cell membrane's stability and reducing EL using HA has been reported by other researchers [Parvazi Shandi et al. 2013].

In all physiological studied traits, mean of traits was different under two environmental conditions. This situation indicates that the environmental conditions studied and the duration of planting in this plant can affect the physiological parameters. The result indicated that salinity can cause severe changes in physiological traits at all stages of plant development. Salinity tolerance depends on plant morpho-physiological features, especially in grasses [Benavides et al. 2000]. Salinity stress may result in the demolition of ion toxicity and osmotic equilibrium [Mane et al. 2011]. Studies have shown that the capability of plants to resist the salinity conditions may reduce the harmful effects of stresses such as salinity [Uddin et al. 2012]. The plants can improve the stresses by synthesizing metabolites such as protein to hamper enzyme destruction through the decline of turgor conversions within the cells [Uddin et al. 2012]. Therefore, in the resistant plant compared to sensitive plants, limited variations occurred in growth rate.

NS and HA cannot prevent oxidant damages *via* increasing of antioxidant enzymes activity and decreasing of free radicals. Due to the lack of information about the main mechanism of silica, the effects are unknown yet, more studies are needed to assay the uptake and transportation of nanoparticles within plants. Humic substances influence several metabolic processes, such as photosynthesis, respiration, nucleic acid synthesis and ion uptake. HA influences the production of RNA, which is essential for many biochemical processes in the cell [Vaughan and Malcolm 1979]. Several researchers have indicated that HA stimulated activities of some enzymes [Neri et al. 2002].

CONCLUSİONS

Results of the present study revealed that application of nano-silica and humic acid was effective in salinity tolerance of Bermuda grass; the best protection was obtained for plants treated with 3 mm/l nano-silica and 150 mg/l humic acid.

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