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ORIGINAL ARTICLE

Response of two halophyte plants (Nitraria schoberi and Halocnemum strobilaceum) to potassium sulfate under saline condition

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Due to the desertification development and consequently the increase of saline lands in Iran, the use of resistant plants in such conditions to reduce its effects seems necessary. Hence, this study has tried to investigate the effect of potassium sulfate treatment on dry matter and Na and K content and physiological properties of foliar tissue of two halophyte species (Nitraria schoberi and Halocnemum strobilaceum) under different saline conditions. According to the results all studied parameters were significantly affected by plant species, potassium sulfate and NaCl treatments. Plant species and NaCl interaction and triple interaction showed significant effect on dry weight, sodium and potassium content of shoot and leaf chlorophyll content, also plant species and K_2SO_4 interaction has significantly effect on plant height and sodium, potassium and flavonoid content of shoots. High levels of K_2SO_4 treatment (135 Kg ha^{-1}) significantly affected potassium content of N. schoberi in 100 and 200 mM NaCl treatments and increased its content more than 27 and 28% respectively compared with control. Also, application of K_2SO_4 affected accumulation of flavonoids in both plant but the response of N. schoberi still significant. According to the mean comparison, N. schoberi showed more than 12% scavenging activity than the H. strobilaceum. It seems that H. strobilaceum has strategies beyond the antioxidant capacity to adapt to the stressful environment condition, which are seemed much more efficient than increasing antioxidant capacity.

Key words: Halochnemum strobilaceum; Nitraria schoberi; Potassium sulfate; Salinity stress

Introduction

In terms of climatology, the main area of Iran is considered as arid and semi-arid regions. In these regions, the concentration of salt is moderate or relatively high because of low precipitation and high evapotranspiration. According to the FAO global map, salinity levels in Iran are 34 million hectares (FAO 2015). The characteristic of these regions is high evaporation rate and low and scattered precipitation, which causes an accumulation of salts in the surface layer of soil (Vijayvargiya and Kumar 2011). The continuity of this condition will reduce crop production, destruction and loss of agricultural land in a short time (Ranjbar and Banakar 2013; Ranjbar and Pirasteh-Anosheh 2015). In this condition, the use of resistant herbaceous species that is capable to survive in tough environmental conditions, such as that occurring in many area in Iran, seems be necessary. Nitraria schoberi is a drought-resistant shrub with numerous ramifications. Nitraria schoberi is a halophic and suffered to high salt concentrations. Plants that can survive on high concentration of salt and grow well are called halophytes (Parida and Das 2005). Despite our county is a favorable condition for Nitraria schoberi cultivation but unfortunately for unknown reasons, there is no evidence about its conventional cultivation (Mojiri and Jalalian 2011). Halocnemum strobilaceum (Chenopodiaceae) is another halophyte plant which habitually occurs in saline soils from Mediterranean to western Asia (Qu et al., 2008).

Under high salinity environment, more toxic ions will uptake by plant as compared to the normal conditions, overload of these toxic ions lead to the destruction of plant tissue structure and osmotic stress (Ashraf et al., 2013). Increasing in Na^+ concentration will reduce the absorption of essential ions such as Mg, Ca and K (Gupta and Huang 2014). On the other hand Mitigating effects of K fertilization under saline condition have been well documented during the previous studies (Schachtman and Liu 1999; Sherif et al., 1998; Hussain and Khattak 2005; Cakmak 2010; Hussain et al., 2013; Kausar and Gull 2014). They suggested that application of potassium can be a useful method to compensate its deficiency under saline condition. Hussain et al., (2013) reported that, K2SO4 increased crop yield by alleviating the negative effects of sodium, so they suggested that potassium sulphate could be an effective source of K for crop production in saline and saline-sodic soils. In similar study Kausar

and Gull (2014) reported that, the highest wheat biomass in saline environment was related to application of 200 mM (K_2SO_4). They were also observed that the use of potassium sulphate has also increased the uptake of essential nutrients like potassium, calcium, magnesium and phosphorus in saline soils.

Hence, in this research, we have tried to investigate the effect of potassium sulfate treatment on dry matter and Na and K content and physiological properties of foliar tissue of two halophyte species (Nitraria schoberi and Halocnemum strobilaceum) under different saline conditions.

Materials and methods

In order to investigation the response N. schoberi and H. strobilaceum to different saline condition and different levels of potassium sulfate a potted experiment was conducted in factorial arrangement based on completely randomized design with 4 replications in Sari Agricultural Sciences and Natural Resources University in north of Iran during the spring of 2016. The mature seeds of two studied halophyte species (N. schoberi and H. strobilaceum) were purchased from Pakan Bazr Esfahan Institute (Esfahan, Iran). The pure seeds were surface sterilized in a 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 min. and rinsed several times with distilled water. Then seeds were allowed to imbibe on moistened paper towels for 2 h. After germination, the uniform seedlings of two studied species (N. schoberi and H. strobilaceum) were transferred to the pots separately. Potassium sulfates at four levels (0, 45, 90 and 135 kg ha^{-1}) were added to pots. After uniformly establishment of the seedlings, the pots irrigated by different concentration of salt solutions (0, 100, 200, 300 and 400 mM).

Plant sampling: Studied parameters consist of: dry weight, plant height, Na^+, K^+, chlorophyll and flavonoid contents were determined 2 month after seedling emerges.

Five plants from each plant species were harvested and data for shoots weight and heights and were recorded.

Plants shoot Na and K analysis

0.5 g ground tissues were extracted by digesting in pure H_2SO_4. The solution was diluted to 50 mL and filtered to determine Na and K by using flame photometer (Jenway-PFP 7, UK) (Wolf 1982).

Chlorophyll content

Lichtenthaler and wellburn (1985) method was used for green pigments determination. So fresh leaf samples (0.025 gr for each treatment) were Freeze Dried and homogenized and poured into a 15 mm polycarbonate tubes, and then 8 cc pure methanol was added to each tube. All tubes were stored at a dark place for 24 hours. The chlorophyll content was determined by spectrophotometer (UNICO UV-2100, USA) by the following equation:

$Chl\ a+b = (15.65\ A^{666} - 7.34\ A^{653}) + (27.05\ A^{653} - 11.21\ A^{666})$

Total flavonoid content

According to the Chang et al., (2007) the colorimetric method was used with slight modification for flavonoid content determination based on quercetin calibration curve. 0.5 ml of Plant extract was mixed with 1.5 ml methanol (80%), 0.1 ml of 10% aluminum chloride, 0.1 ml potassium acetate (1M) and 2.8 ml of distilled water. Then the solution was incubated for 40 minutes at room temperature. The absorbance was measured at 415 nm using spectrophotometer (UNICO UV-2100, USA) against a blank. The blank samples containing all the above compounds with the exception of extract sample, which instead added 80% methanol at the same amount.

Determination of DPPH free radical scavenging activity

By using the Burits and Bucar (2000) method (with slight method) free radical scavenging activities from methanolic extracts of foliar part of above studied plants were analyzed. Methanolic extract of each sample (0.5 ml) was added to 0.5 ml of 0.15 mM DPPH. The mixture was incubated in a dark place at room temperature for 30 min and the absorbance was measured at 517 nm. The blank sample contains all above compounds but 0.5 ml methanol was added instead of tissue extract before absorbance measurement. The Percentage scavenging activity was calculated by using the following equation:

$I\% = (Ablank - Asample / Ablank) \times 100$

Statistical analysis

General linear model (PROC GLM) was performed for Analysis of variance by using the procedure in Statistical Analysis System (SAS) and the mean comparisons were evaluated based on Least Significant Differences (LSD).

Table 1. Physical and mechanical properties of soil.

Depth	Texture	EC ($_{dS\ m^{-1}}$)	pH	T.N.V (%)	O.C%	P (mg $l^{-1)}$	K (mg l^{-1})	S (mg l^{-1})
0-30cm	Clay silt	1.8	7.7	18.1	3.1	7.3	316.1	17.3

Results and discussion

All studied parameters were significantly affected by plant species, potassium sulfate and NaCl treatments (Table 2). Plant species and NaCl interaction (A×B) and triple interaction (A×B×C) showed significant effect on dry weight, sodium and potassium content of shoot and leaf chlorophyll content, also Plant species and K_2SO_4 interaction (A×C) has significantly effect on plant height and sodium, potassium and flavonoid content of shoot (table 2). Meanwhile the interaction of NaCl with potassium sulfate (B×C) was significantly affected shoot dry weight and potassium contents and leaf chlorophyll content (Table2).

Table 2. ANOVA of two studied halophytes response to K_2SO_4 under different levels of NaCl

S.O.V	df	Mean Squares (MS)						
		Shoot dry weight	Plant height	Na^+	K^+	Free radical scavenging	Flavonoid content	Chl (a+b)
Plant sp. (A)	1	4624.65^{**}	8037.22^{**}	17044.92^{**}	2629.10^{**}	5614.05^{**}	1886.09^{**}	326.61^{**}
NaCl (B)	4	118.22^{**}	94.92^{**}	1521.71^{**}	581.26^{**}	340.90^{**}	23.54^{**}	17.93^{**}
K_2SO_4 (C)	3	13.57^{*}	108.66^{**}	450.98^{**}	207.98^{**}	156.32^{*}	29.21^{**}	2.27^{*}
A×B	4	28.51^{**}	29.39^{ns}	265.82^{**}	55.73^{**}	6.96^{ns}	3.44^{ns}	2.38^{*}
A×C	3	9.19^{ns}	70.18^{*}	301.24^{**}	170.20^{*}	83.91^{ns}	21.80^{**}	1.55^{ns}
B×C	12	14.94^{**}	6.16^{ns}	105.89^{ns}	49.84^{**}	32.40^{ns}	3.65^{ns}	1.88^{**}
A×B×C	12	19.00^{**}	20.05^{ns}	161.52^{*}	55.64^{**}	61.95^{ns}	5.43^{ns}	2.49^{**}
Error	120	5.04	20.23	72.88	13.10	49.82	3.86	0.71
Total	159							
(CV %)		19.86	17.43	20.78	14.64	18.19	15.40	19.07

*, ** and ns; significantly at 5 and 1 % probability levels, not significant, respectively.

Shoot dry weight

According to the mean comparisons (table 3) different levels of NaCl and its interaction with potassium sulfate significantly affected both plant species. So that, by increasing levels of NaCl the shoot dry weights of two studied plant decreased while by increasing levels of K_2SO_4 dry weights of both plants increase compared with control but in some cases it's not significant. Meanwhile, the highest shoot dry weights of N. schoberi were related to 0 and 100 mM of NaCl treatments both in interaction with 135 kg of K_2SO_4 (with 7.25 and 7.26 gr, respectively). The lowest amount of dry weight of N. schoberi Observed in high concentration of NaCl. The highest shoot dry weights of H. strobilaceum were related to 0 mM of NaCl treatment × 90 and 135 kg of K_2SO_4 (with 21.64 and 21.66 gr, respectively). The lowest amount of dry weight of H. strobilaceum observed in high concentration of NaCl treatments (table 3). Several evidences have been well documented about the role of potassium in plant functional mechanism under depressing conditions. In plant the sufficient amounts of potassium are essential for enzymes functions, metabolites and both high (such as proteins, starches and cellulos) and low (soluble sugars organic acids, amino acids and amides) molecular weight compounds synthesis (Marschner 2012; Mengel and Kirkby 2001; Sarwar 2012; Prasad et al., 2010). Under salinity environment condition the K^+ deficiency can usually be observed, due to presences of high levels of Na^+ which inhibit K^+ activity in the soil solution (especially in low K^+ status) (Botella et al., 1997). Negative effects of salt on photosynthesis of barley significantly increased due to K^+ deficiency (Degl'Innocenti et al., 2009). In maize, similar results reported by Qu et al., (2012), they founded that potassium deficiency significantly inhibited nitrogen and carbon assimilation under salt stress.

Ions concentration (K^+ and Na^+)

Potassium content of leaves decreased significantly by increasing NaCl levels in both plant species and also increased by increasing the levels of potassium sulfate treatment. High levels of K_2SO_4 treatment (135 Kg ha^{-1}) significantly affected potassium content of N. schoberi in 100 and 200 mM NaCl treatments and increased its content more than 27 and 28% respectively compared with control. Sodium content increased by increasing NaCl levels and decreased due to increasing in K_2SO_4 levels, but it was nevertheless significant during the 0, 200 and 400 mM NaCl treatments (table 3). Response of H. strobilaceum to the mentioned treatment was partially similar to the N. schoberi so that, all levels of K_2SO_4 showed significant effects on potassium content from 100 to 400 mM NaCl with the exception of 0 mM. Sodium content of H. strobilaceum increased significantly by increasing NaCl levels but in contrast by increasing K_2SO_4 levels its content significantly decreased (table 3).

Under salinity (especially NaCl) stress conditions deficiency of K^+ usually be common. Toxic levels of Na^+ inhibit K^+ uptake in the soil solution, due to reduction its availability. Also, Na^+ disorder translocation of K^+ from root to shoot (Botella et al., 1997) and completion with K^+ for uptake (Coskun et al., 2013). On the other hand more uptakes and accumulation of toxic ions (such as Na^+) affected osmotic potential of plant tissues, which cause the reduction in photosynthesis and plant growth rate (Irshad et al., 2002; Ali et al., 2012, Kausar et al., 2012). As a result the plant development depends upon the availability of suitable amounts of potassium especially under saline conditions (Wang et al., 2013). Mojiri and Jalalian (2011) investigated the relationships between growth of N. schoberi and some soil properties. They reported that the sodium ions along with other studied treatments had negative effects on plant physiological parameters.

Chlorophyll (a+b) content

Total green pigments (a+b) content negatively and positively affected by increasing salt (Nacl) and K_2SO_4 levels, respectively in both studied plants (table 3). The highest chlorophyll content of N. schoberi and H. strobilaceum with 7.65 and 4.14 mg g^{-1} were related to 0 mM $_{NaCl}$ × 135 Kg ha^{-1} $_{K2SO4}$ treatment and the lowest with 3.54 and 1.85 mg g^{-1} belonged to 400 mM $_{NaCl}$ × 0 Kg ha^{-1} $_{K2SO4}$ variant. Chlorophyll is the main photosynthesis pigment plays a vital role in moving through the tough environmental conditions. Furdi et al., (2013) found that, chlorophyll content decreased under adverse salt stress due to chlorophyll degradation. Salinity stress Impacts on photosynthesis function and chlorophyll content of N. schoberi was investigated by Ranjba-Fordoei and Dehghani-Bidghol (2016). They indicated that, by increasing salinity levels (especially in high concentration) the chlorophyll content of leaves significantly decreased.

The chlorophyll content decreased due to activity of ROS; thereby photosynthetic activity is reduced (Cakmak 2000). On the other hand, increasing in Na^+ concentration will reduce the absorption of essential ions such as K (Gupta and Huang 2014). Thereby K fertilization under saline condition is suitable (Schachtman and Liu 1999; Garg and Gupta 1998; Sherif et al., 1998; Hussain and Khattak 2005; Cakmak 2010; Hussain et al., 2013; Kausar and Gull 2014).

Table 3. Mean value ± standard deviation of two studied halophytes under interaction of NaCl × K_2SO_4

NaCl (mM)	K_2SO_4 (Kg ha^{-1})	Shoot Dry Weight (gr)		K^+		Na^+		Chl (mg g^{-1})	
		N. schoberi	*Halocnemum Str*	*N. schoberi*	*Halocnemum Str*	*N. schoberi*	*Halocnemum Str*	*N. schoberi*	*Halocnemum Str*
0	0	5.98±0.46	14.73±3.9	35.14±2.1	58.17±2.7	18.15±3.5	32.27±2.4	5.95±0.56	3.23±0.3
	45	6.91±1.56	17.75±3.3	37.51±3.0	62.31±3.8	15.11±2.6	28.20±1.1	7.29±1.16	3.46±0.21
	90	7.07±1.91	21.64±3.9	38.96±3.5	69.70±9.9	13.89±2.9	27.00±3.4	7.58±1.38	3.88±0.88
	135	7.25±0.96	21.66±3.1	39.00±2.7	66.20±9.7	12.64±2.2	16.90±1.4	7.65±0.17	4.14±1.06
100	0	5.94±2.01	14.99±3.7	29.25±3.5	46.67±9.8	21.27±2.1	26.21±2.3	5.69±1.35	2.91±0.77
	45	4.94±1.79	17.02±1.6	33.03±3.1	55.58±4.9	20.40±1.6	23.83±1.8	6.83±0.80	3.35±0.82
	90	6.74±1.77	19.79±1.1	35.62±1.0	65.26±8.6	18.41±2.9	19.26±2.2	6.93±0.39	3.46±0.78
	135	7.26±2.11	20.92±1.9	37.66±1.7	60.29±7.7	17.43±1.4	15.92±3.2	7.32±0.66	3.63±0.48
200	0	5.91±1.08	13.77±2.3	25.09±1.3	41.80±8.2	22.18±3.1	32.32±3.0	4.82±0.80	1.69±014
	45	5.30±1.85	14.80±2.5	29.78±1.7	53.19±9.7	18.74±2.3	31.29±1.9	5.15±1.38	2.35±0.89
	90	5.79±1.41	19.52±2.3	29.37±1.4	50.88±10.4	18.50±3.1	30.88±2.7	5.71±0.68	3.00±0.23
	135	5.61±1.59	20.84±3.3	31.94±3.1	53.89±4.1	17.21±2.9	29.94±2.3	6.21±1.15	3.13±0.19
300	0	4.70±1.77	13.94±0.3	24.78±2.1	39.78±2.5	26.15±2.4	36.26±2.4	4.88±1.12	2.59±1.21
	45	5.18±2.42	16.25±3.7	26.51±3.5	35.85±9.3	24.74±1.3	33.50±3.2	5.18±0.87	2.81±0.79
	90	5.71±1.16	16.32±1.5	26.64±2.2	50.51±14.3	22.94±3.3	34.62±2.1	5.24±1.28	2.90±0.7
	135	5.31±0.98	18.35±3.5	30.62±1.4	52.11±8.7	20.31±2.4	22.10±2.8	6.42±1.22	2.96±0.54
400	0	3.88±0.08	10.12±1.8	18.21±1.6	30.42±4.7	27.08±2.8	42.05±2.6	3.54±0.62	1.85±0.60
	45	4.19±1.20	11.11±2.3	21.95±1.7	33.20±9.8	27.06±2.7	36.78±2.4	3.89±0.81	2.33±0.90
	90	5.65±1.27	12.09±2.1	21.76±1.9	38.81±8.8	26.05±2.1	29.55±2.4	4.23±0.73	2.36±0.58
	135	5.70±1.64	14.52±2.8	23.14±2.5	47.18±4.1	24.40±2.9	25.93±1.1	5.08±0.99	2.39±0.18
LSD		2.20	3.91	6.70	9.72	4.89	5.34	1.37	0.99

Total flavonoid content

Application of K_2SO_4 affected accumulation of flavonoids in both plant but the response of N. schoberi still significant (table 4). So that, by increasing the levels of potassium sulfate flavonoid accumulation was stimulated (table 4). Accumulation of flavonoids and many other secondary metabolites in plants in response to the abiotic stress can lead to serve as antioxidants under stress conditions (Agati et al., 2011). Increasing in content of specific secondary metabolites under osmotic stressful condition is protecting the plants cellular structures from oxidative damages (Jaleel et al., 2007). Meanwhile, flavonoids play an important role to enhance the plant defense system against ROS activity, especially under salt stress (Reginato et al., 2014a). The accumulation of osmolytes and antioxidant compounds (e.g. flavonoids) in different cultivars of pepper (Capsicum annuum L.) was studied by Hand et al., (2017) under salt stress condition. They found that, salinity alters flavonoids accumulation and its content positively correlated with resistance of studied cultivars to salinity. H. strobilaceum did not show significant response.

Table 4. Mean value ± standard deviation of two studied halophytes

Plant Species	K_2SO_4 (Kg ha^{-1})	Flavonoid content (mg $_{Quersetin}$ g^{-1})
N. schoberi	0	14.58±2.45
	45	15.43±1.82
	90	16.52±3.12
	135	18.18±3.02
LSD		1.03
H. strobilaceum	0	8.75±1.58
	45	9.37±1.53
	90	9.50±1.08
	135	9.49±1.30
LSD		0.86

DPPH radical scavenging assay

In present study the DPPH scavenging assay of both studied plants were affected significantly by all treatment with the exception of their interactions (figure 1A, B and C). According to the mean comparison, N. schoberi showed more than 12% scavenging activity than the H. strobilaceum (figure 1A). By increasing levels of K_2SO_4 the response to DPPH radical scavenging capability also increased (figure 1B). In contrast, increasing in salinity levels caused the main reduction in DPPH scavenging

capability among he both studied plant (figure 1C). Previously many studies has been well reported close and positive correlation between antioxidant capacity and crops salinity tolerance (Hernandez et al., 2010, Rezazadeh et al., 2012; Reginato et al., 2014b; Hand et al., 2017). Also application of potassium enhances plant vitality under stressful condition by increasing antioxidant levels and reducing ROS production (Cakmak 2005; Devi et al., 2012). In terms of the flavonoids contents and free radical scavenging capability, the poorer response of H. strobilaceum than the N. schoberi species necessarily does not mean that N. schoberi more resistance to salinity than H. strobilaceum while numerous sources have been reported that H. strobilaceum is one of the most resistant plant species to salinity (Zorb et al., 2013).

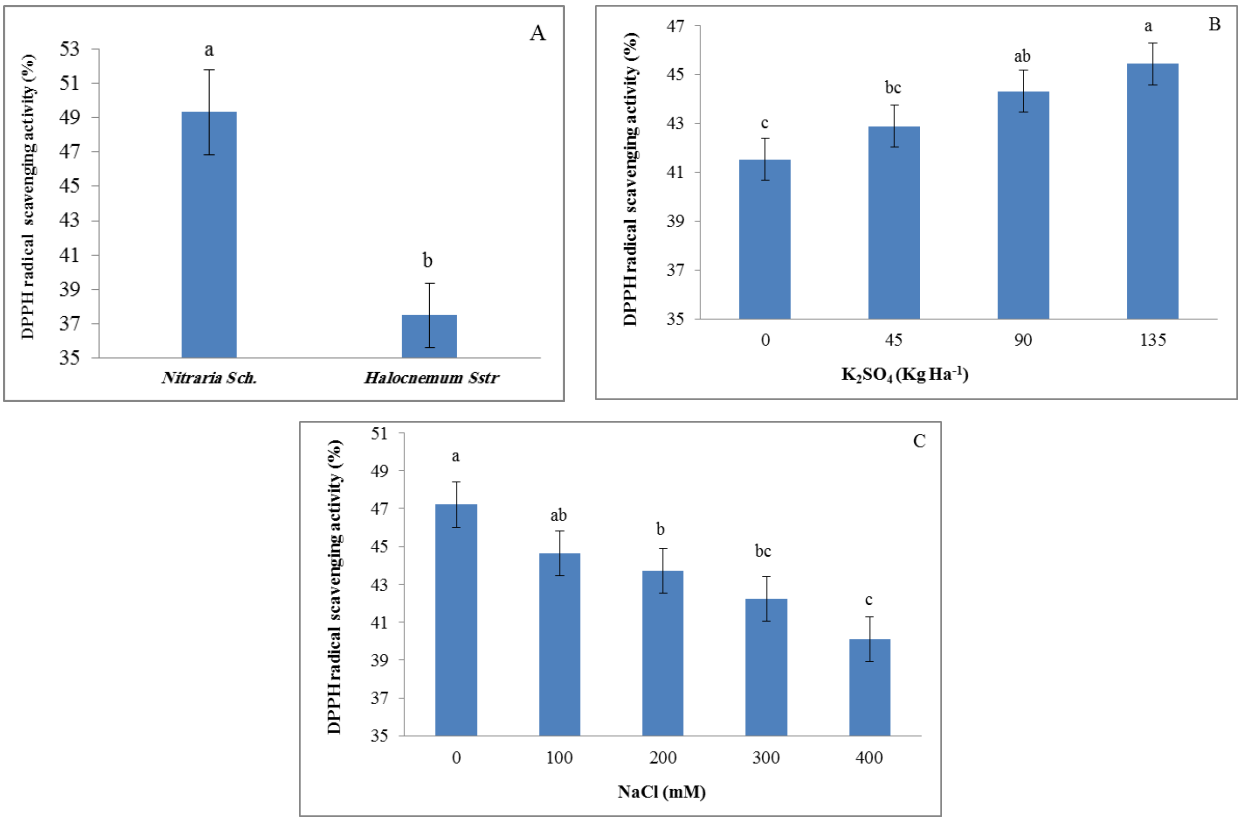

Fig. 1. Mean comparison of main effect in DPPH radical scavenging assay

Conclusion

It seems that H. strobilaceum has strategies beyond the antioxidant capacity to adapt to the stressful environment condition, which are much more efficient than increasing antioxidant capacity. The high water content in leaves of H. strobilaceum allows them to adapt to high-salt conditions by diluting the high Na^+ levels via water retention in vacuoles and high concentration of K^+ in leaves, in contrast N. schoberi adaptation to saline condition was supported by high antioxidant capacity (such as flavonoids content) and free radical scavenging activity.

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