



Research Article

Influence of diluted seawater irrigation on the Physiological and biochemical characteristics of common Egyptian turfgrass

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The objectives of the present study were to exploitation of seawater in turfgrass irrigation, thus providing fresh water for drinking and essential crops irrigation. Pots experiment was conducted at Kafrelsheikh University Farm during the warm season (May-September) of 2014 and 2015 seasons on Seashore paspalum (*Paspalum vaginatum* Swartz) and Bermudagrass (*Cynodon dactylon* × *Cynodon transvaalensis*) turfgrass. Seawater was diluted with freshwater to obtain the required percentages (0, 10, 20, 30, 40, 50, 60, 70 and 80% seawater). Each pot was received 200 ml of the suitable diluted seawater twice a weekly throughout the study course. Data were collected on turf vegetation, plant succulence, leaf firing and turf quality beside some bio and chemical constituents. The results showed that, low percentages (10-30%) of seawater surpassed the higher percentages or control, in most studied characters.

Key words: Seashore paspalum, Bermudagrass, Turfgrass, diluted seawater, irrigation.

INTRODUCTION

The demand for salinity tolerant turfgrass is increasing due to augmented use of low quality water or seawater for turf irrigation and the growing turfgrass industry in coastal areas mainly with the diffusion of tourist pastures and beaches. Drought spread and irrigation water lack became the most urgent global problems especially in Egypt after the latest developments in the upstream of the River Nile. So, the availability of adequate water in terms of quality and quantity will be the number one issue affecting turfgrass management in the 21st century. Since Egypt, overlooking on both Mediterranean Sea and Red Sea, where they could be diluted and used as accessory source for irrigation of some salt-tolerant plant species. Therefore, there is a great need for enhancing salt tolerant turfgrass to survive under such stressful conditions (Glenn *et al.*, 1999). Turfgrass must maintain cosmetic appeal, adequate growth, and persistence

under variable salinity levels in both soil and irrigation water (Lee *et al.*, 2004).

Seashore paspalum (*Paspalum vaginatum* Swartz) belongs to the family Poaceae. It grows along the coastline as strand vegetation in many tropical and subtropical areas of the world. It is a perennial creeping grass that is stoloniferous and rhizomatous. It forms a thick mat of growth and has dark-green leaves with shiny waxy leaf coat (Zinn, 2004).

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Table A. Initial physical and chemical analysis of the experimental soil (average of both seasons).

Sand	Silt	Clay	Soil texture	EC	pH	Tot al N	Tot al P	O M
				mmhos/cm		ppm		
8.13	60.01	25.63	Silty clay	0.90	7.85	23	11	0.45
Soluble cations (meq/l)				Soluble anions (meq/l)				
Na ⁺	K ⁺	Ca ⁺	Mg ⁺⁺	CO ₃ ⁻	HC O ₃ ⁻	Cl ⁻	SO 4 ⁻	
5.60	0.18	3.46	3.30	-	20.04	29.00	5.60	

Table B. Chemical analysis of seawater and tap water (average of both seasons)

Sample	pH	EC	Na ⁺	Cl ⁻	Co 3 ⁻	Ca ++	Mg ++	N	P	K
		dS m ⁻¹	pp m	Meq/l			%	ppm		
Seawater	8.20	50.91	116.00	578.2	1.7	27	189	1.8	113.32	24.7
Freshwater	7.15	3.8	333.5	1.8	0.9	15.5	7.4	0.6	0.4	4.88

Bermudagrass (*Cynodon dactylon* × *transvaalensis*) cv. Tifway 419 belongs to family Poaceae and originated in the Middle East. It is the primary warm-season turfgrass for golf and is tolerant of low mowing heights; therefore, some cultivars are used on golf course greens (Teuton *et al.*, 2005). Seashore paspalum is a relatively new turfgrass for the desert southwest and has greater salt tolerance than bermudagrass (Marcum and Murdoch, 1990). However, far less is known regarding the performance of paspalum under optimal as well as deficit irrigation regimes. To date there have been no studies that have examined the performance of paspalum during extended periods of deficit irrigation.

The main toxic components of seawater is Na⁺ and Cl⁻, which interferes with the normal physiological processes, such as enzyme activities and protein synthesis, as well as causing osmotic imbalances (Munns and Tester, 2008). Salinity cause many impositions as ion toxicities (e.g., Na and Cl), ionic imbalances, osmotic stress and soil permeability problems (Ashraf *et al.*, 2008).

In most of the glycophytic grasses like *Cynodon*, salinity tolerance is positively correlated with Na⁺ and Cl⁻ secretion through salt glands (Marcum, 1999). Halophytic grasses are highly salt tolerant due to their ability to exclude salt from the internal tissues. Tolerance to salinity in the halophytic grasses is facilitated by the development of adventitious roots and a superior ability to maintain negative membrane potential in root cells, resulting in greater retention of K⁺ in shoots (Teakle *et al.*, 2013).

Salt tolerance in plants is generally associated with low uptake and accumulation of Na⁺, which is mediated through the control of influx and/ or by active efflux from the cytoplasm to the vacuoles and also back to the growth medium (Jacoby, 1999).

Managers for perennial turfgrass must deal with problems of reduced growth, tissue dehydration, nutritional imbalances and specific ion toxicities, slow recovery from injury, and poor long term persistence that can be caused by salinity stress (Carrow and Duncan, 1998). Sodium chloride (NaCl) is the major compound contributing salinity in soils and more salt-tolerant turfgrass are required to cope this problem (Harivandi *et al.*, 1992).

Biochemical pathways leading to products and processes that improve salt tolerance are likely to act additively and probably synergistically (Lyengar and Reddy, 1996). Totals protein contents of leaves changes and strongly affected in response to salinity (Alamgir and Ali, 1999).

This study aimed to measure the tolerances extent of common Egyptian warm season turfgrass {(Bermudagrass (*Cynodon dactylon*, L.), cv. Tifway 419 and Seashore paspalum (*Paspalum vaginatum* Swartz)) to diluted seawater irrigation.

MATERIAL AND METHODS

Pots experiment was conducted at Kaferelsheikh University Farm during warm seasons (May-September of 2014 and 2015) to study the effect of diluted seawater

irrigation on Seashore paspalum (*Paspalum vaginatum* Swartz) and Bermudagrass (*Cynodon dactylon* × *Cynodon transvaalensis*) Turfgrass.

Sods (5 cm × 5 cm) of studied turfgrass were transplanted into 16 cm plastic pots filled with silty clay soil (local costal area soil) and allowed to grow four weeks under freshwater irrigation to reach uniform and equal size.

Turfgrass were clipped by scissors biweekly throughout the experiment at the cutting height of 15 mm. The required quantity of seawater was collected from Baltem beach, Kafer El-Sheikh, Egypt. Seawater was diluted with freshwater to obtain the required percentages of seawater / freshwater (0, 10, 20, 30, 40, 50, 60, 70 and 80 %). High seawater percentages (80, 70 and sometimes 60% seawater) were excluded later due to poor results or plants die. Irrigation was repeated twice a weekly at rate 200 ml per pot throughout the study course. Each pot monthly received 2.5 g of NPK (15:15:15) beginning from one month after sodding.

At the end of the experiment, data were collected on shoot growth, leaf area, plant succulence (ratios of the shoot fresh weights to dry weights). Leaf firing and turf quality were visually measured by ten evaluators as follow: Leaf firing was estimated as the total percentage of chlorotic leaf area, with 0 % corresponding to no leaf firing, and 100 % as totally brown leaves. Likewise, turf quality was visually estimated based on a scale of 1-9, with 9 as green, dense and uniform turf, and 1 as thin and completely brown turf (Alshammary *et al.*, 2003). Leaf area was measured by portable laser leaf area meter (CI-202 model). The canopy total green color was measured using a portable chlorophyll meter (Minolta SPAD-502, Minolta co., Ltd. Japan). Plant roots were harvested, washed with fresh water and roots characters were recorded. Both shoots and roots were oven dried at 70° C for 72 h to determine both roots and shoots dry weights. Shoots growth rate was calculated as follows: (Dry weight of each treatment / Dry weight of fresh water irrigated plants (control) × 100. The experimental design was a Randomized Complete Block Design with five replications .

Proline content, catalase and peroxidase activity

Leaf samples (500 mg) were crushed with 10 ml of 3 % 5-sulfosalicylic acid. Proline content was determined spectrophotometrically at 520 nm (Bates, 1973). The method as described by Sadasivam and Manickam (1996) was used for the assay of catalase activity, 1 g of freshly leaf sample was homogenized in 3 ml of 0.067 M phosphate buffer (pH 7.0) The catalase (CAT) activity was determined in the homogenates by measuring the decrease in absorption at 240 nm in a 3 ml of reaction mixture containing (0.16 ml of 10 % W/V H₂O₂ diluted to

100 ml with 0.067 M phosphate buffer) and 0.1 ml of enzyme extract. Peroxidase (POD) activity was carried out spectrophotometrically using guaiacol / H₂O₂ as substrate (Loborzewski *et al.*, 1990).

SDS-protein electrophoresis

Leaves (~0.5 g) were homogenized to obtain protein extracts by mechanically grinding in 500 µl of the protein extraction buffer (62.5 mM Tris-HCl, pH 6.8, 2 % SDS, 10 % glycerol, 5 % β-mercaptoethanol, 5 M Urea and 0.01 % bromo-phenol blue) and mixed well by vortexing. Protein extracts were centrifuged at 14,000 rpm for 10 min at 4°C and apply in 12 % (SDS-PAGE) according to (Laemmli, 1970), Molecular weights of different bands were calibrated with a mixture of standard protein markers (Molecular Weight Marker, M. W. 14.000 - 66.000). The banding profile was stained by Coomase blue dye then photographed and scored.

RESULTS AND DISCUSSION

Effect of diluted seawater irrigation on Seashore paspalum and Bermudagrass turfgrass

Turf vegetation

All aerial parts growth characters recorded a steadily increase whenever seawater percentage increased till 10 % and sometimes 20 %, then it has been declining till 50 % which recorded the absolutely lowest values (Table 1 and Fig., 1). The highest shoot growth rate (1.23 and 0.78 g dry wt./week) and shoot fresh and dry weights (2.58 and 2.49 g) and (0.62 and 0.45 g) were resulted from 10 % seawater for seashore paspalum and Bermudagrass, respectively. Seashore paspalum treated with 20 % seawater and Bermudagrass at 10 % gave the widest leaf area (6.03 and 4.92 mm²). Higher salinity caused larger decreases in growth characters than lower salinity (Qian *et al.*, 2000). Likewise, (Dudeck and Peacock, 1993) obtained 50 % growth reduction when Tifway Bermudagrass treated with salinity. At low percentages of seawater, plant may be benefits of the seawater minerals as a fertigation (Phuntshoa *et al.*, 2011). Seawater (10-30%) can be used as a fertigation of *Conocarpus erectus* plant (El-Mahrouk *et al.*, 2010).

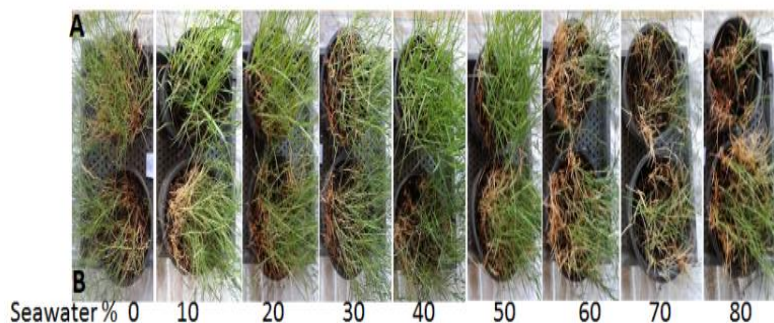
As for roots, both root length and shoots/ roots % followed the same behavior of aerial parts (Table 2). Seawater at 10 % surpassed both control treatment and high seawater percentages (20, 30, 40 and 50%). Conversely, control treatment excelled others in roots fresh and dry weights for both of seashore paspalum or Bermudagrass. Presence of excessive salt (NaCl) outside the cell can induce an osmotic stress which may adversely affect all plant growth Marcum (2006).

Table 1. Effect of diluted seawater irrigation on shoot growth rate, leaf area, shoots fresh and dry weight (average of both seasons).

Seawater %	Shoot growth rate (g dry wt./week)		Leaf area (mm) ²		Shoots F.W. (g)		Shoots F.W. (g)	
	P	B	P	B	P	B	P	B
0	0.94b	0.65f	4.56g	3.74i	2.43bc	2.37bcd	0.57b	0.40f
10	1.23a	0.78d	5.88b	4.92d	2.58ab	2.49a	0.62a	0.45e
20	0.86c	0.49h	6.03a	4.81e	2.44bc	2.30bcd	0.55c	0.36g
30	0.67e	0.27j	5.42c	4.78f	2.25cd	2.13d	0.48d	0.30i
40	0.51g	0.13l	4.11h	3.63j	1.86e	1.63e	0.34h	0.21k
20	0.38i	0.08m	3.39k	3.08m	1.67e	0.92f	0.25j	0.07m
60	0.17k	0.07n	3.22l	2.65n	0.83f	0.77f	0.09l	0.06n

P= Seashore paspalum, B= Bermudagrass

Means within a column having the same letters are not significantly different in Duncan's Multiple Range Test.

**Figure 1.** Effect of diluted seawater irrigation on (A) Seashore paspalum (B) Bermudagrass turfgrass.**Table 2.** Effect of diluted seawater irrigation on roots measurements (average of both seasons).

Seawater %	Root length (cm)		Roots F.W. (g)		Roots D.W. (g)		Shoots/ Roots (%)	
	P	B	P	B	P	B	P	B
0	14.08e	15.26d	4.21a	3.64d	2.96a	2.24e	19.33e	21.47c
10	16.13a	15.78b	3.96b	3.27e	2.85b	2.04g	21.78b	22.10a
20	15.50c	11.67g	3.88c	3.18f	2.80c	2.01h	20.16d	17.94g
30	12.27f	7.05i	3.11g	2.67i	2.30d	1.75j	19.03f	17.23h
40	8.44h	3.55k	2.82h	2.45k	2.05f	1.52k	16.79i	13.72j
20	5.82j	1.73m	2.48j	1.06m	1.83i	0.52l	13.36k	8.71l
60	3.44l	1.00n	1.42l	1.01n	0.50m	0.37n	8.46n	8.50m

P= Seashore paspalum, B= Bermudagrass

Means within a column having the same letters are not significantly different in Duncan's Multiple Range Test.

Plant succulence

Turf succulence increased steadily greater the seawater percentage, as it reached a peak at 30 % seawater then began to gradually decrease in Bermudagrass whereas, in Seashore paspalum the succulence continued to increase till reached the peak at 50 % seawater then it began to gradually decline (Fig., 2). Increasing succulence in the presence of salt is an adaptive mechanism for ion dilution (Debez *et al.*, 2004 and

Pessaraki and Touchane, 2006). Salinity tolerance of turfgrass plants may be due to its ability to retain cell turgor despite fluctuations in water availability. (Marcum and Murdoch, 1994).

Turf quality

Turf quality under salt stress as indicated by visual ratings is presented in Figure 2. It was noticed at the outset that, there was an increase in turf quality (either

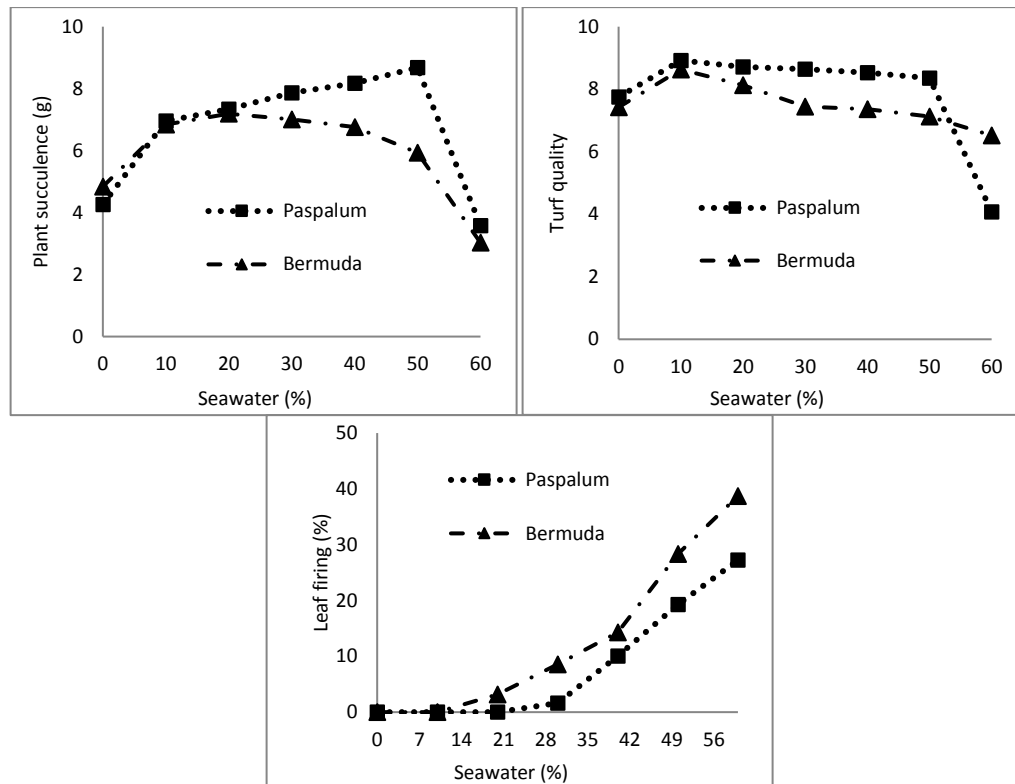


Figure 2. Effect of diluted seawater irrigation on plant succulence, turf quality and leaf firing of Seashore paspalum and Bermudagrass.

turf texture, color and uniformity) as seawater percentage increased till it reached the peak at 10 % seawater, then started to gradually decline with increasing seawater percentage, where turf texture became more coarsely and unacceptable. Generally, turf texture was much better than control especially in seashore paspalum as it continued to be decent till 40 % seawater before that strongly affected compared to Tifway bermudagrass which was more affected and lower turf quality at the same seawater percentage. This results are in harmony with those of Uddin *et al.*(2009) how stated that, turf quality decreased with increasing salinity level. Local *P. vaginatum* was unaffected at the lowest salinity levels (EC at 0-50 dSm⁻¹). A large reduction in visual quality ratings and chlorophyll fluorescence was expected because of the physiological drought expected from the salinity treatments (Dudeck and Peacock, 1993)

Leaf firing

Data revealed that, leaf firing constantly increased as seawater percentage increased although, the salinity appeared to be less harmful for *P. vaginatum* comparing to Tifway bermudagrass at all seawater percentages (Fig. 2). At low seawater ratios (10-20 %), *P. vaginatum* leaves did not show any effect, then they start to gradually affected when seawater ratio increased. Leaf firing was

moderate and turf quality was acceptable in the beginning till 40 % seawater in Tifway bermudagrass and 50 % seawater in seashore *P. vaginatum* then, it reached unreasonable degree of less turf quality. This may be due to that seashore *P. vaginatum* more tolerant to high salinity rates than Tifway bermudagrass and the adverse effect of higher seawater rates on turf quality (Uddin *et al.*, 2009). In this way, it can disrupt the water balance of plants and cause necrosis or loss of leaves, resulting in growth reduction (Sykes and Wilson, 1988 and Tominaga and Ueki, 1991).

Chemical constituents

Characterization of germplasm using biochemical has got special attention due to its increased use in crop improvement and the selection of desirable genotypes for plant crops (Farshadfar and Farshadfar, 2008). A biotic stresses like salinity severely reduce the productivity of most plants. Salt tolerance is a polygenic, highly intricate trait dependent on genotype and plant developmental stage. Activities of antioxidant enzymes have been reported to increase in most plants (Ashraf, 2009). These enhanced activities of antioxidant enzymes (CAT and POD) helps to protect the plants from damages caused by salt induced reactive oxygen species (ROS) (Yasar *et al.*,2008).

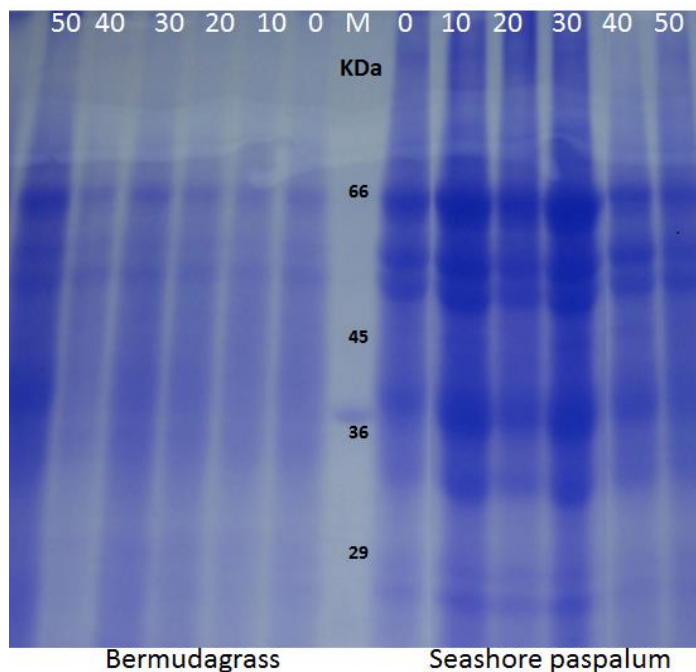


Figure 3. Protein banding patterns of Bermudagrass and Seashore paspalum lanes from 0 to 50 represent seawater dilution, M : marker.

SDS-protein electrophoresis

SDS-PAGE, total proteins were extracted from leaves of seawater-treated and salt-untreated plants. Comparing the protein profiles between control plants and those treated with different salt concentrations using SDS-PAGE showed that treatment induced only few changes in the pattern of proteins. Bands intensity was much different in treated genotypes. Protein SDS-PAGE show differences among the studied two common grasses in the intensity and number of bands (Fig. 3.). This result might be attributed to that the study of the resistant and susceptible genotypes differs under seawater stress. The results of SDS-PAGE proteins leaf revealed a total number of 11 bands in seashore showed that all bands are common (monomorphic), however they differed in density and intensity based on salt stress since most of bands show higher expression than control, e.g. band around 29KDa was very faint in control and gave dark under salt (10, 20 and 30) and become less density under (40 and 50). On the other hand in bermudagrass only five bands and gave different affect to salt those bands doesn't affected under salt stress in lower concentrations till 40 % but it affected and gives higher expression under high salt (50 %) these finding in harmony with (Henry, 2007), who suggested paspalum may be better able to survive prolonged periods of stress. In order to distinguish stress responses from developmental changes in protein accumulation, both control and treated leaves were harvested at the same time to minimize experimental error. It is necessary to study the salt stress responses at the protein level

(Malviya *et al.* 2008). Since, most of proteins undergo post-translational modification, which is extremely important for protein activities and subcellular localization.

Total green color

It is well known that, chlorophyll contents one of the most important measurements and more expression about plant health. There was upward increase in turf greenness at the low seawater percentages (10-30 %) then it took downward trend with increasing seawater percentage in irrigation water (Fig. 4). Apparently Seashore paspalum was more salt-tolerant as it remained greener at higher salinity levels compared to Tifway Bermudagrass. Total green color of Seashore paspalum was higher than Tifway Bermudagrass at all seawater percentages (Lee *et al.*, 2004 and Marcum *et al.*, 2005). High salt levels in the root zone cause a physiological drought which may be the reason for photosynthesis reduction (Carrow *et al.*, 2001).

Proline contents

Proline accumulation is believed to play adaptive roles in plant stress tolerance and used as a parameter of selection for stress tolerance. Thus, the proline content is a good indicator for screening salt tolerant varieties in under stress condition and plays a vital role in osmotic adjustment under stressful environmental conditions (Bayoumi *et al.*, 2008). Accumulation of organic osmotica like proline in both turfgrass cultivars showed a

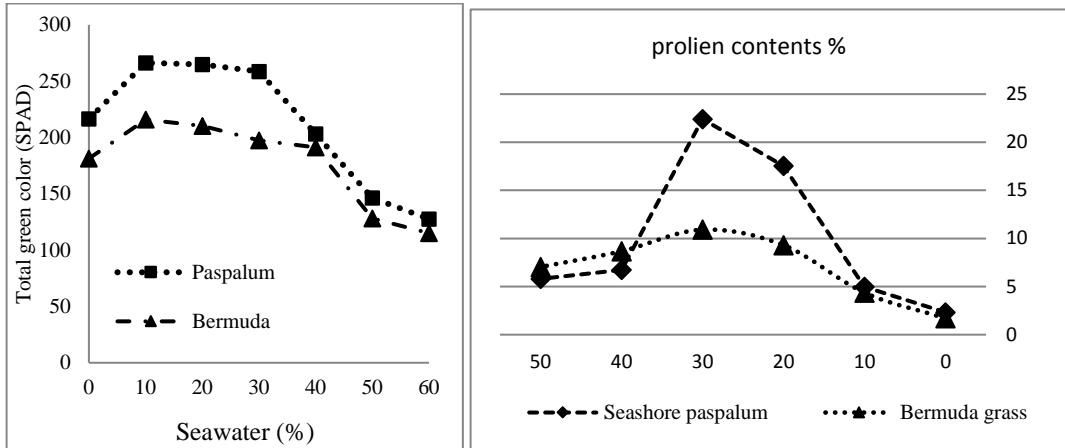


Figure 4. Effect of diluted seawater irrigation on total green color and proline contents of Seashore paspalum and Bermudagrass.

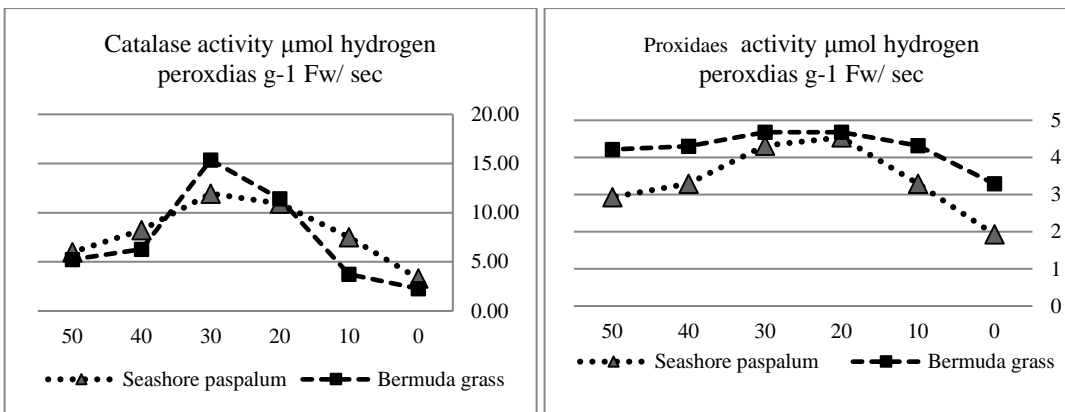


Figure 5. Effect of diluted seawater irrigation on catalase and proxidases activities of Seashore paspalum and Bermudagrass.

significant increase under salt stress (Fig. 4). These organic substances increased with increase in salt stress level till 30% and decreased with increasing the salt concentration, Higher accumulation of proline could be one of the important factors for the adaptation of this grass to saline environments. Such adaptation has also been reported earlier (Ashraf and Harris, 2004; Ashraf *et al.*, 2002; Lu *et al.*, 2007).

OD and POD are the core antioxidant in plants, and play a key role in scavenging superoxide ion and reducing membrane damage. Under stress conditions, the intensity and rise or fall of salt resistance related enzymes activity were related closely to plant species or varieties (Dhindsa *et al.*, 1981). Enzyme activity increased with the stress increasing or first increased and then decreased, the activity first increased and then decreased under salt intercross stress condition, and in the same treatment condition, both SOD and POD activities were decreased along with the extension of treatment time. This study showed that SOD and POD activity had different change patterns under different intercross salt stress; this dynamic progress was related

to that the low concentration of salt stress is able to improve the effects related to salt stress. In contrast, under severe salt stress, protective enzyme system was breached and enzyme activity was inhibited strongly, leading to further reduce in the moderate activity. Activities of SOD and POD decreased so obviously that it was not enough to clear free radicals in the body; then it resulted in lipid peroxidation and the damage of membrane system (Zhao *et al.*, 2010 and Yu *et al.*, 2007)

Mineral constituents

Data presented in Table (3) showed that, some ions (Na⁺, Cl⁻) behaved an upward trend whereas, the others (K⁺, Ca⁺⁺, Mg⁺⁺) turned downward whenever seawater percentage increased. Also, ashes percentage turned upward till 40% seawater then behaved descending direction. While organic matter percentage turned downward direction. Uptake of essential ions (both cations and anions) including Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺ and Cl⁻ have been reported to be suppressed in various species by high concentrations of NaCl, in irrigation waters (Rubinigg *et al.*, 2003).

Table 3. Effect of diluted seawater irrigation on mineral contents (average of both seasons).

Seawater %	Na ⁺		Cl ⁻		K ⁺		Ca ⁺⁺		Mg ⁺⁺		Ashs		Organic mater	
	(mg/g.dry weight)												(%)	
	P	B	P	B	P	B	P	B	P	B	P	B	P	B
0	0.55n	0.59m	1.10m	0.87n	29.33a	16.01g	2.85a	2.12h	4.42b	3.16e	8.42n	9.15m	92.53d	93.02c
10	2.79l	3.62j	9.56h	6.78l	28.06b	15.82h	2.81b	2.09i	4.65a	3.08f	17.45j	18.65h	93.42b	93.71a
20	3.05k	4.50i	9.93g	6.86k	27.53c	14.31j	2.77c	1.89j	3.79c	2.57i	19.04f	19.82d	93.07c	92.49d
30	4.88h	13.54g	12.19e	7.83j	25.82d	12.56k	2.54d	1.73n	3.21d	1.91k	19.25e	20.77b	88.26f	89.55e
40	7.64f	15.07d	13.05c	9.55i	23.60e	10.13l	2.29e	1.78m	3.02g	1.52l	20.13c	22.08a	84.32h	85.06g
20	8.06e	15.61b	15.22b	10.17f	21.22f	8.74m	2.15g	1.83l	2.75h	1.33m	18.61i	19.01g	76.74j	78.12i
60	8.45c	15.77a	16.09a	12.40d	14.86i	6.71n	2.21f	1.88k	2.06j	1.24n	16.03l	17.44k	72.95l	74.68k

P= Seashore paspalum, B= Bermudagrass

Means within a column having the same letters are not significantly different in Duncan's Multiple Range Test.

Soluble salts such as Na⁺, Cl⁻, K⁺, Ca⁺⁺, and Mg⁺⁺ inhibit water movement into plant tissue (Huck *et al.*, 2000)

High salt levels in the root zone cause a condition in plants known as physiological drought. Physiological drought is very similar to normal drought as it causes reductions in growth rate, photosynthesis, cytokinin synthesis and transpiration rate (Carrow *et al.*, 2001).

CONCLUSION

Research results showed a significant improvement in plant growth and most studied characters at low levels of diluted seawater compared to freshwater only or high levels of diluted seawater. So, it viable to use low levels of diluted seawater to irrigate some Egyptian turfgrass as an alternative irrigation source in order to save freshwater for drinking and essential crops irrigation.

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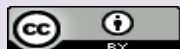
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