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Influence of late season nutrition on seashore paspalum turfgrass (*Paspalum vaginatum*) cold wear

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Abstract

The present research was conducted on Kafer El-Sheikh University garden turfgrass during the winter season (November-May) of 2013/2014 and 2014/2015 to study the possibility of improving cold tolerance of seashore paspalum (*Paspalum vaginatum*) turfgrass during late season through either nutrition by compost alone or in combination with foliar application of potassium, silicon, iron or calcium separately. Compost was applied to all area except control on 1st November of each season at $10m^3$/feddan ($2.38kg/m^2$). Aqueous solutions containing the recommended dose of each element plus 0.1% Tween 20 as a wetting agent were sprayed on the above ground parts until runoff, twice a month. The results showed that compost and Fe combination followed by compost and K_2SO_4 recorded the best results for most growth and anatomical measurements. The least growth values were observed from the compost alone or in combination with silicon and control treatments.

Key words: Seashore Paspalum, *Paspalum vaginatum*, late season nutrition, cold wear.

Introduction

With the rapid weather changes in Egypt, especially in the recent years, the weather tends to be continental, where temperatures rise in summer and fall in winter. Low temperatures in winter unfavorably affect the warm season turfgrass that cause yellowing and withering which badly reverberate on shoot color, shoot density and shoot uniformity due to tissue desiccation. Also, direct low temperature may kill and weak or completely stop plant growth, especially during January and February of each year. So, many physiological practices have been applied to alleviate cold climate adverse effects on normal plant functioning.

Paspalum vaginatum (seashore paspalum) belongs to the family Poaeceae. It grows along the coastline as strand vegetation in many tropical and subtropical areas of the world. It is a perennial, stoloniferous and rhizomatous creeping grass. It forms a thick mat of growth and has dark-green leaves with shinny waxy leaf coat (Zinn, 2004). It is an environmentally compatible warm-season turfgrass that has been used on golf courses and other recreational sites for low requirements of fertilizers and insecticides as well as salt tolerance (Duncan, 1999). Seashore Paspalum has been suggested to have excellent tolerance to high salt levels found in reclaimed water, effluent, salt spray and seawater after it has been established, and also requires less fertilizer and irrigation than many other turfgrasses (Ivan, 2010)

Potassium is classified as the most important components of a turfgrass fertility program. It does not combine with other elements to form plant components such as protoplasm, fats and cellulose, but required for optimal growth, nutrients uptake and root growth. Besides, it regulates stomatal functioning, which enhances shoot water potential of turfgrass plant. Maintenance of turgor potential by K can also help overcome stress effects of cold temperatures, salinity or drought. Turf grass cold resistance, disease resistance, root growth, carbohydrate levels and general hardiness to adverse conditions have been related to potassium nutrition (Matocha and Smith, 1980; Dahisson and Graskonsult, 1993 and Keisling *et al.*, 1979). Potassium is second only to nitrogen in concentration in Bermudagrass (*Cynodon dactylon*) tissue. It ranges between 1 and 3 percent in its clippings (Datnoff *et al.*, 2001).

Silicon is the second most abundant mineral element in soil after oxygen and comprises approximately 28% of the earth's crust. It is presumably not essential for plant growth and development and is therefore not considered a true nutrient element. However, soluble Si has enhanced several plant species growth and development (Epstein, 1999). Silicon fertilization has shown positive effects on alleviating a biotic stress as well as improving plant growth and development in several turfgrass species. Si improves leaf and stem strength through deposition in the cuticle and by maintaining cell wall polysaccharide and lignin polymers (Hull, 2004). The positive influences of silicon on plant are related to the deposition of the element under the leaf epidermis which results a physical mechanism of defense, reduces lodging, increases photosynthesis capacity and decreases transpiration losses (Korndörfer *et al.*, 2004). Many plants are able to uptake Si where they absorb it from the soil solution in the form of monosilicic acid, $Si(OH)_4$, which is carried by the transpiration stream and deposited in plant tissues as amorphous silica gel, SiO_2nH_2O (Savant *et al.*, 1997; Datnoff, 2005).

Calcium is involved in the regulatory mechanisms that activate plants to adjust to adverse environmental conditions such as cold stress (Wang *et al.*, 2009; Zhou and Guo 2009)

Iron is a component of chlorophyll protein complexes in the chloroplast (Taiz and Zeiger, 1998). Iron is also necessary for the synthesis of heme (iron porphyrins) that are incorporated in the plant's cytochromes and enzymes, which are important in chlorophyll synthesis. A cytochrome is an iron containing pigment associated with the electron transport system within the photosynthetic process (McCarty, 2001). Iron functions as an "energy currency" for plants and therefore is essential for plant growth. Because it is required in small quantities, iron is considered a plant micronutrient (Crichton and Ward, 1998; Schmidt, 2003). A number of soil conditions can induce Fe deficiency in turfgrass leaves, including an alkaline pH as well as cold and wet soils (Turner and Hummel, 1992).

Warm-season grasses can benefit from late season application of the aforementioned elements when the metabolism of root system is slow in early spring and during the 30 days before frost in autumn (Schmidt, 2003).

The study aimed to examine the possibility of improving cold tolerance of seashore paspalum turfgrass through late season nutrition by compost or in combination with foliar application of potassium, silicon, iron or calcium separately.

Materials and methods

Plant material and treatments: The present research was conducted at Kafr El-Sheikh University gardens turfgrass during the winter season (November-May) of 2013/2014 and 2014/2015 to study the possibility of improving cold tolerance of seashore paspalum (*Paspalum vaginatum*) turfgrass through late season nutrition by compost alone or in combination with the foliar application of potassium, silicon, iron or calcium separately.

Compost was applied to all area except control on 1st November of each season @ 10m^3/feddan (2.38kg/m^2). Aqueous solution containing the recommended dose of each element and 0.1% Tween 20 were sprayed twice a month on the above ground parts until runoff, . Calcium chloride was applied @ 1.1g/L, iron was applied @ 0.25 mL Fe/L using from the commercial source X-Xtra (10% Fe), potassium sulphate was applied as a source for potassium applied @ 7.5g/L and silicon was applied @ 3mL/L in the form of potassium silicate which contains 32% SiO_2 and 16% potassium. Control plants were sprayed with tap water containing 0.1% Tween 20. A plant mist bottle held about 20 cm from the shoot was used for spraying. Spray treatments began on 1st September 2014 and lasted for 12 weeks. The experiment was laid out in a complete randomized design with three replications.

Turf visual quality: Three turfgrass assessment parameters were visually measured fortnightly through winter season (December 15th - March 15th) of each season as shoots color, turf density (turf texture) and turf uniformity. Turf color was visually measured by ten evaluators on 6 scale assessments as dark green (1), green (2), light green (3), yellow green (4), yellow (5) and straw (6). Whereas, turf density was graded on three grades as highly dense (1), dense (2) and less dense (3). On the other hand, turf quality was evaluated as uniform (1) and not uniform (2), (Waddington *et al.*, 1992; Duble, 1996).

Number of leaves per shoot and shoots number was determined by counting the number of leaves per shoot and shoots per 10 cm^2 area. Mean shoot length was measured from soil level to the terminal bud using a meter rule. Leaf measurements (area, length, width and perim) was measured by portable laser leaf area meter (CI-202 model). Greenness measurements represented in total green color (SAPD) was monthly measured using a portable chlorophyll meter (Minolta SPAD-502, Minolta Co., Ltd., Japan).

Shoots were fortnightly mowed to a height of 2.5 cm during the treatment period then shoots fresh weight data were collected. Also growth rate (GR) was calculated using the commonly used formulae:

$$GR=(W_2-W_1)/SA(t_1-t_2)$$

where, W_2 and W_1 are dry weight at beginning and end of interval, t_1 and t_2 are the corresponding days, and *SA* is the soil area occupied by the plants at each sampling. GR refers to the dry matter accumulation rate per unit of land area.

Before weighing, roots were washed with tap water. Root and shoot fresh weight data were collected separately. Both shoots and roots were dried at 70 ^{o}C for 72 h to determine shoots and roots dry weights (Cooper, 1981).

Anatomical structure: The specimens were taken from the base of seashore paspalum turfgrass stem and leaves. Specimens were taken on day 15th of cutting. Specimens were fixed in formalin, alcohol, acetic acid mixture (FAA, 1: 18: 1; v/v), washed and dehydrated in alcohol series. The dehydrated specimens were infiltrated and embedded in paraffin wax (52-54 °C m. p.). The embedded specimens were sectioned using a rotary microtome (Leica RM 2125) at a thickness of 8-10 µm. Sections were mounted on slides and deparaffinized. Staining was accomplished with safranine and light green combination, cleared in xylene and mounted in Canada balsam (Ruzin, 1999). Ten readings from 3 slides were examined with electric microscope (Lieca DM LS) with digital camera (Lieca DC 300), and then photographed. The investigated histological features of the stem and leaf were: thickness of vascular bundle, phloem tissue, vascular bundle width as well as diameter of stem and xylem vessels. Also, leaf thickness.

Statistical analysis: The experiment was arranged in a completely randomized block design with three replicates. All data were subjected to one-way analysis of variance (ANOVA) and Duncan multiple range comparison test ($P < 0.05$) using the Mstat statistical package.

Results and discussion

Effect on visual color and quality: All treatments had highly significant influence on visual quality scores of turfgrass over control (Fig. 1). Compost and foliar application of Fe at 0.25mL/L treatments significantly increased turf quality, color, and density more than all other treatments and recorded 1.1, 1.2 and 1.0, for the three visual quality parameters (mean turf color, mean turf density and mean turf uniformity, respectively) followed by the treatment of compost + K_2SO_4 (7.5g/L) (2, 1.6 and 1.2, respectively) and compost + $CaCl_2$ (1.1g/L) with the values 2.5, 2.0 and 1.3, respectively. Several researchers have reported that late-season fertilization may extend photosynthetic activity of turfgrass in the fall and stimulate it early in the spring. Taiz and Zeiger (1998) confirmed that, Fe is a component of chlorophyll protein complexes in the chloroplast. Fall applications of Fe may be important for maintaining high turfgrass quality in the fall. A rapid drop in air temperature regularly, causes injury to warm season turfgrasses (McCarty, 2001). Iron fertilization maintained the aesthetic quality of Tifgreen turfgrass after the chilling period and stimulated recovery of day time and night time carbon exchange. Therefore, foliar application of iron maintains desirable

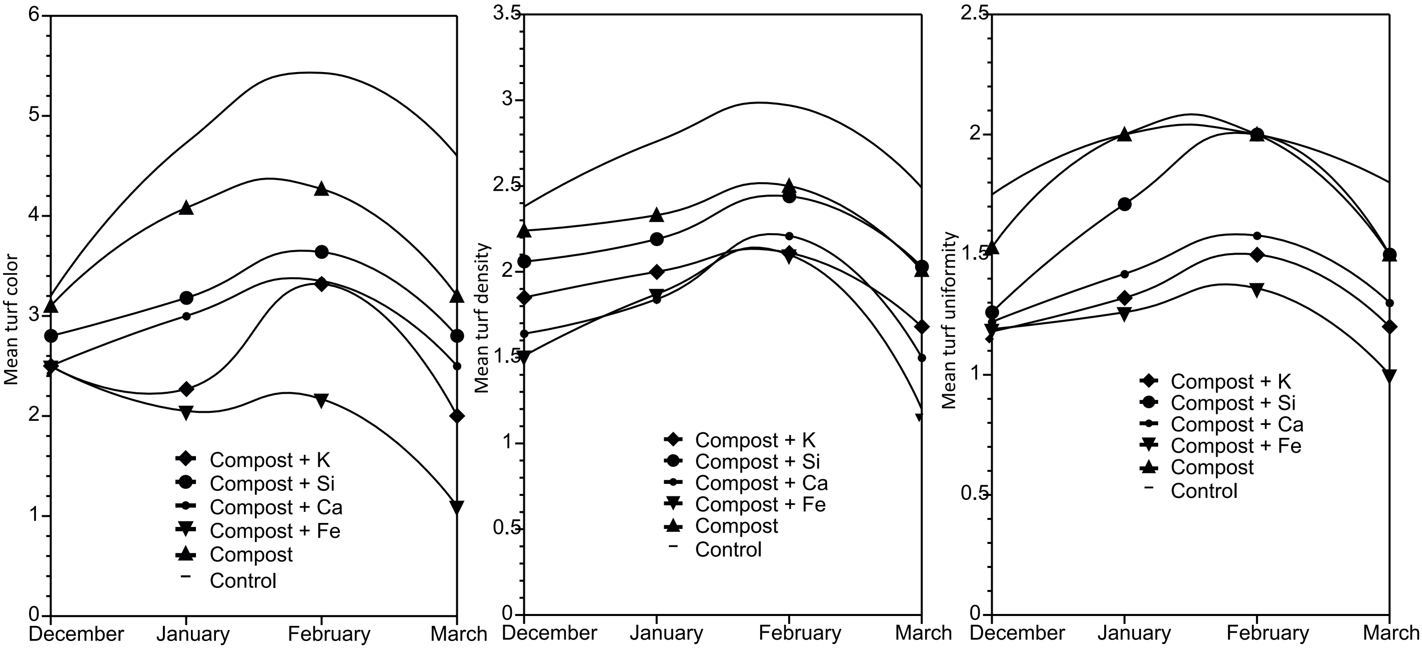

Fig. 1. Effect of late season nutrition on mean turf color, mean turf density and mean turf uniformity of seashore paspalum turfgrass.

Bermuda grass quality exposed to chilling temperatures (Schmidt, 2003). Iron, applied at 4 pounds/acre in late-season, was sufficient to achieve a desirable fall bermudagrass color (Goatley, 1994).

Effect on leaves: Data presented in Table 1 clearly inidcate that compost + Fe (0.25mL/L) recorded the highest leaf number (17.82), tallest leaves (14.87mm) and biggest leaf perim (200.86mm), compost + $CaCl_2$ (1.1g/L) treatment gave highest leaf area (6.86 mm^2) and highest leaf length/width ratio (11.50) whereas the widest leaves (1.63mm) were recorded from compost treatment.

Effect on shoot: Data presented in Table 2 show that all treatments have highly significant influences on all studied measurements (shoots number, length, and fresh and dry weights) over control. Compost + Fe (0.25mL/L) treatment surpassed the other treatments in all studied characters and gave the tallest shoots (41.56cm), highest shoots number (135.70) and heaviest shoots fresh and dry weights (3.11 and 0.41 g /10 cm^2). Compost + K_2SO_4 (7.5g/L) treatment ranked second for most growth characters followed by compost + $CaCl_2$ (1.1g/L) treatment.

Effect on growth rate: In all treatments, there was gradual decrease in plant growth rate per unit area during December

Table 1. Effect of late season nutrition on number and measurements of seashore paspalum turfgrass leaves

Treatments	Number /shoot	Area $(mm)^2$	Length (mm)	Width (mm)	Length/ width ratio	Perim (mm)
Control (Tap water)	5.71f	3.26f	5.98f	1.40b	4.28f	86.84f
Compost	9.47d	4.98d	11.36d	1.63a	6.98d	151.61d
Compost + Fe (0.25 mL/L)	17.82a	6.75b	14.87a	1.36c	10.81b	200.86a
Compost + $CaCl_2$ (1.1 g/L)	14.64b	6.86a	14.64b	1.27d	11.50a	186.49b
Compost + Si (3 mL/L)	8.31e	4.02e	8.24e	1.36c	6.07e	113.77e
Compost + K_2SO_4 (7.5 g/L)	12.57c	5.96c	14.16c	1.26e	10.34c	174.85c

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

Table 2. Effect of late season nutrition on shoots number, shoot length and shoot fresh and dry weights of seashore paspalum turfgrass

Treatments	Number	Length (cm)	Fresh weight g/10cm^2	Dry weight g/10cm^2
Control (Tap water)	107.14f	25.02f	2.02f	0.17f
Compost	120.04e	33.81d	2.25e	0.21e
Compost + Fe (0.25 mL/L)	135.70a	41.56a	3.11a	0.41b
Compost + $CaCl_2$ (1.1 g/L)	122.61c	35.70c	2.42d	0.37c
Compost + Si (3 mL/L)	120.54d	33.50e	2.89c	0.28d
Compost + K_2SO_4 (7.5 g/L)	128.37b	38.33b	3.07b	0.45a

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

to February then it began to increase (Fig. 2). In general, the treatment of compost + Fe (0.25mL/L) and compost + K_2SO_4 (7.5g/L) had higher growth rate followed by compost + $CaCl_2$ (1.1g/L). In general, compost and control recorded the lowest growth rate. This may be due to a steady decrease in the temperature especially in February then increase beginning from March. This is thought to be because of the non-structural carbohydrates which is converted to soluble sugars that promote bud break to form new leaves and roots and help turfgrass to survive the winter (McCarty, 2001). Hale and Orcutt (1987) reported that at low temperature, it appears to be a membrane transition from a fluid to a gel state in the cold sensitive plants. Warm season turfgrasses are best adapted for growth between 27 and 32 ^oC and the optimum soil temperature for Bermudagrass root, rhizome, and stolon growth is 24 ^oC (Duble, 1989). There is a rapid reduction in growth rate and development of Bermuda grass plants when temperatures drops below 12 ^oC (Buchanan *et al.*, 2000).

Effect on root parameters: It was noticed that, combination of compost and K_2SO_4 treatments increased all root measurements values (roots number, length and fresh and dry weights) compared to other treatments (Table 3). Compost +

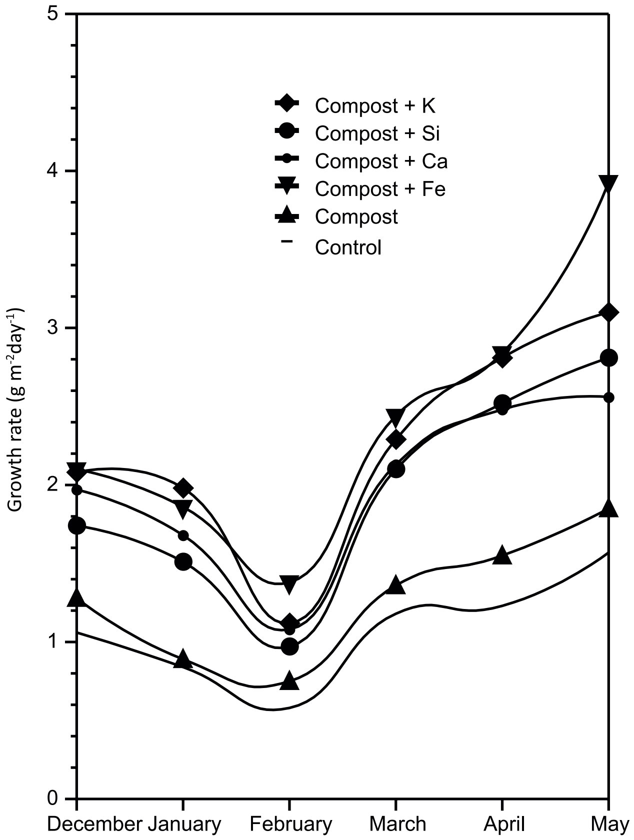

Fig. 2. Effect of late season nutrition on growth rate of seashore paspalum turfgrass

Fe (0.25mL/L) and compost + $CaCl_2$ (1.1g/L) treatments occupied the second and third ranks, respectively. Control recorded the lowest values for all root measurements. These findings are in conformity with many of the researchers such as Keisling *et al.* (1979), who stated that Bermudagrass rhizomes and roots were positively influenced by potassium application. Late-season iron treatments had enhanced root mass. Fall and spring greenness and turf quality were enhanced when iron was applied biweekly for 30 to 40 days before dormancy at 0.5 pound / acre (Schmidt, 2003).

Anatomical features

Leaves anatomy: The leaf blade internal structure of seashore paspalum turfgrass is similar to other monocotyledons plants. The bifacial leaf consists of three major regions: the epidermis, the outer layer of cells which extends all over the leaf surfaces. The mesophyll tissue, the interior of the leaf between the upper and lower epidermis and undifferentiated into palisade and spongy parenchyma. The histological features of leaf included thickness of leaf, phloem tissue, vascular bundle, vascular bundle width and xylem vessels diameter (Ruzin, 1999).

The effect of late season nutrition on the anatomical measurements and counts of the turfgrass leaf as shown in transverse sections are presented in Table 4 and illustrated in Fig. 3. It was noticed that, relative to control treatment, compost alone or in combination with Fe, $CaCl_2$, K_2SO_4 or Si caused a vital increase in the anatomical features of turfgrass leaves. Compost and Fe combination treatment recorded the highest value of histological features (xylem vessels diameter, phloem tissue thickness, phloem tissue thickness, vascular bundle thickness, vascular bundle width and leaf thickness). This may be due to that iron enhances

Table 3. Effect of late season nutrition on roots number, length and fresh and dry weights of seashore paspalum turfgrass

Treatments	Number (10 cm)$^{-1}$	Length (cm)	Fresh weight (g cm^{-2})	Dry weight (g cm^{-2})
Control (Tap water)	43.20f	14.68f	1.73f	1.08f
Compost	48.36e	17.40d	1.77e	1.11e
Compost + Fe (0.25 mL/L)	65.71b	18.51b	2.84b	1.70b
Compost + $CaCl_2$ (1.1 g/L)	50.32d	18.07c	2.05c	1.22c
Compost + Si (3 mL/L)	53.83c	16.22e	1.96d	1.17d
Compost + K_2SO_4 (7.5 g/L)	70.19a	20.04a	3.02a	1.84a

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

both level and activity of certain important enzymes that are very necessary for plant vital processes (Zaharieva and Abadia, 2003). Also, soil compost represents a rich source of macro and micronutrients which improve both plant growth and production (Parr and Hornick, 1990).

Stem anatomy: The internal structure of seashore paspalum turfgrass stem is similar to the other monocotyledon plants and built up essentially of parenchyma ground tissue, lignified vascular bundles and fibers. Data presented in Table 5 and illustrated in Fig. 4 indicate that, compost treatment whether individually or in combination with Fe, K_2SO_4, $CaCl_2$ and Si treatments showed a remarkable increase in all investigated histological measurements compared to their respective controls. Histological measurements (thickness of vascular bundle length and width, phloem tissue and diameter of metaxylem vessels as well as stem diameter) were appreciably increased with remarkably different extents. Generally, compost combination with each of Fe and K_2SO_4, respectively surpassed all other treatments. The thickest vascular bundle (μm) and ground tissue recorded with the combination of compost and K_2SO_4. Fe has an essential role for many important enzymes involved in electron transport chain, synthesizing chlorophyll maintaining the structure of chloroplasts and enzyme activity (Mamatha, 2007, Ziaeian and Malakouti, 2006; Welch, 2002).

Effect on chemical constituents

Total green color: It was noticed that foliar applications of Fe markedly increased and prolonged turfgrass quality and total green color compared to other treatments (Fig. 5). Greener plants throughout the experiment months were obtained from compost + Fe (0.25mL/L) treatment followed by compost + K_2SO_4 (7.5g/L),

Table 4. Effect of late season nutrition on anatomical features as shown in the transverse sections of seashore paspalum turf grass leaves

Treatments	Xylem vessels diameter (μm)	Phloem tissue thickness (μm)	Vascular bundle thickness (μm)	Vascular bundle width (μm)	Leaf thickness (μm)
Control (Tap water)	6.00b	15.67d	35.00d	23.67b	109.33c
Compost	11.00a	19.33abc	39.67c	30.00a	128.67a
Compost + Fe (0.25 mL/L)	11.33a	21.00a	50.00a	31.67a	129.67a
Compost + $CaCl_2$ (1.1 g/L)	10.33a	18.67bc	39..67c	29.33a	122.67b
Compost + Si (3 mL/L)	9.67a	20.00ab	40.33c	30.00a	122.00b
Compost + K_2SO_4 (7.5 g/L)	10.00a	18.00c	46.33b	31.00a	120.00b

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

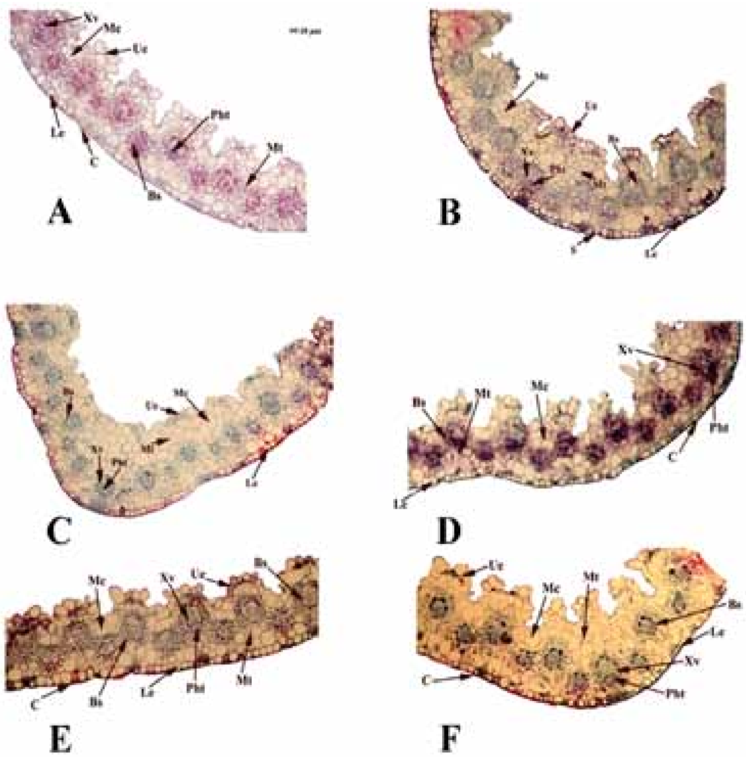

Fig. 3. Transverse sections through the leaves of seashore paspalum turfgrass as affected by late season nutrition. A=Control, B=Compost, C= Compost + Si (3mL/L), D=Compost + K_2SO_4 (7.5 g/l), E= Compost + $CaCl_2$ (1g/l), F=Compost + Fe (0.25 g/l). Ue: Upper epidermis. Le: Lower epidermis. Xv: Xylem vessel. Pht: Phloem tissue. Bs: Bundle sheath. Mc: Motor cells. Mt: Mesophyll tissue. S: Stomata

compost + $CaCl_2$ (1.1g/L) and compost + Si (3mL/L) treatments, while paler plants resulted from control treatment. Iron is a component of chlorophyll protein complexes in the chloroplast (Taiz and Zeiger, 1998). Fe applications can increase the period of time in which the grass stays green, as could increase plant levels of cytokinins by interfering with ethylene synthesis (Schmidt, 2003; Munshaw *et al.*, 2006). Reducing photosynthesis is the common effect of cold injury on turfgrass plants which may be due to membrane dysfunction (Samala *et al.*, 1998).

Nutrient constituents: Data presented in Table 6 revealed that, plants treated with compost + Fe (0.25mL/L) recorded significantly highest contents of Mg^{++}, Fe^{++}, N and organic percentage compared to other treatments. The highest content of K^+ was observed in plants treated with compost + K_2SO_4 (7.5g/L). The significantly higher values of Na^+ and Cl^- resulted from the plants treated with compost + $CaCl_2$ (1.1g/L). Control treatments recorded significantly lowest values for all estimated contents. In this context, Feng *et al.* (2010) reported that macro and micro-nutrients contents showed a significant difference and the extent is affected by silicon foliar application. Maize leaves Fe^{++} content decreased with Si foliar application. Calcium strongly competes with Mg^{++} at the binding sites on the root plasma membrane which appear to have less affinity for the highly hydrated Mg^{++} (Grattan and Grieve, 1999; Rashad and Hussien, 2014).

The present research indicate that cold tolerance of seashore paspalum gardens turfgrass can be improved through nutrition. Compost and Fe combination or compost with K2SO4 can be used for better growth of turf grass. Both of these interventions improved colour values and growth rates.

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Table 5. Effect of late season nutrition on anatomical features as shown in the transverse sections in seashore paspalum turf grass stems

Treatments	Xylem vessels diameter	Phloem thickness	Vascular bundle length	Vascular bundle width	Ground tissue thickness	Stem diameter
			(µm)			
Control (Tap water)	6.67c	16.67d	41.67c	35.67c	140.67c	1072.33e
Compost	10.00b	19.33c	51.00b	40.33b	154.67ab	1094.33d
Compost + Fe (0.25mL/L)	12.33a	23.00a	59.33a	45.33a	154.67ab	1211.00a
Compost + $CaCl_2$ (1.1g/L)	10.00b	20.33bc	54.00b	39.33b	146.67bc	1157.00c
Compost + Si (3mL/L)	10.67ab	20.33bc	52.67b	41.33b	157.33a	1193.33b
Compost + K_2SO_4 (7.5g/L)	11.67ab	21.00b	60.00a	41.00b	158.33a	1196.67b

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

Table 6. Effect of late season nutrition on nutrient content of seashore paspalum turfgrass

Treatments	Ca^{++}	Mg^{++}	Na^+	K^+	Cl^-	Fe^{++}	N (%)	Organic (%)
				(mmol/g dry weight)				
Control (Tap water)	0.63e	0.24e	8.14c	0.80e	4.21f	0.19e	1.61f	79.03f
Compost	0.88b	0.32d	6.04f	0.87d	4.52b	0.27b	4.34d	94.41b
Compost + Fe (0.25mL/L)	0.86c	0.36a	8.22b	0.90b	5.84b	0.42a	4.92a	97.76a
Compost + $CaCl_2$ (1.1g/L)	0.95a	0.33c	10.60a	0.87d	8.31a	0.22d	4.55c	93.05d
Compost + Si (3mL/L)	0.86c	0.32d	8.05d	0.89c	4.88d	0.19e	4.21e	92.38e
Compost + K_2SO_4 (7.5g/L)	0.81d	0.35b	6.33e	0.98a	5.70c	0.25c	4.89b	94.07c

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

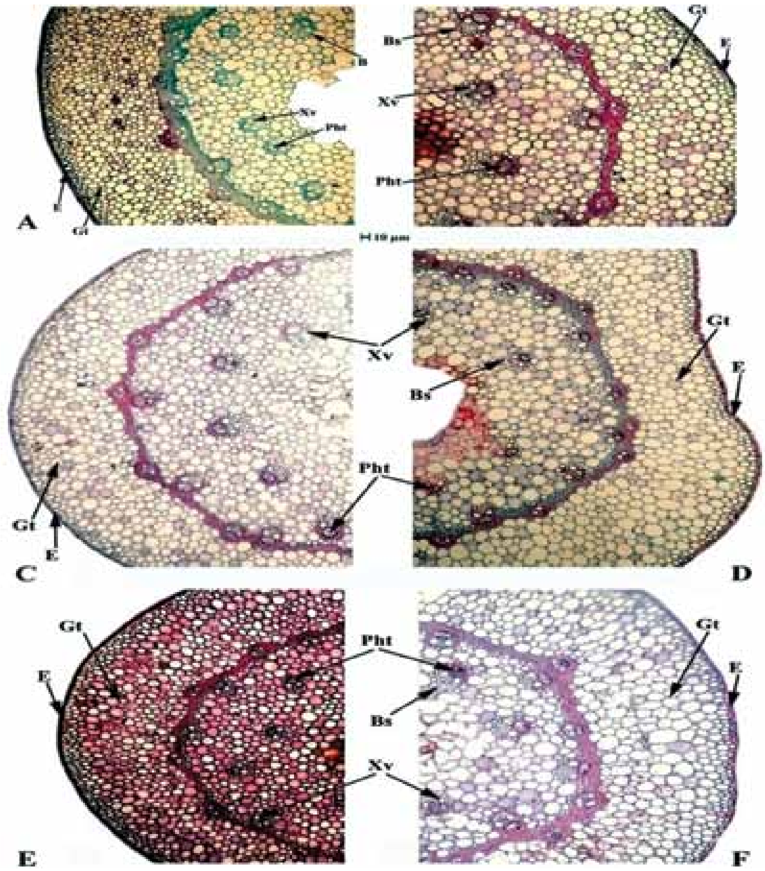

Fig. 4. Transverse sections through the stems of seashore paspalum turfgrass as affected by late season nutrition.

Where: A=Control, B=Compost, C= Compost + Si (3mL/L), D=Compost + K_2SO_4 (7.5 g/L), E= Compost + $CaCl_2$ (1g/L), F=Compost + Fe (0.25 g/L).

E: Epidermis, Xv: Xylem vessel, Pht: Phloem tissue, Bs: Bundle sheath, Gt: Ground tissue.

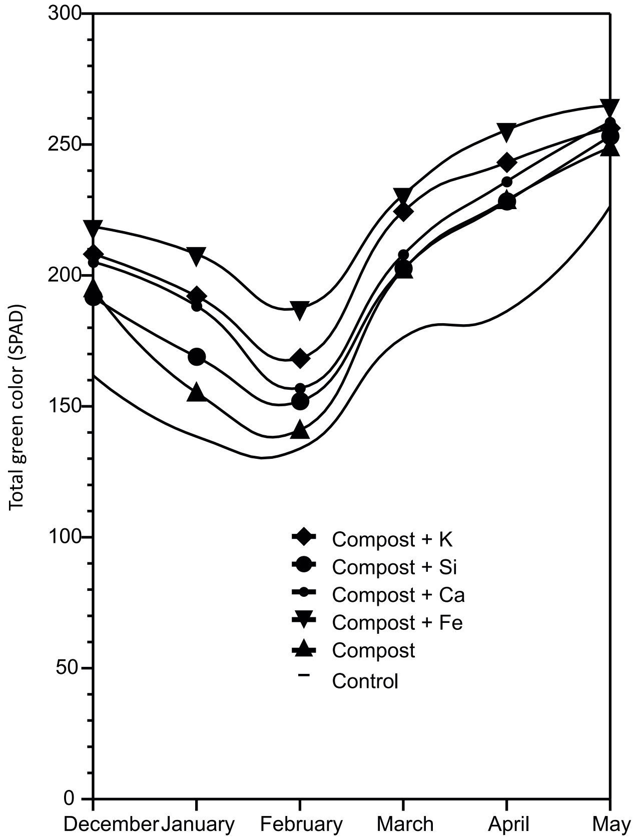

Fig. 5. Effect of late season nutrition on total green color of seashore paspalum turfgrass.

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