NATURAL PLANT EXTRACTS INDUCED-ALTERATIONS IN GROWTH, PHYSIOLOGY AND QUALITY OF TURF GRASS (*Cynodon dactylon*) UNDER SUMMER STRESS

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ABSTRACT: Natural plant extracts may alleviate negative effects of abiotic stresses. To test this hypothesis, a field trial was executed to evaluate the effect of natural plant extracts on growth, physiology and quality of *Cynodon dactylon* under summer stress by following Randomized Complete Block Design (RCBD) having three replicates. When turf established properly, extracts of moringa leaves (3%), seaweed (0.2%), sorghum water (3%), and sunflower water (3%) were foliar applied (two times with an interval of 15 days) while water spray served as control. Results showed that plant extracts had variable but positive effect on tiller height, Inter-nodal distance, leaf area index, root length, root-shoot fresh weight, and root dry weight. However, root-shoot fresh weight ratio and shoot dry weight did not affect significantly by the application of plant extracts under study. Moreover, moringa and seaweed extracts proved most effective equally than sorghum and sunflower extract regarding leaf area index, tiller height, shoot fresh weight and root length. Physiological attributes like chlorophyll contents, cell membrane thermo stability, relative water and K-contents as well as turf quality was also improvd. Conclusively, *Moringa oleifera* and seaweed extracts can be used to enhance aesthete value of turf by improving its growth and quality.

Key words: Cytokinins, Cell membrane stability, Natural extracts, Osmotic adjustment, Turfgrass.

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INTRODUCTION

Turf grass is a multimillion industry that involves business of many groups such as golf courses, play grounds, landscaping, seed and sod production etc. Only in USA there are millions of home lawns beautified with turf, more than 700,000 athletic fields, and 17,000 golf courses provide thousands of economic opportunities (Anonymous, 2007). In addition, turfgrass areas play an important role in our way of life due to its adorned and gorgeous character. The universal choice of people is secure, aesthetically delightful, and wholesome attractive grassy areas to sight, sit on, play and walk in (Behe *et al.*, 2005). In Pakistan, this industry is progressively emerging and has a great scope in future.

Beside the great importance of grasses in situation and promising future, turf industry is facing different challenges such as heavy use of commercial fertilizers and pesticide that are costly as well as hazardous to the environment, contributing in changing climate both seasonal as well as globally, water issue, rise in temperature and environmental constraints (Morris, 2006).

Under summer conditions, water shortage with high temperature drastically affects growth and quality of turfgrass. Increased root mortality rate was recorded in bentgrass when exposed to higher soil temperature (Liu and Huang, 2003). Heat stress due to prolonged sun exposure, compact soil, or stones just under the soil surface collect sun heat. Moreover, heat stress also relates to soil moisture status. During hot weather, increased evapo-transpiration rates reduced soil and plant water status thus prone to high temperature injury (Berndt and Vargas, 2010). Summer is the critical for turf growth due to high temperature which may halt the plant physiological functions due to imbalance of internal water status of plant tissue. This condition may lead to rise in leaf temperature to lethal level that can be injurious for plant growth (Noon, 2012). Further, DiPaola and Beard (1992) state that prevalence of high temperature even below limits (~120 ºF) may also retard turf quality.

On the other hand, the capability of plant endurance in summer stress involves structural and physiological adaptations, which help plants to stay alive under prolonged stress periods i.e. deep and extensive root systems, osmotic adjustment (Huang, 2004), compartmentalization of solutes in cell, modification in minerals ratio especially potassium and sodium, evapo-transpiration adjustment by reducing leaf area, change in photosynthetic pigment, plant hormone regulation and improved scavengers for antioxidants (Sairam and Tyagi,

2004) are associated with plant heat stress adaptations. The other techniques are managemental practices including exogenous application of different plant extracts, hormones, mineral elements and other growth promoting substances.

To meet above mentioned challenges we should find some cheaper rescue methods to save our turf. Plant extracts such as seaweed, moringa, sunflower and sorghum etc., which are rich in bio-stimulant active ingredients can be helpful to mitigate harmful effects of heat stress. These extracts are easily available at economical prices and can be proved helpful to relieve various abiotic stresses including heat stress by promoting the production of auxin or cytokinins. Therefore, present trial was conducted to assess the high temperature reliving effect of natural plant extracts on turf grass (*Cynodon dactylon*) under summer stress conditions at Faisalabad, Pakistan.

MATERIALS AND METHODS

The present experiment was conducted at Floriculture Research Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan during the year 2013. Turf fine Dhaka (*Cynodon dactylon*) was planted on a well prepared and weed free land on 15th March 2013. The experiment was laid out in Randomized Complete Block Design (RCBD) taking four replications**.** The area for each experimental plot was 10 m^2. The prevailing climatic conditions of the experimental site are presented in the table 1.

When turf was established properly then sunflower and sorghum extract were prepared by overnight water soaking as 100 g dry material of respective treatment in 1L of water. Moringa extract was extracted from fresh leaves while the seaweed extract "wokozim crop plus" was taken from an authorized supplier. The experiment consisted of following treatments; T_1: moringa leaf extract (MLE) 3%, T_2: Seaweed extract 0.2%, T_3: sorghum water extracts 3%, T_4: sunflower water extracts 3% and T_0 water spray as control. After mowing and irrigation, 1st foliar spray of extracts was applied on 26th June 2013. After 7 days interval again grass was irrigated and grass samples were taken to determine non stressed osmotic potential on 11th of July 2013. Second foliar spray of treatments was applied on 12th July 2013. No irrigation was done for next 15 days to develop water stress conditions for the grass and after that final sampling was done to record the morpo-physiological and quality parameters.

Turf quality was visually evaluated on a scale of 1 to 10, with 1 being brown or dead turf grass and 9 being ideal turf grass while of 6 was the minimum acceptable. Quality was based on turf grass color, density, uniformity, texture and disease incidence (Skogley and Sawyer, 1992).

The plant Chlorophyll contents were determined by the method described by Davies (1976). The absorbance reading for extracted solution was recorded at 663, 645 nm by using spectrophotometer. Both chlorophyll '*a*' and '*b'* were calculated as: Chl *a* = [12.7 (OD $_{663}$) – 2.69 (OD $_{645}$)] × V/1000XW; Chl *b* = [22.9 (OD $_{645}$) – 4.68 (OD $_{663}$)] × V/1000 × W.

The cell membrane thermo-stability (CMT) was assessed as described in the method proposed by Sullivan (1972). Percentage relative cell injury (RCI %), an indicator of CMT, was estimated as; RCI %=1– [{1–(T_1/T_2)}/{1–(C_1/C_2)}] ×100.

For ascorbic acid, 10 g leaves samples (W) were taken and homogenized with 50 ml 0.4% oxalic acid solution. After grinding, mixture was transferred into a 100 ml volumetric flask and diluted to the mark with 0.4 % oxalic acid solution (V). Out of this 5 ml filtrated aliquot (V_1) was taken and titrated against 2,6-dichlorophenol indophenol dye (R_1), until light pink color appeared. Ascorbic acid was calculated as: Ascorbic acid = [(1× R_1× V× 100) / (R×W×V1)]

Where R = standard reading

Osmotic adjustment was calculated as difference in measured osmotic potential (Ψs) between non stressed and stressed leaves (Turner *et al*., 1986 and Blum, 1989). For this purpose leave samples were collected before and after stress. Then sample leave were sealed in a thermocouple psychrometer cup (2 ml) and freezed at –20 °C. Prior to measurement, samples were thawed for 30 min at room temperature. The sap was extracted by pressing it with glass rod. The sap was put on osmometer (Model; OSMOMAT 030, Cryoscopic osmometer, Gonatee) for the measurement of solute in osmolkg^{-1}. Then osmotic potential was calculated by using formula of (Jones, 1989) as an osmotic pressure of 1 MPa is approximately equivalent to 410 mosmol kg^{-1} at 20 °C.

For estimation of K contents, the sample digestion was made as standard protocol (Yoshida *et al*., 1976). Potassium was finally estimated by using flame photometer, as described by Chapman and parker (1961). The potassium contents were assessed in parts per million (ppm) by estimating the emission of flame photometer through standard curve which was then converted into percentage by using following formula:

K (%) = (ppm on graph ×dilution ×100) / 10^6

For estimation of relative water contents (RWC) 0.5 g fresh leaves were rinsed in water and then put into a tube containing distilled water. Fully saturated leaves were weighed (Ws) and then dried for 24 hour at 80 ºC to determine dry weight (Wd). Relative water contents were calculated as:

RWC (%) = ((Wf-Wd)/(Ws-Wd)) ×100

The collected data was evaluated statistically by using Fisher's analysis of variance (ANOVA) technique (Steel *et al*., 1997). The mean values were compared with

Least Significance Difference (LSD) test (Snedecor and Cochran, 1980).

RESULTS

Morphological characteristics: Natural plant extracts significantly improved morphological characteristics of turf grass except shoot fresh weight (Table 2a and b). Compared with control, maximum number of tillers/ft^2 (2012), shoot fresh weight (205.13 g), root dry weight (14.47 g), and root/shoot dry weight ratio (0.122) was recorded highest where moringa leaves extract was applied whereas effect of seaweed extract remained prominent on tiller height (38.42 mm), number of leaves per tiller (6.51), leaf area index (31.38) and root length (112.85) but found statistically similar with moringa leaves extract. Moreover, intermodal distance (10.55 mm) of turfgrass was recorded maximum in case of exogenous application of sunflower extract. However, sorghum extract showed the least effective regarding turfgrass improvement than all other tested extracts. Shoot dry and root/shoot fresh weight ratio did not affected substantially by any of the extracts applied.

Physiological response: Moringa and seaweed extracts proved effective regarding physiological and biochemical parameters of turfgrass than sorghum and sunflower extracts. Maximum chlorophyll contents were 0.781 mg/g, values of osmotic adjustment were 0.753 –MPa, relative water and potassium (K) contents were 0.432 and 76.47%, respectively. Whereas, 0.2% foliar applied seaweed extract showed the maximum values for all the physiological parameters. However, moringa leaves extract remained prominent regarding cell membrane thermo stability as well as ascorbic acid contents 38.24% and 15.66%, respectively. Regarding the overall efficacy of these natural extracts, the seaweed and moringa leaf extract showed more positive response on observed physiological characteristics followed by sorghum and sunflower water extracts respectively (Table 4). Moreover, the control treatment proved to be least influential for these physiological parameters.

Turfgrass quality: Plant extracts caused considerable improvement in turfgrass quality than control. Attractable color, uniform density, texture, and disease free turfgrass was observed where moringa leaves and seaweed extracts were applied. Both were statistically at par with each other with the values of 9.31 and 9.30, correspondingly. Sorghum and sunflower also improved turfgrass quality but the grass was not as fine in color and texture as in case of moringa and seaweed extracts, so their rank was kept lower with the values of 7.39 and 7.28, respectively. Non-uniform, browny and deformed leaved grass was observed in control (Fig. 1).

Table 1. Prevailing local climatic conditions during the experiment for the year 2013.

Months	Mean monthly						
	Temperature (°C)			Relative Humidity (%)	Sunshine (Hours)	Wind speed (km/h)	Total Rainfall (mm)
	Max.	Min.	Avg.				
March	27.0	13.0	20.0	61.2	8.6	5.4	1.3
April	33.5	19.7	26.6	36.7	8.9	6.2	21.6
May	39.7	24.4	32.0	24.5	10.4	6.8	4.6
June	39.5	27.9	33.7	43.3	9.4	6.8	67.5
July	37.4	28.6	33.0	58.5	9.0	7.3	4.7
August	35.3	27.2	31.3	65.6	7.2	6.0	114.8
September	36.2	25.4	31.0	53.7	9.4	4.7	3.3

Table 2a. Effect of different natural plant extracts on morphological characteristics of turf grass under summer stress.

Treatments	Tiller height (mm)	Tiller/ft^2	Leaves/ tiller	Inter-nodal distance (mm)	Leaf area index	Root length (mm)
Moringa leaf extract (3%)	37.84 A	2012 A	6.06 A	8.05 C	30.67 A	107.08 AB
Seaweed extract (0.2%)	38.42 A	1954 A	6.51 A	7.76 C	31.38 A	112.85 A
Sorghum water extract (3%)	32.70 AB	1347 B	5.98 A	9.09 B	23.26 B	111.40 AB
Sunflower water extract (3%)	35.36 BC	1185 B	5.89 A	10.55 A	24.22 B	103.15 BC
Control (water)	29.46 C	1062 B	5.16 B	9.47 B	21.50 B	96.57 C
LSD($p \leq 0.05$)	4.65	304.69	0.67	0.77	4.66	8.43

NS: Non-significant; Treatments not showing the similar letters within a column differ significantly at 5% probability level.

Table 2b. Effect of different natural plant extracts on morphological characteristics of turf grass under summer stress.

Treatments	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root weight (g)	Fresh Root/shoot weight ratio	Dry Root/shoot weight ratio
Moringa Leaf Extract (3%)	205.13 A	20.80 A	118.61	14.47 A	0.101	0.122 A
Seaweed Extract (0.2%)	195.90 A	17.77 B	107.93	12.99 B	0.095	0.120 A
Sorghum water Extract (3%)	160.55 B	14.85 C	102.89	9.22 C	0.092	0.090 B
Sunflower water Extract (3%)	164.20 B	16.00 BC	101.28	9.68 C	0.097	0.095 B
Control (water)	146.90 B	13.50 C	98.75	8.38 C	0.092	0.087 B
LSD(p≤0.05)	**31.28**	**2.80**	**NS**	**1.32**	**NS**	**0.024**

NS: Non-significant; Treatments not showing the similar letters within a column differ significantly at 5% probability level.

Table 3. Effect of different natural plant extracts on physiological characteristics of turf grass under summer stress.

Treatments	Chlorophyll Contents (mg/g)	Cell membrane thermo stability (%)	Ascorbic acid (mg/100 g)	Osmotic adjustment (-MPa)	Relative water contents	K-contents (%)
Moringa Leaf Extract (3%)	0.768 AB	38.24 A	15.66 A	0.663 A	0.417 AB	75.50 A
Seaweed Extract (0.2%)	0.781 A	37.79 A	15.06 A	0.753 A	0.432 A	76.47 A
Sorghum water Extract (3%)	0.738 BC	33.42 B	13.25 B	0.351 B	0.390 BC	68.17 B
Sunflower water Extract (3%)	0.708 CD	31.71 B	12.65 B	0.307 B	0.411 AB	68.20 B
Control (water)	0.676 D	16.97 C	10.24 C	0.262 B	0.364 C	53.98 C
LSD(p≤0.05)	**0.035**	**4.01**	**1.69**	**0.099**	**4.511**	**0.027**

NS: Non-significant; Treatments not showing the similar letters within a column differ significantly at 5% probability level.

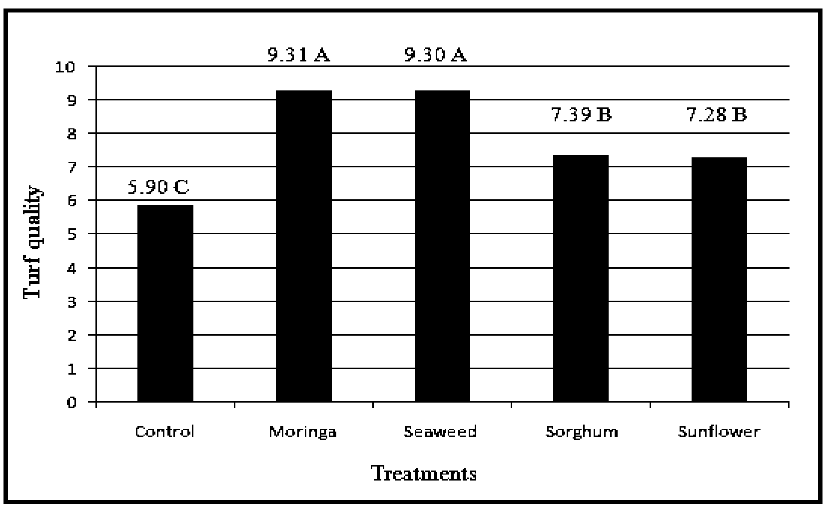

Fig. 1. Effect of different natural plant extracts on turf quality under summer stress. Bars having different letters differ significantly (p≤0.05). Name of plants represents their respective extracts applied.

DISCUSSION

Maximum tiller height, number of tiller, number of leaves per tiller and leaf area index of grass were observed highly significant with moringa and seaweed application followed by sorghum and sunflower application. Results indicated that moringa and sorghum extract containing growth promoting substances like zeatin, which delayed the process of aging and enhanced plant height (Yasmeen *et al.* 2011; Jahangeer, 2011). Increase in tiller height of grass may be due to presence of biological active plant growth regulators *i.e.* auxin and cytokinins in the extract of seaweed (Stirk *et al.,* 2004) and moringa (Nagar *et al.,* 2006). Moreover, both of these hormones played vital role in cell expansion and cell division process which helped to increase the plant height (Xu and Huang, 2010).Yasmeen *et al*. (2011) also stated that moringa extract increased the number of tiller per unit area in wheat. Number of tillers and leaves were affected by drought and heat stress because water deficient plant cell was unable to expand or divide and consequently have low regenerative capacity. While moringa and seaweed extracts were rich in potassium, calcium, ascorbic acid, auxin and zeatin (Stirk *et al.,* 2004 and Basra *et al.,* 2009). Under stress they played a vital role in maintenance of cell expansion by osmoregulation and cell division process consequently increased regenerative division capacity of grass (Xu and Huang, 2010), likewise. In a study Yasmeen *et al.* (2013) also observed increase in leaf area by the use of moringa extract in wheat, this increase in leaf area resulted in increased photosynthetic activity which promoted high biomass production. Further, (Elliott and prevatte, 1996) noted that seaweed had no effect on shoot dry weight when applied on turf grass.

Root/shoot fresh weight ratio showed insignificant difference among treatments while significant improvement in root/shoot dry weight ratio was observed when moringa and seaweed extract were applied (Table 2 and 3) on the grass under summer stress. However, root length, root fresh and dry weight showed significantly positive effect of bio-stimulants especially seaweed and moringa followed by sorghum and sunflower. These results were corroborated with Nouman *et al.* (2012a) who stated that when moringa leaf extract was applied, it increased root length in rangeland grasses. Zhang and Ervin (2008) also observed long roots in creeping bent grass under heat stress when seaweed extract was applied and also noted that internal cytokinin contents enhanced by this foliar application. This increase in fresh and dry weight of root may be due to the presence of cytokinin, polyamine and betains in moringa and seaweed that increased the root dry matter by saving root from desiccation and promoting rooting. These results are also in line with the findings of Zhang *et al.* (2002; 2003) who found increased turf root biomass with application of seaweed under heat and drought stress.

Among different bio-chemical factors the chlorophyll contents, cell membrane thermo stability and contents of ascorbic acid were found significantly higher by the application of seaweed and moringa followed by sorghum and sunflower (Table 3). Zhang *et al.* (2003), also found that seaweed enhance photochemical activity by increasing the chlorophyll content in turf. Moreover, Jahangeer (2011) while working on maize and Akram (2012) on wheat reported that that foliar application of moringa and sorghum extracts increased the chlorophyll contents in respective crops, significantly. Moringa extract application improved cell membrane thermo stability in maize (Mehboob, 2011). Osmotic adjustment, potassium contents and relative water contents were significantly greater with moringa and seaweed extract application (Table 3) than all other treatments. The seaweed and moringa was rich source of phytohormone, minerals, polyamine and betaines (Zhang, 1997 and Basra *et al.,* 2009) which promoted the reducible sugars accumulation and enhanced resistance against wilting, though improving the plant cell internal osmotic pressure. Beckett and Van Staden (1989) observed that the seaweed extract improved the yield and growth related parameters in potassium stressed wheat. Nouman *et al.* (2012b) found that priming with MLE was helpful in seedling emergence and increased potassium contents in moringa seedling as compared with other priming treatments. The application of moringa and seaweed significantly increased the relative water contents followed by sorghum and sunflower which were statistically greater than control. Akram (2012) reported that moringa leaf and sorghum extract application increased the relative water contents in wheat by increasing proline, phenolic and ascorbic acid contents through osmotic adjustment. It was evident from the results of study that moringa and seaweed extract application improved the turf quality significantly, followed by sorghum and sunflower. Similar findings were also reported by Zhang *et al.* (2003) in turfgrass that exogenous application of seaweed extract increased superoxide dismutase activity in chloroplast which saved plant from heat burning.

Overall, *Moringa oleifera* and seaweed extract proved best in order to support the growth and quality of turf grass under summer stress condition. Therefore, these extracts can be used in the golf, amateur and public parks. Further it was an economical and bio-friendly approach as compared to synthetic substances to improve growth and quality of turf grass.

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