J. Agric. Sci. Mansoura Univ., 28 (6): 4943 – 4957, 2003

NITROGENOUS NUTRITION OF KIKUYU TURFGRASS USING CHEMICAL AND BIOFERTILIZERS

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ABSTRACT

This study was conducted at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the two successive seasons of 2000/2001 and 2001/2002, with the aim of investigating the response of Kikuyugrass (*Pennisetum clandestinum* Hochst. ex Chiov.) to two N sources (conventional chemical fertilization and biofertilization), and assessing the possibility of using biofertilizers to reduce the need for chemical N fertilization. Kikuyu turfgrass received pre-planting and post-planting inoculation with Cerealin (a commercial · product containing *Bacillus polymyxa* and *Azotobacter chroococcum* bacteria) only, or post-planting chemical N fertilization using ammonium nitrate at rates equivalent to 2, 3 or 4 g N/m^2/month (with or without Cerealin inoculation). In addition, unfertilized plants were used as the control.

All treatments increased turf density, plant height, the fresh and dry weights of clippings/m^2 and underground parts/m^2, as well as the leaf contents of pigments (total chlorophylls and carotenoids), total carbohydrates, N, P and K. In general, raising the rate of chemical N fertilization caused steady increases in the values recorded for most of these parameters. These increases were more pronounced when chemical N fertilization was combined with the use of Cerealin. In both seasons, Cerealin + N at 3 or 4 g/m^2/month gave the highest values for most of the vegetative growth parameters as well as the different chemical constituents, whereas inoculation with Cerealin alone was the least effective treatment in most cases.

In many cases, the data recorded on some vegetative growth characteristics (including the fresh and dry weights of clippings) show that combining Cerealin with chemical N fertilization reduced the need for chemical N fertilization by approximately 25-33%. It can be concluded that inoculation of *Pennisetum clandestinum* plugs with Cerealin, followed by chemical N fertilization of the turf at the rate of 3 g N/m^2/month was sufficient to maintain high quality for the above-ground turf, and relatively good characteristics for the underground parts and chemical composition.

Keywords: Kikuyugrass, *Pennisetum clandestinum*, NPK, fertilization, biofertilization, *Bacillus polymyxa*, *Azotobacter chroococcum*

INTRODUCTION

The genus *Pennisetum* includes about eighty species, of which only one (Kikuyugrass, *Pennisetum clandestinum* Hochst. ex Chiov.) is cultured as a desirable turfgrass. Kikuyugrass is a vigorous, medium-textured, light green, warm-season grass that rather resembles Bermudagrass and, like it, must be mowed frequently. It stands wear well, and spreads by vigorous rhizomes and stolons, forming a tough, dense, springy turf which grows well in moist, medium-textured soils of high fertility. Its cultural requirements have not been adequately characterized [Rockwell and Grayson (1956), Aldous (1999) and Turgeon (1999)].

It is commonly known among landscape horticulturists that a healthy turfgrass requires adequate fertilization in order to compensate the nutrients lost by mowing. Chemical fertilization (especial. N fertilization) is the most common means of supplying turfgrasses with their nutritional requirements [Aldous (1999) and Turgeon (1999)]. However, factors such as escalating N fertilizer costs, soil structural degradation, environmental pollution, and sustainable land use have generated a growing interest in natural N fixation as a method of providing plants with their N requirements (Subba Rao, 1984). The current trend is to replace a significant portion of mineral fertilizers by biofertilizers which consist mostly of N-fixing bacteria .

Biofertilizers are safe for the environment, and they reduce the need for chemical fertilization, thus reducing the cost of fertilizers and labour. The possibility of using biofertilizers as an alternative to chemical N fertilization was tested by Day *et al.* (1975) who reported that non-symbiotic N_2 fixing bacteria are capable of significant rates of N accumulation. In addition to their main function of fixing molecular nitrogen, the enhancing effect of biofertilizers on some plant growth characters was explained by Subba Rao (1984) who attributed this effect to other factors such as (a) the ability of biofertilizers to synthesize and secrete indole acetic acid, cytokinins, gibberellins and cytokinin- or gibberellin-like substances, (b) increasing amino acid content, and (c) producing anti-fungal antibiotics which inhibit a variety of soil fungi. Also, Okon (1984) mentioned that biofertilizers promote the synthesis of some vitamins, including B_{12}.

Among the large number of bacteria which have been tested for their nitrogen fixation ability, *Azotobacter chroococcum* and *Bacillus polymyxa* have yielded very good results. *A. chroococcum* bacteria are strictly aerobic, whereas *B. polymyxa* bacteria are facultative anaerobes (Schlegel, 1993).

Inoculation of wheat with *Azotobacter*, combined with a moderate N-fertilization rate (40 kg N/fed.) had a beneficial effect on plant growth and productivity (Badawy *et al.*, 1998). This favourable effect of *Azotobacter* bacteria was attributed to an increase in the water and mineral uptake from the soil, which might be ascribed to increase in root surface area, root hairs and root elongation (Hanafy Ahmed *et al.*, 1997). On the other hand, the beneficial effect of *Bacillus polymyxa* may be attributed not only to its N-fixation ability, but also to its P-solubilizing action (Subba Rao, 1984). Other explanations were suggested by Holl *et al.* (1988) who mentioned that crested wheatgrass plants showed a positive response to inoculation with *B. polymyxa*, which may be attributed to suppression of pathogenic organisms in the rhizosphere, root-associated nitrogen fixation, solubilization of organic phosphate compounds, or the bacterial production of indoleacetic acid. A different explanation to the mode of action of *B. polymyxa* was proposed by Gouzou *et al.* (1993), who reported that inoculation of the soil with *B. polymyxa* caused an increase in aggregated soil particles by 57% which led to a more porous structure within the rhizosphere soil and, consequently, enhanced water retention and nutrient transfer in the rhizosphere of wheat. Generally, Omar *et al.* (1991) reported that the inoculation of wheat with *Bacillus polymyxa* can save 41.6% of the nitrogen fertilizer, and El-Haddad *et al.* (2000) reported that *B. polymyxa* was quite effective for increasing the productivity of wheat.

Cerealin biofertilizers are a group of commercial products that contain different species of bacteria, depending on the crop. Omar and Hamouda

(1998) concluded that wheat plants inoculated with Cerealin (containing *Bacillus polymyxa*) obtained 50.4% of the nitrogen in their tissues through nitrogen fixation. In some cases, maximum effectiveness of Cerealin was achieved when Cerealin (containing *Azospirillum lipoferum* and *B. polymyxa*) application was combined with chemical N fertilization [Kotb (1998) on wheat]. A similar conclusion was reached by El-Hawary *et al.* (1998), who mentioned that plant growth, grain yield and straw yield of wheat were increased remarkably due to the interaction between bacterial inoculation (using *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus spp.*) and N-fertilization, especially at the highest level.

This study was conducted with the aim of investigating the effect of the Cerealin (a biofertilizer containing *Azotobacter chroococcum* and *Bacillus polymyxa* bacteria) on the growth and chemical composition of Kikuyugrass, and to assess the possibility of using Cerealin to reduce the need for chemical N fertilization.

MATERIALS AND METHODS

This study was conducted at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the two successive seasons of 2000/2001 and 2001/2002, with the aim of investigating the response of Kikuyugrass (*Pennisetum clandestinum* Hochst. ex Chiov.) to two N sources (conventional chemical fertilization and biofertilization), and assessing the possibility of using Cerealin to reduce the need for chemical N fertilization.

Thirty two square beds (1 m x 1 m) were prepared in a clay-loam soil at the site of the experiment during the month of February, 2000 and 2001 (in the first and second seasons, respectively). The physical and chemical characteristics of the soil are presented in Table (1). The beds were watered, and a non-selective herbicide (Round-Up) was sprayed two weeks later at a rate equivalent to 1 litre/fed. to eliminate all vegetation prior to planting. During soil preparation, all the planting beds were supplied with calcium superphosphate (15.5% P_2O_5) at the rate of 30 g/m^2. On March 20th, 2000 and 2001 (in the two seasons, respectively), plugs of *Pennisetun clandestinum* were planted in the beds at a spacing of 15 x 15 cm (one square metre of sod gave enough plugs to plant about 8 m^2). The planting beds were irrigated daily (at the rate of 5 litres/m^2) from planting till May 30th, at which time the turfgrass had become well-established. Thereafter, they were irrigated every 2 days at the rate of 14 litres/m^2/2 days till October 15th, then at the rate of 8 litres/m^2/2 days till the end of each season (on January 15th 2001 and 2002, in the first and second seasons, respectively).

Prior to plugging, the plugs used for planting sixteen beds were inoculated with the commercial biofertilizer "Cerealin" [produced by Egyptian Ministry of Agriculture, and containing *Bacillus polymyxa* and *Azotobacter chroococcum* bacteria, each at 10^7 colony forming units (CFU)/g carrier] as a source of nitrogen. Prior to inoculation, 40 g of Cerealin (sufficint for inoculation of 40 m^2) were mixed with 160 g of vermiculite in order to obtain a Cerealin/vermiculite mixture with 5 times the original volume of Cerealin. The plugs were inoculated by wetting their bases, then immersing them in the Cerealin/vermiculite mixture, whereas the other plugs were inoculated with vermiculite only. A basal dressing of the Cerealin/vermiculite mixture was added after 20 days from planting, at the rate of 5 g/m^2, and the turfgrass was irrigated immediately thereafter, while vermiculite was added alone at the same rate to beds receiving no biofertilization. In each season, the turf was mowed to a height of 5 cm when necessary till May 30th (when coverage was complete), then the chemical fertilization treatments were initiated. Chemical N fertilization was added using ammonium nitrate (33.5% N) at rates of 6, 9 or 12 g/m^2/month (equivalent to approximately 2, 3 or 4 g N/m^2/month). Each of the chemical N fertilization rates was applied to 4 inoculated and 4 un-inoculated planting beds (replicates). Also, four inoculated beds and four un-inoculated beds were left with no chemical fertilization. Also, all plants were supplied with potassium sulphate (48% K_2O) at the rate of 20 g/m^2, divided into two equal doses. The first dose was applied after 45 days from planting, and the second dose one month later.

The layout of the experiment was a randomized complete blocks design, with 4 blocks (replicates), each consisting of 8 planting beds (one for each treatment).

The turf was mowed every 10 days to a height of 5 cm, starting May 30th (2000 and 2001 in the first and second seasons, respectively), till the end of each season. The plant height was recorded before mowing, starting June 10th. The fresh and dry weights of the clippings were also recorded after each mowing. For each fertilization treatment, the values recorded every 10 days were averaged to determine the average plant height before mowing, as well as the average fresh and dry weights of clippings. At the termination of each season, turf density (number of tillers/100 cm^2), as well as the fresh and dry weights of underground parts (to a depth of 20 cm) were also recorded. In addition, fresh clipping samples were chemically analyzed to determine their total chlorophylls and total carotenoids contents (using the method described by Nornai, 1982), while the total carbohydrates content was determined in dried clipping samples (using the method recommended by Herbert *et al.*, 1971).

Table (1): Physical and chemical characteristics of the soil used for growing *Pennisetum clandestinum* during the 2000/2001 and 2001/2002 seasons.

Physical characteristics								
Soil texture	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)	EC (dS/m)	Field capacity (%)	CEC (meq/100 g)
Clay loam	8.2	31.6	26.7	33.5	2.4	0.34	45.9	34.5

Chemical characteristics				
pH	Organic matter (%)	Available macro-nutrients (mg/kg)		
		N	P	K
7.6	1.34	42.0	9.2	387.0

Also, dried clipping samples were digested to extract nutrients (as described by Piper, 1947), and the extract was analyzed to determine its contents of N (using the modified micro-Kjeldahl method as described by Pregl, 1945), phosphorus (according to King, 1951) and potassium (using a Pye Unicam Model SP 1900 Atomic Absorption Spectrophotometer).

Data collected on vegetative growth were subjected to an analysis of variance, and the means were compared using the "Least Significant Difference (LSD)" test at the 0.05 level, as recommended by Steel and Torrie (1980).

RESULTS AND DISCUSSION

I- Vegetative growth

1- Turf density (number of tillers/100 cm^2)

The results recorded in both seasons (Table 2) show that *Pennisetum clandestinum* plants receiving the different fertilization treatments had significantly more tillers than the unfertilized (control) plants. Moreover, in plants supplied with chemical nitrogenous fertilization alone, raising the application rate caused a steady increase in the number of tillers. Accordingly, plants fertilized with 4 g N/m^2/month gave significantly more tillers than those receiving lower N rates (2 or 3 g N/m^2/month). This increase in turf density as a result of increasing N rate is in agreement with the findings of Hossni (1993) on *Cynodon dactylon*, Razmjoo and Kaneko (1993) on *Lolium perenne*, Razmjoo *et al.* (1996) on creeping bentgrass [*Agrostis palustris* (*A. stolonifera* var. palustris)], and the findings of Oral and Acikgoz (2001) on a turfgrass mixture consisting of perennial ryegrass (*Lolium perenne*), Kentucky bluegrass (*Poa pratensis*), creeping red fescue (*Festuca rubra* var. rubra) and chewing fescue (*Festuca rubra* var. commutata).

Table (2): Effect of nitrogenous chemical and biofertilization on the turf density (number of tillers/100cm^2) and plant height of *Pennisetum clandestinum* turfgrass in the 2000/2001 and 2001/2002 seasons.

*Fertilization treatments	Turf density (number of tillers/100 cm^2)		Plant height (cm)	
	First season (2000/2001)	Second season (2001/2002)	First season (2000/2001)	Second season (2001/2002)
Control	170.3	151.3	6.20	5.91
N1	186.7	172.7	7.43	6.85
N2	191.7	176.3	7.84	7.28
N3	204.0	187.0	8.09	7.89
Cerealin	181.3	170.3	7.11	6.46
Cerealin + N1	188.7	181.3	7.68	6.96
Cerealin + N2	198.0	194.7	8.57	7.98
Cerealin + N3	209.3	198.7	8.59	8.57
LSD (0.05)	**9.5**	**8.1**	**0.41**	**0.35**

* N1, N2 and N3 = N at 2, 3 or 4 g/m^2/month, respectively.

Combining chemical N fertilization with the use of the biofertilizer (Cerealin) caused a further increase in the number of tillers produced by *Pennisetum clandestinum*. In both seasons, the highest number of tillers was formed on plants fertilized with 4 g N/m^2/month + Cerealin. On the other hand, the application of Cerealin (with no chemical fertilization) was less effective in increasing the number of tillers, compared to treatments which included chemical N fertilization. This conclusion is in agreement with the findings of Barea *et al.* (1983) who mentioned that shoot production by ryegrass (*Lolium perenne*) was greater with N+P fertilization than with dual inoculation by *Azospirillum brasilense* and *Glomus mosseae*.

It is also worth mentioning that in many cases, the use of Cerealin reduced the need for chemical N fertilization. For example, results recorded in the first season show that plants fertilized with Cerealin only produced insignificantly fewer tillers than those produced by plants fertilized with 2 g N/m^2/month. Also, plants fertilized with 2 g N/m^2/month + Cerealin gave insignificantly fewer tillers than those produced by plants fertilized with 3 g N/m^2/month alone, while the number of tillers formed on plants fertilized with 3 g N/m^2/month + Cerealin was insignificantly fewer than those formed on plants fertilized with 4 g N/m^2/month alone. The beneficial effect of biofertilization on the formation of tillers was even more evident in the second season, with plants fertilized using Cerealin only forming insignificantly fewer tillers than those produced by plants fertilized with 2 or 3 g N/m^2/month. Also, the number of tillers formed on plants treated using 2 g N/m^2/month + Cerealin was insignificantly different than that formed on plants fertilized with 3 or 4 g N/m^2/month (with no Cerealin). Moreover, when Cerealin was used in the second season, raising the chemical fertilization rate from 3 to 4 g N/m^2/month caused no significant increase in turf density.

2- Plant height

In both seasons, plants receiving any of the tested fertilization treatments were significantly taller than the control plants (Table 2). Moreover, plant height was increased steadily with increasing the rate of chemical nitrogenous fertilization (with or without Cerealin). Similar increases in plant height as a result of increasing N fertilization rates have been reported by Allam (1993) on *Lolium perenne*, and Hossni (1993) on Bermudagrass (*Cynodon dactylon*).

The beneficial effect of Cerealin on plant elongation was clear in both seasons, especially when combined with chemical fertilization at the rates of 3 or 4 g N/m^2/month, since these combinations of treatments gave the tallest plants (with no significant difference between them in the first season). In both seasons, Cerealin + 3 g N/m^2/month gave significantly taller plants, as compared to those fertilized with 3 g N/m^2/month (with no biofertilization). Moreover, this treatment gave significantly taller plants than those receiving 4 g N/m^2/month in the first season. In both seasons, Cerealin + 4 g N/m^2/month gave significantly higher values than those recorded with chemical fertilization only. In contrast, the least effective treatment for increasing plant height was the application of Cerealin alone. The favourable effect of treatments including Cerealin on plant height may be explained by the ability of biofertilizers to synthesize and secrete indole acetic acid and gibberellins or gibberellin-like substances [Subba Rao (1984) and Holl *et al.* (1988)].

3- Fresh and dry weights of clippings/m^2

The results recorded in the two seasons (Table 3) show that in most cases, the fresh and dry weights of clippings/m^2 were significantly increased by the different fertilization treatments, compared to the control. The only exception to this general trend was detected in the second season, with plants that received biofertilization only having an insignificantly higher dry weight of clippings/m^2, compared to the control. These results are in agreement with the findings of Haahtela (1986) who mentioned that inoculation of *Poa pratensis* with *Klebsiella* and *Enterobacter* increased the dry matter yield significantly, especially with N fertilization. Also, inoculation with *Azospirillum brasilense* has been found to increase the yields of *Paspalum notatum* (Menze, 1985), *Lolium perenne*, *Festuca arundinacea* (Scotti *et al.*, 1987) and *F. pratensis* (Yurko, 1997).

The fresh and dry weights of clippings were increased steadily by raising the rate of chemical nitrogenous fertilization, with or without biofertilization. In both seasons, the highest values for fresh and dry weights of clippings/m^2 were obtained from plants supplied with 4 g N/m^2/month (without biofertilization), or with biofertilization + 3 or 4 g N/m^2/month (with no significant difference among these three treatments in most cases).

Table (3): Effect of nitrogenous chemical and biofertilization on the fresh and dry weights of clippings/m^2 of *Pennisetum clandestinum* turfgrass in the 2000/2001 and 2001/2002 seasons.

*Fertilization treatments	Fresh weight of clippings (g/m^2)		Dry weight of clippings (g/m^2)	
	First season (2000/2001)	Second season (2001/2002)	First season (2000/2001)	Second season (2001/2002)
Control	15.90	28.00	4.35	6.65
N1	34.00	40.83	8.28	8.62
N2	44.27	49.15	9.80	10.44
N3	49.10	58.45	11.09	13.50
Cerealin	24.80	32.85	6.17	7.14
Cerealin + N1	43.18	45.76	10.45	11.52
Cerealin + N2	48.50	57.59	11.33	14.84
Cerealin + N3	52.12	60.02	12.75	14.90
LSD (0.05)	**4.01**	**3.88**	**1.51**	**1.39**

*** N1, N2 and N3 = N at 2, 3 or 4 g/m^2/month, respectively.**

The increase in fresh and dry weights of clippings as a result of raising the N fertilization rate is in agreement with the findings of Hossni (1993) on *Cynodon dactylon*, Razmjoo and Kaneko (1993) on *Lolium perenne*, Soni *et al.* (1993) on *Zoysia matrella*, as well as Paswan and Machahary (2000) and

Sistana and Mays (2001) on bahiagrass (*Paspalum notatum*). On the other hand, inoculation with Cerealin only was the least effective treatment for increasing the fresh and dry weights of clippings/m^2. This treatment gave significantly lower fresh and dry weights of clippings, as compared with plants supplied with any other fertilization treatment. In this respect, Kapustka *et al.* (1985) mentioned that nitrogen-fixing bacteria (including *Azotobacter* and *Bacillus* bacteria) are not capable of supplying sufficient N to *Lolium perenne* plants.

The data presented in Table (3) also show that in many cases, the use of biofertilization decreased the amounts of chemical nitrogenous fertilizers needed for the production of new tissues between mowings. In both seasons, fertilization with Cerealin + 2 g N/m^2/month gave insignificantly different values, compared to those obtained from plants fertilized with 3 g N/m^2/month (without Cerealin). Moreover, plants fertilized with Cerealin + 3 g N/m^2/month gave fresh and dry weights of clippings that were insignificantly different than those obtained with fertilization using the highest N rate (4 g N/m^2/month), without the use of biofertilization. These results show that inoculation with Cerealin reduced the need for chemical N fertilization by 25-33%. In this respect, Omar *et al.* (1991) reported that inoculation of wheat with *Bacillus polymyxa* can save 41.6% of the nitrogen fertilizer. Also, Quarles (1996) concluded that application of biofertilization treatments to golf courses (using *Azospirillum brasilense*) reduced the need for fertilizer applications.

3- Fresh and dry weights of underground parts/m^2

In most cases, the fresh and dry weights of underground parts were significantly increased by the different fertilization treatments, compared to the unfertilized control (Table 4). The results recorded in the two seasons also show that raising the rate of chemical nitrogenous fertilization (with or without Cerealin) caused a steady increase in the recorded values.

Table (4): Effect of nitrogenous chemical and biofertilization on the fresh and dry weights of underground parts/m^2 of *Pennisetum clandestinum* turfgrass in the 2000/2001 and 2001/2002 seasons.

*Fertilization treatments	Fresh weight of underground parts (g/m^2)		Dry weight of underground parts (g/m^2)	
	First season (2000/2001)	Second season (2001/2002)	First season (2000/2001)	Second season (2001/2002)
Control	275.5	240.1	100.4	94.1
N1	400.7	302.2	122.8	112.3
N2	480.4	337.3	165.9	128.2
N3	501.3	400.6	202.7	150.7
Cerealin	362.7	251.9	110.6	98.9
Cerealin + N1	486.0	370.1	169.5	135.0
Cerealin + N2	520.2	420.0	210.3	165.0
Cerealin + N3	561.5	454.1	218.2	183.2
LSD (0.05)	9.9	8.5	6.5	4.9

* N1, N2 and N3 = N at 2, 3 or 4 g/m^2/month, respectively.

Although the least effective treatment for increasing the fresh and dry weights of underground parts was the inoculation with Cerealin only (with no chemical fertilization), but the favourable effect of biofertilization on the growth of underground parts was very clear in both seasons when the use of Cerealin was combined with the addition of chemical N fertilization, especially at the relatively high rates of 3 or 4 g N/ m^2/month. In both seasons, plants receiving Cerealin + 3 g N/m^2/month gave significantly higher values than those obtained from plants receiving chemical N fertilization only, even at the highest level (4 g N/ m^2/month). Moreover, combining Cerealin with highest rate of chemical fertilization (4 g N/m^2/month) gave significantly higher values than any other treatment. The favourable effect of biofertilization on root growth is in agreement with the findings of Hanafy Ahmed *et al.* (1997) on *Corchorus olitorius* and *Raphanus sativus*.

II- Chemical composition

1- Leaf pigments content (total chlorophylls and carotenoids)

The synthesis and accumulation of leaf pigments (total chlorophylls and carotenoids) were generally enhanced by the application of the different fertilization treatments (Table 5). In both seasons, the lowest contents of leaf pigments were obtained in the leaves of unfertilized plants, whereas raising the rate of chemical N fertilization caused a steady increase in the recorded values. This increase in the content of chlorophylls and carotenoids as a result of raising the rate of N fertilization is in agreement with the findings of Allam (1993) on ryegrass (*Lolium perenne*). Also, increasing the rate of N fertilization caused an increase in the chlorophylls content of *Cynodon dactylon* (Hossni, 1993) and *Paspalum notatum* (Paswan and Machahary, 2000).

Table (5): Effect of nitrogenous chemical and biofertilization on the total chlorophylls, total carotenoids and total carbohydrates contents of *Pennisetum clandestinum* turfgrass in the 2000/2001 and 2001/2002 seasons.

*Fertilization treatments	Total chlorophylls content (mg/g fresh matter)		Total carotenoids content (mg/g fresh matter)		Total carbohydrates content (% of dry matter)	
	First season (2000/2001)	Second season (2001/2002)	First season (2000/2001)	Second season (2001/2002)	First season (2000/2001)	Second season (2001/2002)
Control	1.08	0.95	0.23	0.16	9.51	10.89
N1	1.18	1.07	0.31	0.24	10.83	11.32
N2	1.30	1.23	0.36	0.26	10.94	11.76
N3	1.48	1.35	0.44	0.33	11.32	12.04
Cerealin	1.11	1.05	0.27	0.21	10.90	10.97
Cerealin + N1	1.33	1.22	0.36	0.25	11.40	11.88
Cerealin + N2	1.55	1.30	0.54	0.28	11.73	12.89
Cerealin + N3	1.60	1.40	0.59	0.36	11.91	12.96

* N1, N2 and N3 = N at 2, 3 or 4 g/m^2/month, respectively.

Biofertilization alone (using Cerealin) was the least effective treatment for increasing the leaf pigments content compared to the other fertilization treatments. Combining biofertilization with chemical N fertilization gave higher values than those obtained with chemical fertilization alone (at the same rates). Among the different fertilization treatments, the application of Cerealin + 4 g N/m^2/month gave the highest contents of total chlorophylls and carotenoids.

The promotion in the synthesis and accumulation of chlorophyll as a result of chemical N fertilization or the use of N-fixing bacteria may be attributed to the role played by nitrogen as an essential component in the structure of porphyrines, which are found in many metabolically active compounds, including chlorophylls. Chlorophylls are bound to, and perhaps even embedded within protein molecules (Devlin, 1975).

The results presented in Table (5) also show that in both seasons, the pigment contents of plants fertilized with Cerealin + 2 g N/m^2/month were approximately equal to those of plants fertilized with 3 g N/m^2/month (with no Cerealin). Moreover, results recorded in the first season show that plants fertilized with Cerealin + 3 g N/m^2/month had higher pigment contents, compared to plants fertilized with 4 g N/m^2/month (with no Cerealin). These results indicate that biofertilization may cause some reduction in the requirements of chemical N fertilization needed by Kikuyugrass for the synthesis of pigments.

2- Total carbohydrates content

Fertilization of Kikuyugrass with the different biofertilizer and chemical N fertilizer treatments increased the total carbohydrates content, compared to that of control plants (Table 5). Raising the rate of chemical N fertilization caused a steady increase in the recorded values, especially when combined with biofertilization. The increase in the total carbohydrates content of plants receiving chemical N fertilization treatments is in agreement with the findings of Allam (1993) on *Lolium perenne*. Also, Hossni (1993) detected an increase in the total sugars content of *Cynodon dactylon* as a result of increasing the rate of chemical N fertilization.

The favourable effect of combining biofertilization with chemical N fertilization was clear in both seasons, with plants fertilized using Cerealin + the highest chemical N fertilization rate (4 g N/m^2/month) giving the highest values, followed by plants supplied with Cerealin + 3 g N/m^2/month, with only a slight difference between these two treatments. Moreover, plants fertilized with Cerealin + 2 g N/m^2/month had a higher carbohydrates content in the first season, compared to plants which received chemical N fertilization only, even at the highest rate (4 g N/m^2/month). On the other hand, the least effective treatments for increasing the total carbohydrates content were the application of N at 2 g N/m^2/month only (in the first season), or Cerealin only (in the second season).

The favourable effect of the different fertilization treatments on the synthesis and accumulation of carbohydrates may be attributed to the increase in the chlorophylls content of fertilized plants, and to the role played by nitrogen in the structure of porphyrine molecules (as previously mentioned), which are found in the cytochrome enzymes essential in photosynthesis. This increase in the contents of chlorophylls and cytochrome enzymes results in an increase in the rate of photosynthesis, and a promotion in carbohydrate synthesis and accumulation (Devlin, 1975).

3- Contents of nutrients (N, P and K)

The contents of N, P and K in clippings of Kikuyugrass were increased as a result of the different fertilization treatments, compared to the control (Table 6). In both seasons, raising the rate of chemical N fertilization resulted in a steady increase in the contents of the three nutrients (in most cases). A similar increase in the N content of *Zoysia matrella* as a result of increasing the rate of N application (from 0 to 40 g N/m^2/year) was reported by Soni *et al.* (1993). Also, Razmjoo *et al.* (1996) found that the N, P and K contents of creeping bentgrass [*Agrostis palustris* (*A. stolonifera* var. palustris)] were increased as a result of high N fertilization rates.

Table (6): Effect of nitrogenous chemical and biofertilization on the N, P and K contents of *Pennisetum clandestinum* turfgrass in the 2000/2001 and 2001/2002 seasons.

*Fertilization treatments	N content (% of dry matter)		P content (% of dry matter)		K content (% of dry matter)	
	First season (2000/2001)	Second season (2001/2002)	First season (2000/2001)	Second season (2001/2002)	First season (2000/2001)	Second season (2001/2002)
Control	1.38	1.30	0.09	0.11	1.11	1.08
N1	1.59	1.41	0.14	0.13	1.22	1.49
N2	1.73	1.89	0.15	0.15	1.47	1.60
N3	2.23	2.24	0.21	0.25	1.58	1.79
Cerealin	1.45	1.77	0.17	0.14	1.22	1.30
Cerealin + N1	2.15	1.94	0.26	0.22	1.42	1.66
Cerealin + N2	2.66	2.48	0.28	0.21	1.69	1.93
Cerealin + N3	2.69	2.42	0.35	0.24	1.99	2.12

*** N1, N2 and N3 = N at 2, 3 or 4 g/m^2/month, respectively.**

The effect of fertilization on the nutrients content was generally more pronounced when chemical N fertilization was combined with the application of biofertilization. Accordingly, the highest values (in most cases) were obtained from plants fertilized with Cerealin + 4 g N/m^2/month. The favourable effect of treatments including biofertilization on the N content is in agreement with the results recorded on *Festuca pratensis*, which showed an increase in the N content as a result of inoculation with *Klebsiella*, *Enterobacter* (Haahtela, 1986) or *Azospirillum brasilense* (Yurko, 1997). The effect of combining biofertilization with chemical N fertilization was generally clear on the N and P contents in both seasons, and on the K content in the second season, since plants fertilized with Cerealin + 2 g N/m^2/month had higher contents than those fertilized with 2 or 3 g N/m^2/month (without Cerealin). Also, plants fertilized with Cerealin + 3 g N/m^2/month had higher N and K contents in both seasons, and P contents in the first season, than those supplied with chemical N fertilization alone, even at the highest rate (4 g N/m^2/month). These results are in agreement with the findings of Kotb (1998), who reported that N and P uptake by wheat plants fertilized with 50 kg N/fed. in combination with bacterial inoculation was more or less similar to that of plants fertilized with 75 kg N/fed. alone.

On the other hand, inoculation with Cerealin only was less effective than the other fertilization treatments for increasing the N content in the first season, and the K content in the second one, whereas application of chemical fertilization at 2 g N/m^2/month was the least effective treatment in increasing the P content in both seasons, and the N content in the second season. These two treatments gave equally low K contents in the first season.

CONCLUSION

From the above results, it can be concluded that in many cases, combining Cerealin with chemical N fertilization treatments reduced the need for chemical N fertilization by approximately 25-33%. Thus, inoculation of *Pennisetum clandestinum* plugs with Cerealin, followed by chemical N fertilization of the turf at the rate of 3 g N/m^2/month, is recommended. This was sufficient to maintain high quality for the above-ground turf, and relatively good characteristics for the underground parts and chemical composition.

REFERENCES

Aldous, D.E. (1999). International Turf Management. Butterworth-Henemann Ltd., 306-307 & 139-157.

Allam, S.S.A. (1993). Effect of nitrogenous fertilization and shading on ryegrass lawns in Egypt. M.Sc. Thesis, Fac. Agric., Cairo Univ., p. 194.

Badawy, F.H.; S.M. Mahmoud; M.M. El-Desouky and H.M. El-Rewainy (1998). Inoculation with N_2-fixing microbes as a practice to improve plant growth and crop yield. In: Proceedings of "The Regional Symposium on Agro-Technologies Based on Biological Nitrogen Fixation for Desert Agriculture". April 14-16, 1998, El-Arish, North Sinai Governorate, 197-208.

Barea, J.M.; A.F. Bonis and J. Olivares (1983). Interactions between *Azospirillum* and VA-mycorrhiza and their effects on growth and nutrition of maize and ryegrass. Soil Biology and Biochemistry, 15 (6): 705-709.

Day, I.M.; D. Harris; P.J. Dart and P. Van Berkum (1975). The broadback experiment. An investigation of nitrogen gains from non-symbiotic fixation. I.B.P. Series, Vol. 6, Cambridge, 71-84.

Devlin, R.M. (1975). Plant Physiology. 3rd Ed., Affiliated East-West Press Pvt. Ltd., New Delhi.

El-Haddad, M.E.; M.N.A. Omar; M.I. Mostafa and A.A. Hassan (2000). Bio-diversity of *Bacillus polymyxa* colonizing soil and roots of wheat. II. Serological diversity and possible manipulation in biofertilization. Arab. Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 8 (2): 557-571.

El-Hawary, F.; I. Ibrahim and F. Hammouda (1998). Effect of integrated bacterial fertilization on yield and yield component of wheat in sandy soil. In: Proceedings of "The Regional Symposium on Agro-Technologies Based on Biological Nitrogen Fixation for Desert Agriculture". April 14-16, 1998, El-Arish, North Sinai Governorate, 235-243.

Gouzou, L.; G.Burti; R. Philippy; F. Bartoli and T. Heulin (1993). Effect of inoculation with *Bacillus polymyxa* on soil aggregation in the wheat rhizosphere (preliminary examination). Geoderma, 56: 479-491.

Haahtela, K. (1986). Root-associated nitrogen fixation in grasses and cereals. Dissertation Abstracts International. C- European Abstracts, 47 (1): 86. [C.F. Field Crops Abst., 40 (1): 582].

Hanafy Ahmed, A.H.; N.F. Kheir and N.B. Talaat (1997). Physiological studies on reducing the accumulation of nitrate in jew's mallow (*Corchorus olitorius*) and radish (*Raphanus sativus* L.). Bull. Fac. Agric. Cairo Univ., 48: 25-64.

Herbert, D.; P.J. Philipps and R.E. Strange (1971). Determination of total carbohydrates. Methods in Microbiology, 5(8): 204-244.

Holl, F.B.; C.P. Chanway; R. Turkington and R. Radley (1988). Response of crested wheat-grass (*Agropyron cristatum* L.), perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) to inoculation with *Bacillus polymyxa*. Soil Biol Biochem., 20: 19-24.

Hossni, Y.A. (1993). Effect of soil media, chemical fertilization and salinity on growth and chemical composition of Bermuda grass (*Cynodon dactylon* L.). Ph.D. Thesis, Fac. Agric., Cairo Univ., 216-218.

Kapustka, L.A.; P.T. Arnold and P.T. Lattimore (1985). Interactive responses of associative diazatrophs from a Nebraska Sand Hills grassland. In: Ecological Interactions in Soil: Plants, Microbes and Animals (edited by Fitter A.H., Atkinson D., Read D.J. and Usher M.B.), Blackwell Scientific Publications, pp. 149-158.

King, E.J. (1951). Micro-Analysis in Medical Biochemistry. 2nd Ed, Churchill Publishing Co., London.

Kotb, M.Th.A. (1998). Response of wheat to biofertilizer and inorganic N and P levels. In: Proceedings of "The Regional Symposium on Agro-Technologies Based on Biological Nitrogen Fixation for Desert Agriculture". April 14-16, 1998, El-Arish, North Sinai Governorate, 291-301.

Menze, H. (1985). Interactions between *Azospirillum* and VA-mycorrhiza in Graminae at different soil pH levels. Zeitschrift für Acker und Pflanzenbau, 155 (4): 232-237. [C.F. Soils and Fertilizers, 50 (1): 284].

Nornai, R. (1982). Formula for determination of chlorophyll pigments extracted with N.N. dimethyl formamide. Plant Physiol., 69: 1371-1381.

Okon, Y. (1984). Response of cereal and forrage grasses to inoculation with N_2-fixing bacteria. In: Advances in Nitrogen Fixation Research, (Veeger,C. and Newton, W.E., Eds.), pp. 303-309, Nijboff/Junk, Hague.

Omar, M.N.A. and A.A. Hamouda (1998). Effect of inoculation by Cerealin (*Bacillus polymyxa*) on soil aggregation under wheat plants and nitrogen fixed by N-15 dilution technique. In: Proceedings of "The Regional Symposium on Agro-Technologies Based on Biological Nitrogen Fixation for Desert Agriculture". April 14-16, 1998, El-Arish, North Sinai Governorate, 227-234.

Omar, M.N.A.; M.H. Hegazy; R.A. Abd El-Aziz; M.S.M. Abo Soliman and M.M. Sobh (1991). Effect of inoculation with rhizobacteria on yield of wheat under graded level of nitrogen fertilization. Annals Agric. Sci., Ain Shams Univ., Cairo, 36 (1): 99-104.

Oral, N. and E. Acikgoz (2001). Effects of nitrogen application timing on growth and quality of a turfgrass mixture. J. Plant Nutrition, 24 (1): 101-109.

Paswan, L. and R.K. Machahary (2000). Effect of nitrogen on bahiagrass. J. Ornam. Hort. (New Series), 3 (2): 87-90.

Piper, C. S. (1947). Soil and Plant Analysis. pp. 258 - 275. Univ. of Adelaide, Adelaide.

Pregl, P. (1945). Quantitative Organic Microanalysis. 4th Ed., Churchill Publishing Co., London, pp. 78-82.

Quarles, W. (1996). Fungicide and fertilizer reduction on golf courses. IPM Practitioner, 18 (2): 5-7.

Razmjoo, K.; T. Imada; J. Suguira and S. Kaneko (1996). Effect of nitrogen rates and mowing heights on color, density, uniformity, and chemical composition of creeping bentgrass cultivars in winter. J. Plant Nutrition, 19 (12): 1499-1509.

Razmjoo, K. and S. Kaneko (1993). Effect of fertility ratios on growth and turf quality of perennial ryegrass (*Lolium perenne*) in winter. J. Plant Nutrition, 16 (8): 1531-1538.

Rockwell, F.F. and E.C. Grayson (1956). The Complete Book of Lawns. The American Garden Guild and Doubleday & Company, Inc., Garden City, New York, p. 55.

Schlegel, H.G. (1993). Fixation of molecular nitrogen. In: General Microbiology. 7th Ed., Cambridge University Press, p. 441.

Scotti, C.; M. Molinari and C. Garau (1987). Biological fixation of nitrogen in forage grasses and legumes. Annali dell'Istituto Sperimentale per le Colture Foraggere, 8: 35-48. [C.F. Herbage Abst., 62 (2):1246].

Sistana, K.R. and D.A. Mays (2001). Nutrient requirements of seven plant species with potential use in shoreland erosion control. J. Plant Nutrition, 24 (3): 459-467.

Soni, R.; A.S. Parmar and R. Kumar (1993). Effect of levels and timings of nitrogen application on the growth of turf of *Zoysia matrella* L. Punjab Hort. J., 33 (1/4): 135-141. [C.F. Hort. Abst. 65 (9): 8183].

Steel, R.G. and S.H. Torrie (1980). Principles and Procedures of Statistics. 2nd Ed., McGraw-Hill Inc.

Subba Rao, N.S. (1984). Biofertilzers in Agriculture. 3rd printing. Oxford and IBH Publishing Co., New Delhi, India, pp. 1-13, 83, 132 & 153-165.

Turgeon, A.J. (1999). Turfgrass Management. Prentice-Hall International Ltd., pp. 82-83 & 165-185.

Yurko, L.A. (1997). Effect of *Azospirillum brasilense* on the yield and chemical composition of *Festuca pratensis* on a dernopodzolic loamy soil. In: Soil Research and the Use of Fertilizers. Fleet Publishers, Ontario, Canada. pp. 176-181 & 207.

التغذية النتروجينية للمسطح الأخضر "كيكويو" بإستخدام الأسمدة الكيماويـــة والحيوية

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أجـــرى هذا البحث فى مشـــتل التجارب بقسم بساتين الزينة، كليــــة الزراعـــة، جامعـــة القاهرة، خلال الموسمين المتتاليين ٢٠٠٠/٢٠٠١ و ٢٠٠١/٢٠٠٢، بـــهـــدف دراسة إســـتجـــابة مسطحات الكيكويـــو (Kikuyugrass, *Pennisetum clandestinum* Hochst. ex Chiov.) لمصدرين من النتروجين (التسميد الكيماوى التقليدى، و التســـميد الحيـــوى)، و تحديـــد إمكانية إستخدام الأسمدة الحيوية لتقليل الحاجة للتسميد النتروجينى الكيماوى. حيث لقحت نباتـــــات الكيكويو قبل و بعد الزراعة بالســـيريالين (منتـــج تجـــارى يحتـــوى علـــى بكتريـــا *Bacillus polymyxa* و *Azotobacter chroococcum*) فقط، أو سمدت بعـــد الزراعـــة بإســـتخدام نترات النشادر بمعدلات ٢، ٣ أو ٤ جم نتروجين/م٢/ شهر (مع التلقيح بالســـيريالين أو بدونـــه). بالإضافة إلى نباتات غير مسمدة للمقارنة (كنترول).

أدت جميع المعاملات المستخدمة إلى زيادة كثافة المسطح، و إرتفاع النباتات قبل القص، و الأوزان الطازجة و الجافة لناتج القص و للأجزاء الأرضية لكل متر٢، و كذلك محتـوى الأوراق من الصـــبغات (الكلوروفيلات الكلية و الكاروتينويدات) و الكربوهيدرات الكليـــة و النتروجيـــن والفوسفور و البوتاسيوم. و بصفة عامة فإن زيادة معدلات التسميد النتروجينى الكيماوى أدت إلـــى زيادة طردية فى القيم المسجلة لمعظم هذه الصفات، و كانت هذه الزيادة أكثر وضوحاً عند الجمـــع ما بين التســـميد النتروجينى الكيماوى و إستخدام السيريالين. و فـــى كـــل مـــن الموســـمين أدى إســـتخدام السيريالين + التســـميد الكيماوى بمعدل ٣ أو ٤ جم نتروجين/ م٢/ شهر إلى أعلى القيـــم لمعظم صفات النمو الخضرى و المكونات الكيميائية المختلفة، فى حين كان التلقيح بالسيريالين فقط هو أقل المعاملات فعالية فى معظم الصفات.

فى كثير من الأحيان أظهرت النتائج المسجلة على بعض صفات النمو الخضـــوى (الأوزان الطازجة و الجافة لناتج القص) أن الجمع ما بين إســـتخدام الســـيريالين و التســـميد النـــتروجينى الكيماوى أدى إلى التقليل من إحتياجات التسميد النتروجينى الكيماوى بمقدار ٢٥-٣٣% تقريبـــاً.
و يمكن إستنتاج أن تلقيح غزل الـــ *Pennisetum clandestinum* بالسيريالين ، مع التسميد النتروجينى الكيماوى بعد الزراعة بمعدل ٣ جم نتروجين/م٢/شهر كان كافيا للحفاظ علـــى جـــودة عالية لنمو المجموع الخضرى للمسطح، و كذلك صفات جيدة نسبياً للأجزاء الأرضية و المكونـــات الكيميائية.