

INFLUENCE OF MINERAL AND BIOFERTILIZER ON FORAGE YIELD AND QUALITY TRAITS OF TEOSINTE

Ibrahim, Hoda I. M.¹; B.A.A.kandil² and N.M.Hamed¹

1- Forage Crops Research Department, FCRI, ARC, Giza, Egypt.

2-Agric. Res. Microbiol. Dept., Soils, Water and Envir. Res. Inst., Agric. Res. Center (ARC),Egypt

ABSTRACT

Two field experiments were conducted at two locations; New Valley and Giza Agric. Res. Stations, ARC during the two successive summer seasons of 2007 and 2008 to study the influences of inoculation with Cyanobacteria, *Azospirillum* and *Pseudomonas* individually or in combination on forage yield and quality traits of teosinte (local variety). The experimental design was a randomized complete block with three replications. Combined data over the two seasons at two locations indicated that biofertilizers inoculation significantly increased fresh and dry yields, plant height, crude protein, crude fiber, ash%, ether extract and nitrogen free extract and total digestible nutrient comparing to uninoculated plants through three cuts. Also, data conformed the superiority of inoculation with mixture of inoculants in achieving higher values of all forage yield teosinte characters. Teosinte plants inoculated with Cyanobacteria, *Azospirillum* and *Pseudomonas* gave significant increases in fresh forage yield by about 149 and 140% as compared with the uninoculated plants receiving no nitrogen fertilizer at New Valley and Giza location, respectively.

It is concluded that teosinte inoculated with biofertilizers in dual or in mixture and received the half dose of nitrogen fertilizer gave results near or similar those obtained from uninoculated teosinte and received the recommended dose (120kgNfed⁻¹) of nitrogen fertilizer.

Keywords: Teosinte, *Zea mays* spp. *mexicana*, *Euchlaena mexicana* , Biofertilizers, Plant Growth Promoting Rhizobacteria , Cyanobacteria, *Azospirillum* sp., *Pseudomonas* sp., N₂-fixing , Diazotrophs .

INTRODUCTION

Forage grasses, i.e. sorghum, millet and teosinte are considered to be the most important summer forage crops in Egypt because fresh fodder during summer is of a limited supply. Therefore, great efforts have been directed towards the improvement of summer forage crops.

In Egypt summer forage crops are planted to provide supplementary forage for animals as pasture, silage or green crop. The forage grasses play an important role in plant management of many livestock producers. Among these summer forage grasses are sorghum, pearl millet, maize (Darawa) and teosinte. Nowadays, growing forage sorghum is limited to the resistant varieties to downy mildew disease.

So, many efforts are needed to increase the productivity of the other forage grasses such as teosinte (*Zea mays* ssp. *Mexicana* (Schrader) Iltis or *Euchlaena mexicana* Schrad.) for animal feeding. Panikar (1951) in India recorded that 4.46 percent crude protein, 32.2 percent crude fiber, 10.8 percent ash, 1.2 percent ether extract and 51.34 percent nitrogen free extract for *Euchlaena mexicana* on dry matter basis. However, its Australian analysis

was 7.27 percent crude protein, 27.67 percent crude fiber, 7.03 percent ash, 1.39 percent ether extract and 53.75 percent nitrogen free extract.

Teosinte (*Euchlaena mexicana*) was recently expanded as a summer forage crop in Egypt. It recovers quickly after grazing or clipping and produces highly palatable forage. It is closely related to maize in most allelometric characters. It has also the advantages of tillering and regeneration as a fodder crop under irrigated conditions in addition to its high level of tolerance to excess moisture (Lal *et al.*, 1980) which makes this crop more adaptable to the humid tropics and sub-tropics (Whyte *et al.*, 1975). For forage crops, nitrogen fertilization could determine both the productivity and quality of the herbage.

Gheit *et al.*(1995) indicated that plant height, fresh and dry forage yields as well as crude protein of sorghum hybrid were significantly increased with increasing nitrogen level up to 90Kg N/fed. Gheit (2000) also revealed that plant height, fresh and dry forage yield of teosinte were significantly affected by nitrogen fertilization .He added that 40 kg N/fed/cut gave the highest fresh and dry forage yields (30.363-4.458 ton/fed, respectively). Crude protein content was increased gradually with increasing nitrogen level up to 40 kg N/fed/cut.

The intensive use of nitrogen fertilizers and their cost have comprised expensive charges for the agricultural products, particularly in the developing countries (El-Kholi, 1998). Thus, various alternatives were put forward to account for the benefits of biofertilizers in general and Cyanobacteria, *Azospirillum* and *Pseudomonas* inoculation in particular. Biofertilizers are considered as the most important factor in reducing the application of chemical nitrogen fertilizers and minimizing the induced environmental pollution, such as those resulted from nitrogen losses (volatilized NH₃ and /or leached NO₃⁻). Hence, an increasing attention is being paid to biological N₂-fixation e.g., *Azotobacter* and /or *Azospirillum* inoculated to meet the N requirements and improve the soil fertility status to sustain crop yield (George *et al.*, 1992; Senaratne and Ratnasinghe, 1995 and Wu-Feibo & Omar, 1998). Increased yield response of crops have been observed following seed inoculation with each of N₂-fixing bacteria, i.e., *Azotobacter* and /or *Azospirillum* (Omar *et al.*, 1991; Abbass *et al.*, 1994; Soliman *et al.*, 1995; El-Hawary *et al.*, 1998; Rashid *et al.*, 1998 and Tantawey,2001).

Biological N₂-fixation (BNF) by the diazotrophs is a spontaneous process where soil N is limited and adequate C sources are available. A range of diazotrophs plant growth promoting rhizobacteria (PGPR) participates in interactions with most cereal plants increasing their vegetative growth and grain yield. Several investigators showed that inoculation with N₂-fixers have a potential importance to improve growth and increase yield productivity of cereal crops not only due to high N₂-fixation activity, but also due to plant growth promotion by production of auxins, cytokinins, gibberlins, and ethylene, siderophore aiding plant nutrition by chelation P-solubilization, increased nutrient uptake, enhanced stress resistance, vitamin production and biocontrol (Kloepper, 2003). Kennedy *et al.* (2004) proposed that inoculation biofertilizers, particularly N₂-fixing bacterial diazotrophs, can help to ensure that the supply of nutrients contributing to optimized yield is

maintained. Diazotrophic PGPR may hold the key to activating these outcomes as evolutionary advantages in a situation of adequate C-substrates, but of N-deficiency, allowing their selective enrichment in the rhizosphere (Döberiner and Pedrosa, 1987).

Advanced researches have altered the interests of root microbiologists to establish more intimate association of wheat and both N₂-fixing bacteria and Cyanobacteria. The application of N₂-fixing Cyanobacteria biofertilizers in the cultivation of wetland rice has beneficial effect on growth and yield (Mule *et al.*, 1999). Reports on the effect of Cyanobacteria on growth and other crops rather than rice are, however, scarce (Gantar *et al.*, 1995 and Abd El-Rasoul *et al.*, 2003).

Cyanobacteria inoculation along with PGPRs in a multi – strains inoculation caused enhancement for growth and yield of peanut (El-Sawy *et al.*, 2006).

Comparative studies in relation to organic manuring have shown that morphologically and physiologically distinguishable types of particularly *Pseudomonas* types, have been stimulated in the rhizosphere of wheat and rye those found after incorporation of straw in the soil (Höflich, 1989).

The aim of this research work was to study the influences of inoculation with Cyanobacteria (*Anabaena* sp. & *Nostoc* sp.), *Azospirillum* sp., and *Pseudomonas* sp. as N₂-fixing and plant growth promoting rhizobacteria (PGPRs) on yield production and forage quality of teosinte (*Euchlaena mexicana*). In addition to comparing mineral N and biofertilizer treatments and their combinations

MATERIALS AND METHODS

Two field experiments were conducted at two locations; New Valley and Giza Agric. Res. Stations, ARC during the two successive summer season of 2007 and 2008 to study the influences of inoculation with Cyanobacteria, *Azospirillum* and *Pseudomonas* individually or in combination on forage yield and quality traits of teosinte (local variety) in comparison with mineral N- fertilizations and combination of mineral N and biofertilizers.

Bacterial strains:

Cyanobacteria (*Nostoc* sp. & *Anabaena* sp.), *Azospirillum* sp. and *Pseudomonas* sp. were kindly provided by biofertilizers Production Unit; Soils, Water and Environment Research Institute, ARC, Giza, Egypt. They were prepared as inoculants on suitable sterilized carriers, packed into polyethylene bag (300g per bag, each bag content is 10⁹ CFU/g for both inoculants).

Cyanobacteria and *Azospirillum* were used as N₂-fixers bacteria and producers of growth promoters (indol acetic acid, gibberellins and cytokinins) or substances which help in greater absorption of nutrients from the soil. Also, *Pseudomonas* was used as producer of growth promoters or substances which help in greater absorption and antagonism to soil borne root pathogens.

Soil used:

The main physical and chemical properties samples of two soils for the two experimental sites were shown in Table1

Table 1: Mechanical and chemical analysis in soils

Property	Giza		New Valley	
	٢٠٠٧	٢٠٠٨	٢٠٠٧	٢٠٠٨
A-Mechanical analysis				
Sand (%)	24.98	24.00	٥١,٨٠	50.90
Silt (%)	35.87	36.20	٣٤,٦٠	34.80
Clay (%)	39.15	39.80	١٣,٦٠	14.30
Texture grade	Clay loam	Clay loam	Sandy loam	Sandy loam
S.P (%)	43.60	43.30	42.30	42.00
pH	7.70	7.62	7.92	8.00
E.C (dsm ⁻¹ at 25°C)	1.08	1.02	1.92	1.96
Organic matter (%)	0.78	0.8	0.86	0.88
Soluble cations (me/l)				
Ca ⁺⁺	4.00	4.10	2.83	2.90
Mg ⁺⁺	2.91	2.82	1.76	1.75
Na ⁺	3.13	3.02	7.28	7.30
K ⁺	0.81	0.91	7.11	7.14
Soluble anions (meg/l)				
CO ₃ ⁻	---	---	---	---
HCO ₃ ⁻	3.19	3.23	3.12	3.17
Cl ⁻	4.22	4.20	4.96	5.10
SO ₄ ⁻⁻	3.44	3.42	10.90	10.82
Total soluble – N (ppm)	45.17	45.25	67.30	68.0
Available – P (ppm)	13.22	14.00	12.40	12.45
DTPA-extractable (ppm)				
Fe	5.60	5.45	2.18	2.21
Mn	3.13	3.00	0.66	0.70
Zn	1.31	1.34	0.89	0.91
Cu	0.79	0.81	0.41	0.38

The following 17 treatments were conducted:

- 1- Un inoculated without nitrogen fertilizer.
- 2- Un inoculated with 50% nitrogen fertilizer (60 kg N fed⁻¹).
- 3- Un inoculated with 100% nitrogen fertilizer (120 kg N fed⁻¹).
- 4- Inoculated with Cyanobacteria.
- 5- Inoculated with *Azospirillum* sp.
- 6- Inoculated with *Pseudomonas* sp.
- 7- Inoculated with Cyanobacteria + *Azospirillum* sp.
- 8- Inoculated with Cyanobacteria + *Pseudomonas* sp.
- 9- Inoculated with *Azospirillum* sp. + *Pseudomonas* sp.
- 10- Inoculated with Cyanobacteria + *Azospirillum* sp+ *Pseudomonas* sp.
- 11- Inoculated with Cyanobacteria + 50% nitrogen fertilizer.
- 12- Inoculated with *Azospirillum* sp. + 50% nitrogen fertilizer.
- 13- Inoculated with *Pseudomonas* sp. + 50% nitrogen fertilizer.
- 14- Inoculated with Cyanobacteria + *Azospirillum* sp. + 50% nitrogen fertilizer.

- 15- Inoculated with Cyanobacteria + *Pseudomonas* sp. + 50% nitrogen fertilizer.
- 16- Inoculation with *Azospirillum* sp. + *Pseudomonas* sp. + 50% nitrogen fertilizer.
- 17- Inoculated with Cyanobacteria + *Azospirillum* sp. + *Pseudomonas* sp. + 50% nitrogen fertilizer.

Teosinte grains were inoculated with gamma irradiated vermiculite-based inoculants of *Azospirillum* and *Pseudomonas* at the rate of 400g / 20kg grains using Arabic gum solution (16%) as a sticking agent. Cyanobacteria inoculation was carried out at teosinte by broad casting 10kg of soil-based inoculums fed^{-1} over teosinte seeds before covering.

The experiments were sown on the second week of May at both locations and in each growing season. The experimental design was a randomized complete block with three replications and plot size was 12m² consisted of five ridges with 60 cm wide and 4m long. grains were planted in hills 20cm apart with 20kg fed^{-1} seeding rate. The plot unit received 22.5 kg P₂O₅ fed^{-1} at soil preparation. All plots received nitrogen fertilizer at rates of 60 kg N fed^{-1} and 120 kg N fed^{-1} in form of urea (46.5%N). Also, all plots received 25kg K₂O fed^{-1} . The nitrogen and potassium fertilizers were added at three equal doses. The first dose was added after 21 days from sowing, the second and the third doses were added after the first and the second cuts, respectively. Three cuts were taken during each growing season in both locations. The first cut was taken after sixty days from sowing and, the other two cuts were taken subsequently every thirty days.

The studied characters were plant height (cm), fresh and dry forage yields (ton fed^{-1}) Chemical analysis of forage yield was done on dry matter basis (%) at the three cuts for both seasons in both locations to determine crude protein (CP %), crude fiber (CF %), ash%, ether extract (EE %) and nitrogen free extract (NFE %) according to A.O.A.C (1980). Total digestible nutrient (TDN %) was estimated according to prediction equation for grasses (Adams *et al.*, 1964) as: $\text{TDN} = 50.41 + 1.04 \text{ CP} - 0.07 \text{ CF}$. Soil characters were determined according to Page *et al.* (1982).

Data were statistically analyzed according to procedures outlined by Snedecor and Cochran (1980) using MSTAT computer program V.4 (1986). Bartlett's test was done to test the homogeneity of error variances. The test was non significant for all traits, thus combined analysis was carried out for all studied traits.

RESULTS

Results of plant height in local teosinte variety are presented in Table 2. Data showed that the mean second cut was significantly higher than other cuts (88.09, 112.20 and 89.13 cm) at the New Valley and (91.98, 118.55 and 96.82 cm) at the Giza for first, second and third cuts as average of cuts, respectively. With regard to biofertilization, data in Table 2 revealed that Cyanobacteria, *Azospirillum* and *Pseudomonas* individually, dual and in

combinations inoculation treatments showed significant increases in the plant height of each cut and the average over three cuts. Cyanobacteria inoculation increased plant height of teosinte 28 and 24% as compared with the control receiving no fertilizer at New Valley and Giza, respectively. The increases reached to 31 and 26% when the seed plants were inoculated with *Pseudomonas* sp compared with control. On the other hand, the mixtures inoculation (Cyanobacteria, *Azospirillum* and *Pseudomonas*) increased plant height of teosinte by about 65 and 52% compared with the control at New Valley and Giza, respectively. Regarding the interaction between nitrogen fertilizer levels and biofertilizer treatments, the data in Table 2 showed clearly that the mixtures inoculation when combined with 60kg N fed⁻¹ increased plant height by 51 and 49% as compared with the plants received the nitrogen rate, i.e., 60kg N fed⁻¹, while the increases reached to 8 and 9% as compared with the plants received the recommended nitrogen rate (120kg N fed⁻¹) at two locations.

Table 2: Plant height (cm) of teosinte at two locations over the two seasons.

Character Treatments	Plant height (cm)							
	New Valley				Giza			
	Cut1	Cut2	Cut3	Mean	Cut1	Cut2	Cut3	Mean
Control (without N)	70.00	75.33	62.67	69.33	72.67	87.33	74.33	78.11
N 50% (60 kg N fed ⁻¹)	74.67	91.00	71.00	78.89	78.33	96.00	80.00	84.78
N 100% (120 kg N fed ⁻¹)	104.33	120.33	107.33	110.66	110.67	122.50	112.50	115.22
Cyanobacteria	75.33	114.33	77.00	88.89	80.33	120.68	89.00	96.67
<i>Azospirillum</i> sp.	72.67	94.33	71.00	79.33	78.00	101.00	80.67	86.56
<i>Pseudomonas</i> sp.	79.33	112.00	81.00	90.78	83.67	120.33	91.33	98.44
Cyano.+Azo. sp.	76.67	115.33	80.33	90.78	79.00	120.50	84.00	94.50
Cyano.+ Pseu. sp.	87.00	120.00	90.00	99.00	91.67	125.33	95.00	104.00
Azo. sp.+ Pseu. sp.	80.67	113.33	90.67	94.89	85.33	121.00	100.00	102.11
Cyano.+ Azo. sp. +Pseu. sp.	111.67	124.33	107.67	114.56	112.33	128.33	115.50	118.72
Cyano.+ 50%N	86.00	116.67	90.33	97.67	90.33	120.00	96.67	102.33
Azo. sp.+50%N	81.67	101.67	83.00	88.78	86.33	110.00	90.50	95.61
Pseu. sp.+50%N	86.67	115.00	88.33	96.67	87.67	117.00	90.00	98.22
Cyano.+Azo. sp.+50%N	93.67	120.00	96.50	103.39	98.50	127.00	101.00	108.83
Cyano.+Pseu.sp.+50%N	95.67	124.00	100.50	106.72	98.67	132.00	108.50	113.06
Azo. sp.+Pseu. sp.+50%N	104.50	121.00	106.50	110.67	109.67	130.00	116.00	118.56
Cyano. + Azo. sp. + Pseu. sp.+50%N	117.00	128.67	111.33	119.00	120.50	136.33	121.00	125.94
LSD 0.05	5.24	6.04	7.51	4.44	7.90	14.31	10.27	6.68
Mean	88.09	112.20	89.13	96.47	91.98	118.55	96.82	102.45

Fresh forage yield of the tested treatments varied significantly for individual cuttings as well as total fresh forage yield. The results in Table 3 showed that significant differences between treatments in the two locations. Regarding the comparison among cuts; second cut produced the highest fresh yield. Averaged over all treatments, forage yield was 5.79, 7.83 and 5.32 ton fed⁻¹ at New Valley and 6.92, 8.70 and 7.27 ton fed⁻¹ at Giza for the first, second and third cuts, respectively. It is clear from the data presented in Table 3 that the mixture inoculation (Cyanobacteria, *Azospirillum* and *Pseudomonas*) produced the highest fresh forage yield of teosinte by about

149 and 140% as compared with the control at New Valley and Giza, respectively. Regarding the interaction between nitrogen fertilizer levels and biofertilizer treatments, the data in Table 3 showed clearly that the mixtures inoculation when combined with 60kgNfed⁻¹ increased forage yield by 6 and 4% as compared with the plants received the recommended nitrogen rate, i.e., 120kg Nfed⁻¹ at two locations.

Table 3: Fresh forage yield (ton fed⁻¹) of teosinte at two locations over the two seasons.

Character	Fresh yield (ton fed ⁻¹)							
	New Valley				Giza			
	Cut1	Cut2	Cut3	Total	Cut1	Cut2	Cut3	Total
Treatments								
Control (without N)	3,90	3,47	2,70	10.07	4,22	4,88	3,38	12.48
N 50% (60 kg N fed ⁻¹)	4,33	6,00	3,70	14.03	0,26	6,32	0,18	16.76
N 100% (120 kg N fed ⁻¹)	7,30	11,00	6,90	25.70	8,40	12,19	10,82	32.46
Cyanobacteria	4,47	6,87	4,37	15.71	0,66	6,04	0,49	17.69
<i>Azospirillum</i> sp.	4,30	6,00	3,77	14.07	0,07	6,92	4,74	16.73
<i>Pseudomonas</i> sp.	4,87	6,97	0,10	16.94	0,68	7,01	6,60	19.79
Cyano. + Azo. sp.	4,00	7,03	4,87	16.40	0,00	6,83	0,92	18.30
Cyano. + Pseu. sp.	0,23	7,20	0,47	17.90	6,89	7,88	6,07	20.84
Azo. sp. + Pseu. sp.	4,90	7,03	6,00	17.93	6,40	7,27	6,88	20.60
Cyano.+ Azo. sp. +Pseu. sp.	7,70	10,63	6,60	24.93	8,73	11,27	9,94	29.94
Cyano.+ 50%N	6,27	7,80	0,83	19.90	8,04	9,30	8,30	25.69
Azo. sp.+ 50%N	0,00	6,27	4,37	16.14	6,33	6,39	0,90	18.67
Pseu. sp.+ 50%N	6,10	7,13	0,27	18.50	7,72	7,90	8,07	23.74
Cyano.+Azo. sp.+ 50%N	6,67	7,30	0,03	19.50	7,97	9,38	7,90	25.30
Cyano.+Pseu.sp.+ 50%N	6,90	10,37	7,07	24.34	7,93	11,27	9,04	28.92
Azo. sp.+Pseu. sp.+ 50%N	6,97	9,77	6,07	22.81	8,07	9,80	9,18	27.55
Cyano.+ Azo. sp. + Pseu. sp.+ 50%N	8,47	11,73	6,87	27.07	9,18	14,46	10,04	33.68
LSD 0.05	0,33	0,40	0,00	0,83	0,83	1,40	0,66	1,82
Mean	5.79	7.83	5.32	18.84	6.92	8.70	7.27	22.89

Results presented in Table 4 indicated that significant differences between treatments on dry forage yield at all cuts and their total in two locations. Generally, dry forage yield exhibited similar trend as fresh forage yield .It is clear from the data presented in Table 4 that the mixtures inoculation (*Cyanobacteria* , *Azospirillum* and *Pseudomonas*) produced the highest dry forage yield of teosinte by about 155 and 178 % as compared with the control at New Valley and Giza, respectively for total dry yield. Regarding the interaction between nitrogen fertilizer levels and biofertilizer treatments, the data in Table 4 showed clearly that the mixtures inoculation when combined with 60kgNfed⁻¹ increased the dry forage yield by 7 and 6 % as compared with the plants received the recommended nitrogen rate, i.e., 120kgNfed⁻¹ at two locations.

Table 4: Dry forage yield (ton fed⁻¹) of teosinte at two locations over the two seasons.

Character	Dry yield (ton fed ⁻¹)							
	New Valley				Giza			
	Cut1	Cut2	Cut3	Total	Cut1	Cut2	Cut3	Total
Treatments								
Control (without N)	0.74	0.74	0.70	1.88	0.08	0.80	0.32	2.02
N 50% (60 kg N fed ⁻¹)	0.71	1.13	0.79	2.63	0.80	1.13	1.00	3.03
N 100% (120 kg N fed ⁻¹)	1.04	2.09	1.46	4.59	1.21	2.26	2.19	5.66
Cyanobacteria	0.79	1.18	0.89	2.76	0.88	1.06	1.06	3.01
<i>Azospirillum</i> sp.	0.77	1.27	0.84	2.78	0.79	1.40	1.01	3.20
<i>Pseudomonas</i> sp.	0.72	1.30	1.06	3.03	0.72	1.36	1.31	3.41
Cyano. + Azo. sp.	0.81	1.06	1.09	3.46	1.00	1.44	1.26	3.70
Cyano. + <i>Pseu.</i> sp.	0.76	1.08	1.21	3.45	0.86	1.60	1.28	3.74
Azo. sp. + <i>Pseu.</i> sp.	0.81	1.40	1.33	3.59	1.06	1.42	1.40	3.94
Cyano. + Azo. sp. + <i>Pseu.</i> sp.	1.24	2.17	1.41	4.82	1.41	2.18	2.02	5.61
Cyano.+ 50%N	0.86	1.60	1.37	3.83	1.10	1.81	1.88	4.79
Azo. sp.+ 50%N	0.89	1.33	0.98	3.20	1.02	1.30	1.27	3.60
<i>Pseu.</i> sp.+ 50%N	0.80	1.30	1.08	3.23	1.02	1.42	1.07	3.51
Cyano.+Azo. sp.+ 50%N	1.00	1.23	1.20	3.53	1.20	1.48	1.72	4.40
Cyano.+ <i>Pseu.</i> sp.+ 50%N	1.06	2.13	1.69	4.88	1.22	2.30	2.07	5.64
Azo. sp.+ <i>Pseu.</i> sp.+ 50%N	1.01	1.78	1.39	4.18	1.24	1.68	2.00	4.93
Cyano.+ Azo. sp. + <i>Pseu.</i> sp.+ 50%N	1.19	2.26	1.46	4.91	1.29	2.60	2.03	5.93
LSD 0.05	0.07	0.13	0.12	0.19	0.14	0.22	0.10	0.29
Mean	0.87	1.54	1.17	3.57	1.03	1.67	1.50	4.16

Results of crude protein (CP %), crude fiber (CF %), ash%, ether extract (EE %) and nitrogen free extract (NFE %) in teosinte local variety are presented in Tables (5, 6, 7, 8 and 9). Among the tested treatments the statistical analysis indicated the presence of significant differences regarding the CP%, CF%, EE% and NFE%. Concerning the comparison of different cuts, the first cut gave the highest CP% followed by the third and the second cut in Table 5 at the two locations (10.15, 7.72 and 7.32%) at the New Valley (12.21, 10.70 and 10.53%) at the Giza for first, third and second cut as an average of cuts, respectively. Generally, treatment (Cyano. + Azo. Sp. + *Pseu.* sp. +50%N) was superior in crude protein percentage to the other treatments at the first cut (11.53%). Meanwhile, treatment (N100%) gave (9.17%) at the New Valley, while treatment of (Azo. sp. + 50%N) gave the highest CP% (13.67%) compared to (10.27%) from the plants received the recommended dose of nitrogen fertilizer at Giza.

With regard to crude fiber percentage, data in Table 6 revealed that the second cut gave the highest crude fiber percentage followed by the third cut and the first cut at two locations (29.71, 32.35 and 30.92%) at the New Valley (28.44, 31.39 and 29.70%) at the Giza for first, second and third cut as average of cuts, respectively. Treatment (Cyano. + Azo. sp. +50%N) recorded the highest crude fiber content in the first cut (31.20%), in the second cut (33.10%) and third cut (32.30%) at New Valley. Treatment (Cyano. + Azo. sp.) had the highest crude fiber content in the first cut (29.70%) and treatment (*Pseu.* sp. + 50%N) had the highest crude fiber content in the second cut (34.13%) and third cut (32.37%) at Giza.

Table 5: Crude protein percentage of teosinte at two locations over the two seasons.

Character Treatments	Crude protein (%)							
	New Valley				Giza			
	Cut1	Cut2	Cut3	Mean	Cut1	Cut2	Cut3	Mean
Control (without N)	7.43	8.97	8.80	6.40	8.27	7.87	7.30	7.10
N 50% (60 kg N fed ⁻¹)	8.23	7.53	7.23	7.03	9.07	8.00	7.60	8.22
N 100% (120 kg N fed ⁻¹)	9.17	7.20	7.70	7.69	10.27	8.20	7.37	8.78
Cyanobacteria	10.70	7.83	8.70	9.08	13.27	9.77	10.20	11.10
<i>Azospirillum</i> sp.	10.60	7.80	8.73	9.02	13.60	12.23	12.03	12.77
<i>Pseudomonas</i> sp.	11.37	9.03	9.20	9.93	12.10	10.93	12.23	11.70
Cyano. + Azo. sp.	10.23	7.83	8.23	8.83	13.27	11.03	12.00	12.30
Cyano. + Pseu. sp.	10.87	7.20	8.23	8.83	12.20	10.93	12.07	11.73
Azo. sp. + Pseu. sp.	11.27	8.20	7.93	9.20	12.60	8.23	7.90	9.12
Cyano.+ Azo. sp. +Pseu. sp.	11.27	7.20	8.27	8.98	13.20	11.07	12.03	12.27
Cyano.+ 50%N	10.23	7.03	7.13	8.13	13.33	11.83	12.83	12.66
Azo. sp.+ 50%N	9.60	7.03	7.93	7.80	13.77	11.90	12.73	12.77
Pseu. sp.+ 50%N	10.23	7.87	7.83	8.31	12.33	11.00	10.20	11.30
Cyano.+Azo. sp.+ 50%N	9.07	7.03	7.87	7.99	13.17	12.00	12.07	12.41
Cyano.+Pseu.sp.+ 50%N	10.23	7.37	7.37	8.32	13.27	12.20	12.10	12.06
Azo. sp.+Pseu. sp.+ 50%N	9.77	7.20	7.20	8.12	10.73	8.03	8.63	9.30
Cyano.+ Azo. sp. + Pseu. sp.+ 50%	11.03	7.27	8.17	9.06	13.10	12.27	12.73	12.77
LSD 0.05	0.22	0.20	0.20	0.16	0.17	0.16	0.28	0.10
Mean	10.10	7.32	7.72	8.20	12.21	10.03	10.70	11.10

Table 6: Crude fiber percentage of teosinte at two locations over two seasons.

Character Treatments	Crude fiber (%)							
	New Valley				Giza			
	Cut1	Cut2	Cut3	Mean	Cut1	Cut2	Cut3	Mean
Control (without N)	31.70	34.70	32.70	33.00	31.60	32.67	31.90	32.06
N 50% (60 kg N fed ⁻¹)	30.60	32.10	32.00	32.23	29.90	29.40	29.80	29.70
N 100% (120 kg N fed ⁻¹)	30.00	32.30	30.30	31.37	28.50	31.30	28.80	29.53
Cyanobacteria	29.17	32.20	29.80	30.46	27.40	31.50	31.40	30.10
<i>Azospirillum</i> sp.	29.13	32.00	30.10	30.58	29.30	31.60	29.70	30.20
<i>Pseudomonas</i> sp.	28.70	30.70	30.00	29.80	28.50	32.50	29.30	30.10
Cyano. + Azo. sp.	29.10	31.80	30.20	30.37	29.70	30.40	30.30	30.13
Cyano. + Pseu. sp.	29.27	31.60	30.03	30.03	27.50	33.63	32.07	31.07
Azo. sp. + Pseu. sp.	28.80	31.37	29.77	29.98	28.30	31.87	29.77	29.98
Cyano.+ Azo. sp. +Pseu. sp.	28.70	31.70	30.90	30.23	27.50	28.73	26.03	27.42
Cyano.+ 50%N	29.60	31.80	30.10	30.00	28.80	31.00	26.80	28.87
Azo. sp.+ 50%N	30.10	32.60	31.60	31.23	29.40	30.90	27.40	29.23
Pseu. sp.+ 50%N	30.10	32.10	30.93	31.28	27.03	34.13	32.37	31.18
Cyano.+Azo. sp.+ 50%N	31.20	32.10	32.30	32.20	28.50	32.40	30.10	30.33
Cyano.+Pseu.sp.+ 50%N	29.20	31.20	32.00	30.93	26.40	30.40	30.50	29.10
Azo. sp.+Pseu. sp.+ 50%N	30.03	32.10	31.60	31.22	27.63	31.70	31.70	30.34
Cyano.+ Azo. sp. + Pseu. sp.+ 50%	28.70	31.70	30.90	30.23	27.60	29.50	27.03	28.04
LSD 0.05	0.16	0.12	0.12	0.09	0.09	0.21	0.16	0.11
Mean	29.71	32.35	30.92	30.99	28.44	31.39	29.70	29.85

Concerning the ash percentage, the data presented in Table 7 showed that the first cut gave the highest ash content followed by the third and the second cuts in the two location (10.00, 8.90 and 9.32%) at the New Valley and (11.06, 9.13and 9.24%) at the Giza for first, second and third cut

as an average of cuts, respectively. Treatment (Cyano. + Azo. sp.) had the highest ash content in the first cut (10.47%), while treatment (Cyano. + 50%N) gave the highest ash content in the second cut (9.87%) while treatment (Cyano. +Pseu. sp. +50%N) recorded the highest ash content in the third cut (10.53%) at New Valley. Treatment (Azo. sp. + Pseu. sp.+50%N) had the highest ash content in the first cut (13.43%), while treatment (Azo. sp. + Cyano. + Pseu. sp.) gave the highest ash content in the second cut (11.20%) while treatment (Azo. sp.+ Pseu. sp.) recorded the highest ash content in the third cut (10.27%) at Giza.

Table 7: Ash percentage of teosinte at two locations over the two seasons.

Character	Ash (%)							
	New Valley				Giza			
	Cut1	Cut2	Cut3	Mean	Cut1	Cut2	Cut3	Mean
Treatments								
Control (without N)	10.67	8.80	9.63	9.70	11.97	10.23	11.23	11.14
N 50% (60 kg N fed ⁻¹)	10.90	8.47	9.00	9.46	12.23	10.80	11.23	11.42
N 100% (120 kg N fed ⁻¹)	11.10	9.00	9.73	10.11	11.80	9.72	10.17	10.56
Cyanobacteria	9.97	9.37	8.23	9.19	11.03	7.53	8.77	9.11
<i>Azospirillum</i> sp.	10.00	9.10	8.10	9.07	8.93	7.67	8.37	8.32
<i>Pseudomonas</i> sp.	9.73	8.63	9.20	9.19	11.20	8.37	8.97	9.51
Cyano. + Azo. sp.	10.47	8.97	9.13	9.52	8.77	7.80	7.63	8.07
Cyano. + Pseu. sp.	9.00	9.27	9.30	9.36	12.10	8.60	9.00	9.90
Azo. sp. + Pseu. sp.	9.73	8.87	10.00	9.53	10.93	10.43	10.27	10.54
Cyano.+ Azo. sp. +Pseu. sp.	9.63	8.90	9.23	9.29	11.03	11.20	10.10	10.78
Cyano.+ 50%N	9.97	9.87	10.37	10.07	9.73	9.63	9.17	9.51
Azo. sp.+ 50%N	10.13	9.40	8.77	9.43	8.73	9.30	8.73	8.92
Pseu. sp.+ 50%N	9.47	8.23	9.40	9.03	12.63	8.43	8.63	9.90
Cyano.+Azo. sp.+ 50%N	9.03	8.17	8.37	8.52	10.18	8.00	8.57	8.92
Cyano.+Pseu.sp.+ 50%N	10.17	8.63	10.03	9.78	12.10	8.57	8.83	9.83
Azo. sp.+Pseu. sp.+ 50%N	10.00	8.03	9.80	9.44	13.43	9.00	9.63	10.69
Cyano.+ Azo. sp. + Pseu. sp.+ 50%N	9.07	8.63	9.47	9.22	11.20	9.93	7.80	9.64
LSD 0.05	0.37	0.42	0.17	0.16	0.17	0.78	0.47	0.29
Mean	10.00	8.90	9.32	9.41	11.06	9.13	9.24	9.81

Concerning the ether extract (EE %), the data presented in Table 8 showed that the first cut had the highest value followed by the third and the second cuts in the two locations (1.97, 1.25 and 1.81%) at the New Valley and (2.06, 1.88 and 1.92%) at Giza for first, second and third cut as an average of cuts, respectively. Treatment (*Azospirillum* sp.) gave the highest EE% in the first cut (2.23%) at the New Valley while treatment (Cyano. + Pseu. sp.) had the highest value (3.03%) at Giza.

With regard to nitrogen free extract, data in Table 9 revealed that third cut gave the highest nitrogen free extract followed by the second cut and the first cut in the two locations (48.17, 49.62 and 50.79%) at the New Valley and (46.22, 47.07 and 48.43%) at Giza for first, second and third cut as an average of cuts, respectively. Treatment (Azo. sp. + Pseu. sp.) gave the highest NFE% in the first cut (48.50%) at the New Valley while treatment (*Azospirillum* sp.) had the highest value (46.80%) at Giza.

Table 8: Ether extract percentage of teosinte at two locations over the two seasons.

Character	Ether extract (%)							
	New Valley				Giza			
	Cut1	Cut2	Cut3	Mean	Cut1	Cut2	Cut3	Mean
Treatments								
Control (without N)	1.77	1.33	1.33	1.61	1.97	1.50	1.67	1.71
N 50% (60 kg N fed ⁻¹)	2.03	1.13	1.87	1.68	1.30	1.30	1.33	1.31
N 100% (120 kg N fed ⁻¹)	2.07	1.33	1.33	1.71	1.87	1.70	1.57	1.71
Cyanobacteria	1.97	1.23	1.33	1.66	1.47	1.30	1.30	1.36
<i>Azospirillum</i> sp.	2.23	1.10	1.33	1.67	1.37	1.60	1.23	1.40
<i>Pseudomonas</i> sp.	2.03	1.23	1.33	1.72	2.23	2.10	2.13	2.15
Cyano. + Azo. sp.	1.93	1.33	1.33	1.69	2.13	1.80	1.83	1.92
Cyano. + <i>Pseu.</i> sp.	1.73	1.33	1.33	1.64	3.03	2.40	2.60	2.68
Azo. sp. + <i>Pseu.</i> sp.	1.70	1.33	1.33	1.65	2.77	2.10	2.37	2.41
Cyano.+ Azo. sp.+ <i>Pseu.</i> sp.	2.03	1.23	1.33	1.74	2.43	2.30	2.40	2.38
Cyano.+ 50%N	2.03	1.13	1.33	1.64	2.20	2.17	2.07	2.15
Azo. sp.+ 50%N	1.97	1.13	1.33	1.67	1.87	1.80	1.93	1.87
<i>Pseu.</i> sp.+ 50%N	1.93	1.33	1.33	1.73	1.90	1.60	1.80	1.77
Cyano.+Azo. sp.+ 50%N	1.93	1.13	1.33	1.60	1.90	1.90	2.00	1.93
Cyano.+ <i>Pseu.</i> sp.+ 50%N	1.93	1.13	1.33	1.61	2.00	1.90	1.93	1.94
Azo. sp.+ <i>Pseu.</i> sp.+ 50%N	2.00	1.13	1.33	1.63	2.13	2.10	2.07	2.10
Cyano.+ Azo. sp. + <i>Pseu.</i> sp.+ 50%N	2.13	1.33	1.33	1.81	2.47	2.37	2.40	2.41
LSD 0.05	0.12	0.09	0.09	0.06	0.19	0.13	0.20	0.15
Mean	1.97	1.25	1.81	1.67	2.06	1.88	1.92	1.95

Table 9: Nitrogen free extract percentage of teosinte at two locations over the two seasons.

Character	Nitrogen free extract (%)							
	New Valley				Giza			
	Cut1	Cut2	Cut3	Mean	Cut1	Cut2	Cut3	Mean
Treatments								
Control (without N)	58.43	58.80	50.63	49.29	46.19	48.73	48.90	47.94
N 50% (60 kg N fed ⁻¹)	58.13	59.03	51.63	49.60	47.50	50.50	50.04	49.35
N 100% (120 kg N fed ⁻¹)	57.17	58.27	51.93	49.12	47.56	48.88	52.09	49.51
Cyanobacteria	58.20	58.63	52.03	49.62	46.73	50.00	48.13	48.29
<i>Azospirillum</i> sp.	58.03	58.93	51.97	49.64	46.80	46.90	48.67	47.46
<i>Pseudomonas</i> sp.	58.17	59.77	50.13	49.36	45.97	46.10	47.37	46.48
Cyano. + Azo. sp.	58.27	59.63	50.87	49.59	46.03	48.47	48.24	47.58
Cyano. + <i>Pseu.</i> sp.	58.43	50.20	50.27	49.63	45.17	44.44	44.26	44.62
Azo. sp. + <i>Pseu.</i> sp.	58.50	59.60	50.83	49.64	45.40	47.17	49.69	47.42
Cyano.+ Azo. sp. + <i>Pseu.</i> sp.	58.17	50.27	50.23	49.56	45.74	46.20	48.94	46.96
Cyano.+ 50%N	58.17	59.07	51.23	49.66	45.94	45.37	49.13	46.81
Azo. sp.+ 50%N	58.20	50.07	50.07	49.61	46.33	46.10	49.21	47.21
<i>Pseu.</i> sp.+ 50%N	58.27	58.87	51.00	49.55	46.31	44.31	46.80	45.81
Cyano.+Azo. sp.+ 50%N	58.27	50.27	50.23	49.69	46.25	45.70	47.26	46.40
Cyano.+ <i>Pseu.</i> sp.+ 50%N	58.27	50.83	58.97	49.36	46.13	46.93	46.64	46.57
Azo. sp.+ <i>Pseu.</i> sp.+ 50%N	58.20	50.20	50.07	49.56	46.08	48.67	47.97	47.57
Cyano.+ Azo. sp. + <i>Pseu.</i> sp.+ 50%N	58.07	50.23	50.13	49.48	45.63	45.73	50.04	47.13
LSD 0.05	0.22	0.23	0.23	0.15	0.19	0.82	0.53	0.33
Mean	58.17	59.62	50.79	49.53	46.22	47.07	48.43	47.24

Results of total digestible nutrient presented in Table10 showed that the first cut had the highest value followed by the third and the second cuts in the two locations (58.89,55.76 and56.28%) at the New Valley and (61.12,59.16and 59.46) at Giza for first, second and third cut as an average

of cuts, respectively. The treatment of Cyano. + Azo. sp. +Pseu. sp.+50%N had the highest values (60.39%)in the first cut ,while the inoculated plants with (*Azospirillum* sp.) had the highest value (62.50%) of TDN% of the first cut followed by (62.40%) of TDN% obtained from the plant inoculated with Cyanobacteria in the same cut.

Table 10: Total digestible nutrient of teosinte at two locations over the two seasons.

Character	Total digestible nutrient (%)							
	New Valley				Giza			
	Cut1	Cut2	Cut3	Mean	Cut1	Cut2	Cut3	Mean
Treatments								
Control (without N)	55.92	54.19	54.16	54.76	56.80	55.27	54.73	55.60
N 50% (60 kg N fed ⁻¹)	56.93	54.81	54.65	55.46	57.75	56.67	56.23	56.88
N 100% (120 kg N fed ⁻¹)	57.81	55.57	55.26	56.21	59.10	56.96	56.06	57.37
Cyanobacteria	59.50	56.29	57.37	57.72	62.40	58.26	59.03	59.90
<i>Azospirillum</i> sp.	59.39	56.25	57.38	57.67	62.50	60.92	60.84	61.42
<i>Pseudomonas</i> sp.	60.23	57.65	58.09	58.66	61.00	59.50	61.08	60.53
Cyano. + Azo. sp.	59.01	56.33	57.06	57.47	62.24	60.27	60.77	61.09
Cyano. + Pseu. sp.	59.65	55.69	57.04	57.46	61.17	59.42	60.72	60.44
Azo. sp. + Pseu. sp.	60.11	56.95	56.57	57.88	61.53	56.95	56.54	58.34
Cyano.+ Azo. sp. +Pseu. sp..	60.33	55.68	56.85	57.62	62.32	60.43	61.62	61.46
Cyano.+ 50%N	58.98	55.50	55.72	56.73	62.26	60.54	61.88	61.56
Azo. sp.+ 50%N	58.29	54.40	56.45	56.38	62.57	60.62	61.73	61.64
Pseu. sp.+ 50%N	58.94	56.28	55.35	56.86	61.13	60.01	58.96	60.03
Cyano.+Azo.sp.+ 50%N	58.18	54.88	56.33	56.46	62.11	60.62	60.86	61.20
Cyano.+Pseu.sp.+ 50%N	58.99	55.88	55.83	56.90	62.47	60.97	60.86	61.43
Azo. sp.+Pseu.sp.+ 50%N	58.47	55.65	55.89	56.67	59.64	57.06	57.17	57.96
Cyano.+ Azo. sp. + Pseu. sp.+ 50%N	60.39	55.96	56.74	57.70	62.10	61.31	61.76	61.72
LSD 0.05	0.36	0.26	0.11	0.13	0.18	0.17	0.30	0.16
Mean	58.89	55.76	56.28	56.98	61.12	59.16	59.46	59.92

DISCUSSION

Results of the current study assured the significance of biofertilization on teosinte growth and productivity. These results are in agreements with many investigators. Gantar (2000) emphasized significance of Cyanobacteria-wheat association and found that Cyanobacteria penetrated the roots in the form of motile filaments (hormogonia), at once inside, they divided and transformed into a seriate packages, which showed nitrogenase activity. Thus, co-cultivation of wheat with Cyanobacteria could partially meet the wheat nitrogen needs.

Results were almost in accordance with others concerning Cyanobacteria inoculation (Abd El-Rasoul *et al.*, 2003 and 2004; Mussa *et al.*, 2003; Hanna *et al.*, 2004 and El- Sawy 2006) regarding *Pseudomonas* (Abdel -Wahab *et al.*, 2006; Hassanein *et al.*, 2006 and Abo El-Soud *et al.*, 2007) and *Azospirillum* (Saubidet *et al.*, 2002; Meawed & Gebrael, 2002 and Abo El-Soud *et al.*, 2007).

Inoculation of local teosinte variety with (Cyanobacteria, *Azospirillum* and *Pseudomonas*) individually or in dual and in combination and improved

total plant dry weight , fresh yield, crude protein, crude fiber, ash , ether extract, nitrogen extract and total digestible nutrient.

Over the last few years, a diverse array of bacterial species including Cyanobacteria, *Azospirillum*, *Pseudomonas*, *Serratia*, *Azotobacter*, *Bacillus*, *Klebsiella* and *Anterobacter* has been shown to promote plant growth. The mechanisms by which these rhizobacteria enhance plant growth are not clear, but it is postulated that they may be associated with (a) production of secondary metabolites such as antibiotics, cyanide and hormone like substances, (b) production of siderophores (c) dinitrogen fixation, (d) increase phosphate solubilization, (e) enhance mineral uptake and/or (f) antagonism to soil borne root pathogens.

Conclusion

From the previous results of forage teosinte, it could be concluded that combination between PGPRs and N₂- fixer bacteria inoculants increased growth, forage yield and quality traits of teosinte and save about 50% of nitrogen fertilizer with decreasing hazard environmental effects that may be caused by mineral N-fertilizer.

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تأثير التسميد المعدني والحيوي على إنتاجية وجودة الذرة الريانة

- هدى إمام محمد إبراهيم^١, بلال عبد السميع احمد قنديل^٢ و ناصر محمد حامد^١
- ١ - قسم بحوث محاصيل العلف - معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية - جيزة - مصر.
- ٢ - قسم بحوث الميكروبيولوجي - معهد بحوث الأراضي والمياه والبيئة - مركز البحوث الزراعية - جيزة - مصر.

اقيمت تجربتان حقليتان في موقعين مختلفين ببنيها بمحطتى البحوث الزراعية بالوادي الجديد والجيزة خلال الموسمين الزراعيين الصيفيين ٢٠٠٧ و ٢٠٠٨ لدراسة تأثير التلقيح بالسيانوبكتريا والازوسبيريللام والسيديموناس كل بمفرده او فى ازواج او فى خليط على كمية محصول العلف الاخضر والجاف وعلى صفات الجودة فى الذرة الريانة.

وقد أشارت نتائج التحليل التجميى للموسمين فى كل من الموقعين ان التلقيح بالاسمدة الحيوية ادت الى زيادة معنوية فى محصول العلف الاخضر والجاف, طول النبات, نسبة البروتين الخام, نسبة الالياف الخام, نسبة الرماد, نسبة الدهون, نسبة الكربوهيدرات الذائبة ونسبة المواد الغذائية المهضومة الكلية.

وقد اكدت النتائج تفوق التلقيح بخليط الكائنات الدقيقة المستخدمة وذلك فى جميع الصفات المدروسة.

كما ثبتت النتائج ان نباتات الذرة الريانة الملقحة بكل من السيانوبكتريا, والازوسبيريللام, والسيديموناس اعطت زيادات معنوية فى محصول العلف الاخضر حوالى ١٤٠, ١٤٩ % مقارنة بالنباتات غير الملقحة وغير المسمدة بالازوت فى موقعى الوادي الجديد والجيزة على التوالى.

خلصت النتائج الى ان الذرة الريانة الملقحة بالاسمدة الحيوية فى ازواج او مخلوط منها وسمدت بنصف جرعة السماد الازوتى اعطت نتائج قريبة من النتائج المتحصل عليها فى حالة النباتات غير الملقحة والمسمدة بالجرعة الكاملة الموصى بها من التسميد النيتروجينى (٢٠ كجم ازوت للفدان)