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PRODUCTIVITY AND QUALITY OF FORAGE MILLET AS AFFECTED BY NITROGEN AND BIO FERTILIZATION UNDER NEW VALLEY CONDITIONS

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ABSTRACT

Two field experiments were conducted at New Valley Agric. Res. Station, ARC during the two successive summer seasons of 2012 and 2013 to study the effect of nitrogen fertilizer levels (Zero, 60, 90 and 120 kg N fed^{-1}) and inoculation with cyanobacteica and / or *Azospirillum* sp. under the two levels of inorganic nitrogen (60 and 90 kg N fed^{-1}) on growth, forage yield and quality traits of forage millet cv. Shandaweel-1 as well as economic evaluation of studied treatments. The experimental design was a randomized complete block with three replications.

Combined analysis of data over the two seasons revealed that growth parameters forage yield and quality traits were significantly affected by the full recommended nitrogen rate (120 kg N fed^{-1}). The application of cyanobacteria and *Azospirillum* sp. combined with 75% of its recommended nitrogen rate(120 kg N fed^{-1}) led to significantly increase in plant height by 2.5 %, number of tillers plant^{-1} by 11%, stem diameter by 10%, total fresh forage yield by 15% , total dry forage yield by about 8%, crude fiber yield by 7.8%, ash yield by about 20.7%, total digestible nutrient seasonal yield by7.5% and digestible crude protein seasonal yield by about 0.40% as compared with the plants received the recommended nitrogen rate (120 kg N fed^{-1}). Application of 120 kg N fed^{-1} gave crude protein yield similar to that obtained from inoculation with treatment cyanobacteria and *Azospirillum* sp combined with 75% N fertilization (90 kg N fed^{-1}).

The application of cyanobacteria and *Azospirillum* sp. combined with 75% of the recommended nitrogen rate (120 kg fed^{-1}) is the highest in return of invested L.E. which estimated at about 3.7 L.E., meaning that every pound is spent in the cultivation of millet to this the transaction back to the farmer in the pound has been spent in agriculture plus net return, which is estimated at 2.7 pounds and considered the best studied treatment.

From the above mentioned results it could be recommended that the application of cyanobacteria and *Azospirillum* sp. Combined with 75% of the recommended mineral nitrogen gave the highest productivity of forage millet and a higher net return for the farmer under New Valley conditions.

Keywords: Forage millet, *Pennisetum glaucum*, Nitrogen fertilization, Plant Growth Promoting Rhizobacteria, Cyanobacteria, *Azospirillum* sp., Yield, Quality and Economic evaluation

INTRODUCTION

In Egypt, animals suffer from deficiency of green fodder during the summer season. So, increasing forage crop productivity per unit area during the summer season or/and increasing the cultivated area of summer forage crops especially in the newly reclaimed lands become the back bone to solve this problem. Forage millet is a summer forage crop which can be cultivated in the newly reclaimed lands to overcome this problem. To increase the forage production of forage millet, it depends on many factors as mineral nutrition, soil fertility, sowing date, cutting height, etc.

Forage millet (*Pennisetum glaucum*) is the most widely grown type of millet. Pearl millet is well adapted to production systems characterized by drought, low soil fertility, and high temperature. It performs well in soils with high salinity or low pH. Because of its tolerance to difficult growing conditions, it can be grown in areas where other cereal crops, such as maize or wheat, would not survive. It is an important forage crop of the arid and semi-arid regions of the country. It is fed to the cattle either as green or dry (Maiti and Bidinger, 1981).

Increasing nitrogen fertilization rates caused significant effect in many growth attributes of forage millet as well as forage yield such as plant height at the rate of 90 kg $Nfed^{-1}$ (Manohar *et al.*, 1991; El-Houssini and Zeinab Nasser, 1998 and Lakhana *et al*., 2005), 100 kg N ha^{-1}, (Puri and Tiwana, 2005), and 180 kg N ha^{-1} (Ayub *et al.*, 2009 and Pathan *et al.*, 2010), number of tillers at 80 kg N ha^{-1} (Verna *et al*., 2006), 90 kg N ha^{-1} (Lakhana *et al*., 2005), 100 kg N ha^{-1} (Pathan *et al.*, 2010), 180 kg N ha^{-1} (Mesquita and Pinto, 2000), and 470 kg N ha^{-1} (Jinxing *et al.*, 1998). Green forage yield of pearl millet was at rates 120 kg $Nfed^{-1}$ (Mousa, 1991), 60–75 kg $Nfed^{-1}$ (Shahin *et al*., 2013), 90–100 kg N ha^{-1} (Manohar *et al.,* 1991; Sharma *et al.,* 1999 and Tiwana *et al.,* 2003) and 180 kg/ha (Ayub *et al.,* 2009), and dry forage yield was at the rate of 60–75 kg $Nfed^{-1}$ (Shahin *et al*., 2013), 90 kg $Nfed^{-1}$ (El-Houssini and Nasser, 1998); 90–120 kg N ha^{-1} (Tiwana *et al*., 2003; Lakhana *et al*., 2005; Puri and Tiwana, 2005 and Bhilare *et al.*, 2010) and 180 kg N ha^{-1} (Ayub *et al.*, 2009).

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 inorganic nitrogen fertilizers and minimizing the induced environmental pollution, such as those resulted from nitrogen losses (volatilized NH_3 and /or leached $NO_3^{-)}$. Hence, an increasing attention is being paid to biological N_2-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 and Senaratne and Ratnasinghe, 1995). Increased yield response of crops has been observed following seed inoculation with each of N_2-fixing bacteria, i.e., *Azotobacter* and /or Azospirillum (Eman Tantawey, 2001).

The diverse groups of bacteria in close association with roots and capable of stimulating plant growth by any mechanism(s) of action are referred to as plant growth-promoting rhizobacteria (PGPR). They affect plant growth and development directly or indirectly either by releasing plant growth regulators (PGPRs) or other biologically active substances, altering endogenous levels of PGPRs, enhancing availability and uptake of nutrients through fixation and mobilization, reducing harmful effects of pathogenic microorganisms on plants and/or by employing multiple mechanisms of action. Recently, PGPR have received more attention for use as a biofertilizer for the sustainability of agro-ecosystems. Selection of efficient PGPR strains based on well-defined mechanism(s) for the formulation of biofertilizers is vital for achieving consistent and reproducible results under field conditions. Numerous studies have suggested that PGPR-based biofertilizers could be used as effective supplements to chemical fertilizers to promote crop yields on sustainable basis (Khan *et al.*, 2009). The inoculation with N_2-fixers have a potential importance to improve growth and increase yield productivity of cereal crops not only due to high N_2-fixation activity, but also due to plant growth promotion by production of auxins, cytokinins, gibberlins, and ethaylene, 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 and PGPRs 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 (Döberiener and Pedrosa, 1987).

Cyanobacteria are one of the major components of the nitrogen fixing biomass in paddy fields and provides a potential source of nitrogen fixation at no cost. Due to the important characteristic of nitrogen fixation, cyanobacteria have a unique potential to contribute to enhance productivity in a variety of agricultural and ecological situations. The blue green algae (cyanobacteria) play an important role to build-up soil fertility consequently increasing the yield. Biofertilizer being essential components of organic farming play vital role in maintaining long term soil fertility and sustainability by fixing atmospheric dinitrogen (N=N), mobilizing fixed macro and micro nutrients or convert insoluble phosphorus in the soil into forms available to plants, thereby increases their efficiency and availability. Bio-fertilizers are eco-friendly and have been proved to be effective and economical alternate of chemical fertilizers with lesser input of capital and energy (Sahu *et al.,* 2012). Advanced researches have altercated the interests of root microbiologists to establish more intimate association of wheat and both N_2-fixing bacteria and cyanobacteria. The application of N_2-fixing cyanobacteria biofertilizers in the cultivation of wetland rice has beneficial effect on growth and yield. Reports on the effect of cyanobacteria on growth and other crops rather than rice are, however, scarce (Abd El-Rasoul *et al*., 2003). Hoda Ibrahim *et al.* (2009) indicated 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 without any hazard environmental effects caused by inorganic N-fertilizer.

The objective of this study was to evaluate the effect of the application of different levels of mineral N and bio-fertilization on the forage yield and quality of the forage millet, cv. (Shandaweel-1) as well as economic evaluation of studied treatments.

MATERIALS AND METHODS

Two field experiments were carried out at the New Valley Research Station, El-Kharga, New Valley Governorate, Egypt (which is located around the point of 25° 27′ 88.48″ N latitude and 30° 32′ 43.38″ E longitude and at 51 m altitude) during the two successive summer seasons of 2012 and 2013. These experiments were conducted to study the influences of nitrogen fertilizer levels (Zero, 60, 90 and 120 kg N fed^{-1}) and inoculation with composite inocula cyanobacteica and / or *Azospirillum* sp. under the two levels of inorganic nitrogen (60 and 90 kg N fed^{-1}) on growth, forage yield and quality traits of forage millet (cv. Shandaweel-1). The texture of the soil of the experimental site was sandy loam and their physical and chemical analyses shown in Table1 were determined according to Page *et al*. (1982).

Bacterial strains:

Cyanobacteria (*Nostoc* sp. & *Anabaena* sp.) and *Azospirillum* 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 (400g per bag, each bag content is 10^9 CFU/g. for both inoculants).

The following ten treatments were conducted:

1-Un inoculated without nitrogen fertilizer (control).
2-Un inoculated with 50% nitrogen fertilizer (60 kg N fed^{-1}).
3-Un inoculated with 75% nitrogen fertilizer (90 kg N fed^{-1}).
4-Un inoculated with 100% nitrogen fertilizer (120kgN fed^{-1}; recommended dose).
5-Inoculated with cyanobacteria + 50% nitrogen fertilizer.
6-Inoculated with *Azospirillum* sp. + 50% nitrogen fertilizer.
7-Inoculated with cyanobacteria + *Azospirillum* sp. + 50% nitrogen fertilizer.
8-Inoculated with cyanobacteria +75% nitrogen fertilizer.
9-Inoculated with *Azospirillum* sp. +75% nitrogen fertilizer.
10- Inoculated with cyanobacteria + *Azospirillum* sp. + 75% nitrogen fertilizer.

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

The preceding crop in the two seasons was Egyptian clover and sowing date was 10th and 8th May in the first and second seasons respectively. The experiments were laid out in a randomized complete block design with three replications. Each plot size was $12m^2$ (3x4m) consisted of 15 rows. The seeds were hand-drilled in rows 20 cm apart at the seeding rate of 20 kg fed^{-1}. Nitrogen fertilizer was applied in the form of ammonium nitrate (33.5% N) at the different rates under study was divided into 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. Each of calcium superphosphate (15.5% P_2O_5) at the rate of 150 kg fed^{-1} and potassium sulphate (48% K_2O) at the rate of 50 kg fed^{-1} was applied before sowing. The other cultural practices were carried out as recommended .Three cuts were taken during each growing season after 55, 90 and 125days of planting.

Table 1. Physical and chemical soil analysis of the experimental site at the depth of (0-15 cm) in the summer seasons of 2012 and 13.

Physical properties			Chemical properties		
	Season			Season	
	2012	2013		2012	2013
Sand (%)	51.80	50.90	Organic matter (%)	0.86	0.88
Silt (%)	34.60	34.80	Soluble cations mg/l)		
Clay (%)	13.60	14.30	Ca^{++}	2.83	2.90
Texture grade	Sandy loam	Sandy loam	Mg^{++}	1.76	1.75
S.P (%)	42.30	42.00	Na^{+}	7.28	7.30
pH	7.92	8.00	K^{+}	7.11	7.14
E.C (dsm^{-1} at 25°C)	1.92	1.96	Soluble anions (mg/l)		
			CO_3^{-}	---	---
			HCO_3^{-}	3.12	3.17
			Cl^{-}	4.96	5.10
			$SO4^{--}$	10.90	10.82
			Total soluble – N (ppm)	67.30	68.0
			Available – P (ppm)	12.40	12.45
			DTPA-extractable (ppm)		
			Fe	2.18	2.21
			Mn	0.66	0.70
			Zn	0.89	0.91
			Cu	0.41	0.38

Data were recorded at each harvest on five guarded plants $plot^{-1}$ to determine:

1. Plant height (cm); length of the main stem from soil surface to stem-tip.
2. Number of tillers $plant^{-1}$.
3. Stem diameter (cm) at third internode above soil surface.
4. Fresh forage yield (ton fed^{-1}): plants were hand clipped and weighed in kg $polt^{-1}$ then, converted to ton fed^{-1}.
5. Dry forage yield (ton fed^{-1}): 100g plant samples from each plot were dried at 105°C till constant weight and dry matter percentage (DM %) was estimated. The dry forage yield (ton fed^{-1}) was calculated by multiplying fresh forage yield (ton fed^{-1}) X dry matter percentage.

Chemical analysis followed the conventional method recommended by the Association of Official Agricultural Chemists A.O.A.C (1980) on the dried samples at 70 ° C for each cut of the two seasons to determine crude protein (CP %), crude fiber (CF %) and ash%. Total digestible nutrient (TDN %) was estimated as

TDN = 50.41+ 1.04 CP - 0.07 CF, according to Church (1979) and Digestible crude protein (DCP) was calculated as DCP = ((CP X 0.9115)- 3.62) according to Mcdonald *et al.* (1978). Recorded data were used to compute:

1. Crude protein yield (kg fed^{-1}); estimated by multiplying forage dry yield X CP%.

2. Crude fiber yield (kg fed^{-1}); estimated by multiplying forage dry yield X CF%,
3. Ash yield (kg fed^{-1}); estimated by multiplying forage dry yield X Ash%,
4. Total digestible nutrient yield (kg fed^{-1}); estimated by multiplying forage dry yield X TDN %.
5. Digestible crude protein yield (kg fed^{-1}); estimated by multiplying forage dry yield X DCP %.

Economic evaluation:

In the present study, the economic evaluation included three parameters that were estimates as follows:

1-Average input variables as well as total costs of forage millet production as affected by cyanobacteria and / or *Azospirillum* sp under different nitrogen levels, control treatments and the applied different culture practices during the different stages of growth in each season.

2-Net farm income of forage millet production as affected by the different studied treatments. Net farm income is the values of forage yield according to the actual marketing price.

3-Net farm return of forage millet production as affected by the different studied treatments. It is the difference between forage yield value according to the actual price and the total costs. All of the above estimations are based on the official and actual market prices determined by the Ministry of Agriculture and the Agricultural Credit and Development Bank at New valley.

Statistical analysis:

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 in both seasons.

RESULTS

Morphological characters:

Results in Table 2 elucidated the effect of the applied nitrogen levels and cyanobacteica and/or *Azospirillum* sp. inoculation under different levels of inorganic nitrogen on morphological characters, namely plant height, number of tillers $plant^{-1}$and stem diameter of forage peal millet . Data demonstrate the differences among the tested treatment were significant for all studied characters.

Data of morphological characters showed that the mean first cut was significantly higher (135.97cm) than second and third cuts (127.62 and 119.75 cm, respectively) for the plant height. Plants of forage millet produced significantly higher number of tillers $plant^{-1}$ at the first cut (5.37) than second and third cuts (4.58 and 4.45, respectively) and their stems were significantly thicker at first cut (1.69 cm) than second and third cuts (1.59 and 1.30 cm, respectively).

Concerning nitrogen fertilization, data indicated that the increase in nitrogen levels caused significant substantial increase in the growth parameters of each cut and average over three cuts. Regarding plant height, the values of mean over three cuts for the plants fertilized with 60, 90and 120 kg N fed^{-1} increased by 23, 43 and 57% as compared with the control received no fertilizer, respectively. While the increases reached to 60, 95 and 162 % for the number of tillers plant^{-1} and 40, 95 and 170% for stem diameter in the same respect.

Regarding the interaction between nitrogen fertilizer levels and biofertilizer treatments, the data in Table 2 showed clearly that the application of cyanobacteria and *Azospirillum* combined with 75% N fertilization (90 kg N fed^{-1}) produced the tallest plant (154.00, 145.22, 135.33 and 144.85 cm at the 1st, 2nd , 3rd and over cuttings, respectively), maximum number of tillers plant^{-1} (7.33, 6.64, 5.83 and 6.60) and thick stem diameter (2.25, 2.30, 1.95 and 2.17 cm) followed by the application of cyanobacteria and *Azospirillum* combined with 50 % N fertilization (60 kg N fed^{-1}). The mixtures inoculation when combined with 90 kg Nfed^{-1} increased plant height by (16, 14 and 8 %), number of tillers plant^{-1} by (47, 63 and 40%) and stem diameter by (43, 55 and 63%) as compared with the plants received the nitrogen rate, i.e., 90kg Nfed^{-1} ,while increased plant height by (2, 3 and 2 %), number of tillers plant^{-1} by (10, 7 and 17%) and stem diameter by (3, 10 and 20%) as compared with the plants received the recommended nitrogen rate (120 kg N fed^{-1}) for the first, second and third cuts, respectively.

Table 2. Mean performance of morphological characters of forage millet treated with cyanobacteria and / or *Azospirillum* sp under different nitrogen levels. (Combined analysis over the two seasons of 2012 and 2013)

Characters / Treatments	Plant height (cm)				No. of tillers plant^{-1}				Stem diameter (cm)			
	Cut1	Cut2	Cut3	Mean	Cut1	Cut2	Cut3	Mean	Cut1	Cut2	Cut3	Mean
Control (without N addition)	104.17	92.55	73.33	90.02	2.83	1.81	2.17	2.27	0.92	0.77	0.50	0.73
50% N (60 kg N fed^{-1})	123.50	110.72	97.17	110.46	4.17	3.58	3.17	3.64	1.27	1.03	0.75	1.02
75% N (90 kg N fed^{-1})	133.33	127.05	125.67	128.68	5.00	4.08	4.17	4.42	1.57	1.48	1.20	1.42
100% N (120 kg N fed^{-1})	150.50	141.38	132.17	141.35	6.67	6.19	5.00	5.95	2.18	2.10	1.63	1.97
cyanobacteria + 50%N	135.00	126.88	125.00	128.96	4.83	4.03	3.83	4.23	1.45	1.30	1.20	1.32
***Azospirillum* sp. + 50%N**	136.33	129.22	126.33	130.63	5.17	4.58	4.83	4.86	1.67	1.62	1.35	1.54
cyano.+*Azo*. sp. + 50%N	146.17	138.55	130.67	138.46	6.50	5.69	5.50	5.90	2.02	1.93	1.55	1.83
cyanobacteria + 75%N	137.33	131.38	124.50	131.07	5.17	4.36	5.00	4.84	1.70	1.62	1.37	1.56
***Azospirillum* sp. + 75%N**	139.33	133.22	127.33	133.30	6.00	4.86	5.00	5.29	1.85	1.70	1.47	1.67
cyano.+*Azo*. sp. + 75%N	154.00	145.22	135.33	144.85	7.33	6.64	5.83	6.60	2.25	2.30	1.95	2.17
Mean	135.97	127.62	119.75	127.78	5.37	4.58	4.45	4.80	1.69	1.59	1.30	1.52
F-test	*	*	*	*	*	*	*	*	*	*	*	*
LSD at 0.05	3.47	2.94	3.18	2.59	0.63	0.61	0.92	0.50	0.15	0.15	0.20	0.12

Fresh and dry forage yields:

Fresh and dry forage yields of the tested treatments significantly different for individual cuttings as well as total fresh forage yield (Table 3). Regarding the comparison among cuts; first cut produced the highest fresh and dry yields. Averaged over all treatments, fresh forage yield was 17.47, 15.05and 12.13 ton fed^{-1} while dry forage yield was 2.78, 2.74and 2.73 ton fed^{-1} for the first, second and third cuts, respectively. The results of this study demonstrate that the fresh and dry forage yields as affected by different level of nitrogen and cyanobacteica and/or *Azospirillum* sp. inoculation under different levels of mineral nitrogen.

Applying 60 and 90 kg N fed^{-1} led to significantly lower fresh and dry forage yields than those obtained by adding 120 kg N fed^{-1} at all single cuts as well as seasonal yield. It is clear from the data presented in Table 3 indicated the promising role of mixture cyanobacteria and *Azospirillum* sp. combined with 75% N fertilization (90 kg N fed^{-1}) followed by the application of cyanobacteria and *Azospirillum* sp. combined with 50% N fertilization(60kgN fed^{-1}). Moreover, mixture cyanobacteria and *Azospirillum* sp. combined with 75% N fertilization (90 kgNfed^{-1}) increased total fresh yield by 94% and dry forage yield of millet by about 104% as compared

with the treatment 90 kgNfed^{-1}, while increased total fresh yield by 15% and dry forage yield by about 8% as compared with the plants received the recommended nitrogen rate (120kg N fed^{-1}).

Table 3. Fresh and dry forage yields of forage millet treated with cyanobacteria and / or *Azospirillum* sp under different nitrogen levels. (Combined analysis over the two seasons of 2012 and 2013)

Characters / Treatments	Fresh yield (ton fed^{-1})				Dry yield (ton fed^{-1})			
	Cut1	Cut2	Cut3	Total	Cut1	Cut2	Cut3	Total
Control (without N addition)	10.88	7.48	5.55	23.92	1.83	1.32	1.30	4.45
50% N (60 kg N fed^{-1})	12.92	10.65	7.53	31.10	1.97	1.76	1.57	5.30
75% N (90 kg N fed^{-1})	14.17	12.60	9.25	36.02	2.12	2.09	2.04	6.25
100% N (120 kg N fed^{-1})	23.25	20.82	16.53	60.60	3.91	3.97	3.94	11.82
cyanobacteria + 50%N	15.33	11.78	8.43	35.55	2.29	1.98	1.91	6.17
Azospirillum sp. + 50%N	16.62	14.23	11.47	42.32	2.57	2.81	2.71	8.08
cyano.+*Azo*. sp. + 50%N	20.87	19.02	16.48	56.37	3.33	3.39	3.80	10.52
cyanobacteria + 75%N	17.67	14.82	11.93	44.42	2.75	2.94	2.72	8.41
Azospirillum sp. + 75%N	18.02	15.17	13.10	46.28	2.82	2.83	3.12	8.77
cyano.+*Azo*. sp. + 75%N	25.00	23.97	20.97	69.93	4.25	4.32	4.20	12.77
Mean	17.47	15.05	12.13	44.65	2.78	2.74	2.73	8.26
F-test	*	*	*	*	*	*	*	*
LSD at 0.05	1.36	1.16	1.60	3.57	0.25	0.33	0.39	0.79

Forage quality:

Results of crude protein, crude fiber and ash yields in forage millet are presented in Tables 4. Analysis of variance indicated significant differences among test treatments for crude protein, crude fiber and ash yield**s.** Concerning the comparison of different cuts, the first cut gave the highest crude protein yield (288.0 kgfed^{-1}) followed by the third (194.7 kgfed^{-1}) and the second cut (191.4 kgfed^{-1}), while the first cut gave the highest crude fiber yield (874.7 kgfed^{-1}) followed by the second (788.8 kgfed^{-1}) and the third cut (766.1 kg fed^{-1}), as for second cut produced the highest ash yield (350.9 kgfed^{-1}) followed by the first (332.6 kg fed^{-1}) and the third cut (329.7 kg fed^{-1}) as average over all tested treatments.

Data presented in Table 4 indicated that increasing level of nitrogen from zero, 60, 90 and 120kg Nfed^{-1} progressively increased crude protein, crude fiber and ash yields in all single cuts as well as seasonal yield.

Table 4. Crude protein, crude fiber and ash yields of forage millet treated with cyanobacteria and / or *Azospirillum* sp under different nitrogen levels. (Combined analysis over the two seasons of 2012 and 2013)

Characters / Treatments	Crude protein yield (kgfed^{-1})				Crude fiber yield (kgfed^{-1})				Ash yield (kgfed^{-1})			
	Cut1	Cut2	Cut3	Total	Cut1	Cut2	Cut3	Total	Cut1	Cut2	Cut3	Total
Control (without N addition)	167.0	98.0	90.5	355.4	594.0	395.1	397.0	1386.1	189.6	147.3	113.1	449.9
50% N (60 kg N fed^{-1})	222.2	137.7	122.9	482.9	604.6	512.4	430.2	1547.2	204.2	194.6	134.9	533.7
75% N (90 kg N fed^{-1})	256.6	145.3	159.9	561.8	868.9	584.1	623.5	2076.5	313.8	246.6	196.8	757.2
100% N (120 kg N fed^{-1})	408.6	311.0	239.9	959.5	1178.7	1160.1	1098.5	3437.3	518.5	419.4	471.2	1409.1
cyanobacteria + 50%N	238.7	154.8	149.2	542.7	681.4	565.9	539.3	1786.7	263.7	295.9	220.0	779.6
***Azospirillum* sp. + 50%N**	246.1	170.9	211.9	629.0	786.4	838.9	741.9	2367.2	269.8	328.1	354.8	952.7
cyano.+*Azo*. sp. + 50%N	376.2	206.5	264.7	847.4	1011.3	931.2	1084.7	3027.3	351.3	550.9	480.0	1382.2
cyanobacteria + 75%N	286.9	205.0	189.5	681.4	798.9	848.2	726.9	2374.0	383.7	392.9	370.9	1147.4
***Azospirillum* sp. + 75%N**	270.2	221.9	189.7	681.7	878.3	848.9	861.9	2589.1	303.2	328.1	387.8	1019.1
cyano.+*Azo*. sp. + 75%N	407.0	263.3	329.0	999.3	1344.1	1203.6	1157.6	3705.2	528.3	605.7	567.2	1701.2
Mean	288.0	191.4	194.7	674.1	874.7	788.8	766.1	2429.7	332.6	350.9	329.7	1013.2
F-test	*	*	*	*	*	*	*	*	*	*	*	*
LSD at 0.05	25.2	22.0	28.6	59.9	77.4	95.9	107.4	219.2	30.2	43.9	48.1	97.3

Inoculation significantly increased crude protein yield (77.9%), crude fiber yield (78.4%) and ash yield (124.7%) seasonal yield particularly with treatment cyanobacteria and *Azospirillum* sp combined with 75% N fertilization (90 kg N fed^{-1}) as compared with the uninoculated ones that received the same amount of N-fertilizer, while increased crude fiber yield by 7.8% and ash yield by about 20.7% as compared with the plants received the recommended nitrogen rate (120kg N fed^{-1}).

Application of 120kg Nfed^{-1} gave crude protein yield similar to that obtained from inoculation with treatment cyanobacteria and *Azospirillum* sp combined with 75% N fertilization (90 kg N fed^{-1}).

Table 5. Total digestible nutrient and digestible crude protein yield of forage millet treated with cyanobacteria and / or Azospirillum sp under different nitrogen levels. (Combined analysis over the two seasons of 2012 and 2013)

Characters / Treatments	Total digestible nutrient yield (kg fed^{-1})				Digestible crude protein yield (kg fed^{-1})			
	Cut1	Cut2	Cut3	Total	Cut1	Cut2	Cut3	Total
Control (without N addition)	1054.4	739.7	721.6	2515.8	85.9	41.6	35.4	162.8
50% N (60 kg N fed^{-1})	1182.4	994.6	889.2	3066.2	131.4	61.9	55.2	248.5
75% N (90 kg N fed^{-1})	1231.5	1163.9	1151.0	3546.4	136.9	56.9	71.8	265.5
100% N (120 kg N fed^{-1})	2313.2	2243.4	2158.7	6715.3	230.7	139.7	76.1	446.5
cyanobacteria + 50%N	1355.2	1119.7	1080.5	3555.4	134.8	69.5	67.0	271.3
Azospirillum sp. + 50%N	1496.3	1535.7	1534.7	4566.6	131.2	54.2	95.2	280.6
cyano.+*Azo*. sp. + 50%N	1999.3	1858.4	2114.7	5972.4	222.5	65.5	103.6	391.6
cyanobacteria + 75%N	1628.8	1635.5	1517.2	4781.6	162.0	80.2	74.2	316.5
Azospirillum sp. + 75%N	1641.0	1597.8	1710.1	4948.8	144.1	99.7	60.2	303.9
cyano.+*Azo*. sp. + 75%N	2471.4	2366.9	2378.0	7216.3	217.1	83.5	147.8	448.3
Mean	1637.3	1525.6	1525.6	4688.5	159.7	75.3	78.6	313.6
F-test	*	*	*	*	*	*	*	*
LSD at 0.05	147.1	183.9	217.9	431.2	13.9	8.2	12.2	27.4

Table 5 shows the effect of inoculation and nitrogen fertilization on total digestible nutrient and digestible crude protein yields for individual cuttings and seasonal yield. The differences in total digestible nutrient and digestible crude protein yields of forage millet between test treatments were significantly for all cuts as well as seasonal yield.

It is clear from data presented in Table 5 that first cut had the highest value of total digestible nutrient and digestible crude protein yield followed by the second and the third cut. Average over all treatments of total digestible nutrient yield (1637.3, 1525.6 and 1525.6 kg fed^{-1}) and digestible crude protein yield (159.7, 75.3 and 78.6 kg fed^{-1}) for the first, second and third cut, respectively.

Data indicated that application of cyanobacteria and *Azospirillum* sp combined with 75% N fertilization (90 kg N fed^{-1}) led to pronounced increase in total digestible nutrient (103.5%) and digestible crude protein (68.9%) seasonal yield as compared with the treatment (90kg N fed^{-1}), while increased total digestible nutrient seasonal yield by7.5% and digestible crude protein seasonal yield by about 0.40% as compared with the plants received the recommended nitrogen rate (120kg N fed^{-1}).

Economic evaluation:

Costs

Total costs including values of production tools and requirements such as seeds, fertilizers, irrigation, man power, machinery and other general or miscellaneous costs without land rent average summer 2012 and 2013seasons are shown in Table 6.

The price of 50 kilogram ammonium nitrate (33.5%N) was 70 L.E., the price of 50 kilogram calcium superphosphate (15.5% P_2O_5) was 55 L.E., and the price of 50 kilogram potassium sulphate (48% K_2O) was 200 L.E., the price 2 bags inoculants fed^{-1} was 25 L.E., the price of one kilogram seeds (cv. Shandaweel-1) was 11 L.E. the total cost of soil tillage included the cost for first and second plowing by chisel plow was 200 L.E. and present in Table 6.

Data in Table 6 show the total costs of forage millet production per feddan as affected by the applied different treatments (average of 2012 and 2013seasons). From such data, it is clear that the minimum total costs were those of application of control (un- inoculated without addition nitrogen fertilizer zero % N) , being 2445.00 L.E. and the maximum total costs were those of the plants received the recommended nitrogen rate (120kg N fed^{-1}).which was 2935.00 L.E. Average over all treatments of total costs were 2754.00 L.E.

Net return

Results in Table 6 reveal that the highest net farm return was achieved from treatment cyanobacteria and *Azospirillum* sp combined with 75% N fertilization (7652.00 L.E.fed^{-1}) followed by the recommended nitrogen rate 120kg N fed^{-1} (6155.00 L.E.fed^{-1}) and cyanobacteria and *Azospirillum* sp combined with 50% N fertilization (5740.50 L.E.$fed.^{-1}$) . On the other hand, the lowest net farm return were (1143.00 L.E.fed^{-1}) recorded by un-inoculated without nitrogen fertilizer (Zero %N) .But, the highest net return per one invested L.E. was achieved from application cyanobacteria and Azospirillum sp combined with 75% N fertilization (2.70 L.E.) Followed by cyanobacteria and *Azospirillum* sp combined with 50% N fertilization (2.11 L.E.fed^{-1}) and the recommended nitrogen rate 120kg N fed^{-1} (2.10 L.E.) and, while the un- inoculated without nitrogen fertilizer (Zero %N) was 0.47 L.E. fed^{-1}.

T 6

DISCUSSION

Results of the current study assured the significance of biofertilization on forage millet 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.

These results may be due to the effect of nitrogen fertilization in pushing growth of pearl millet and the increments in inter-node length or/and number of internodes, number of tillers plant^{-1}. These findings are in harmony with those obtained by Ayub *et al.* (2009), Pathan *et al.* (2010), Abd El-Lattief (2011) and Shahin(2013).

Nitrogen fixers and phosphate dissolving bacteria was reported to increase protein yield in pearl millet (Mahmoud *et al.,* 1994). Bashan and Levanony (1990) found that enhanced minerals uptake of inoculated plants are possible mechanisms of plant growth enhancement by *Azospirillum*. The major element involved was suggested to be N in the form of nitrate in wheat, sorghum and corn plants (Pacovesky *et al.,* 1985).

Results were almost in accordance with others concerning cyanobacteria inoculation (Abd El-Rasoul *et al*., 2004 and Hoda Ibrahim *et al*., 2009) and regarding *Azospirillum* sp (Amal Helmy, 2003; Abdel-Galil *et al*., 2006 and Hoda Ibrahim *et al*., 2009).

Inoculation with composite inocula cyanobacteica and *Azospirillum* sp. combined with 75% N fertilization (90 kg fed^{-1}) and improved growth, fresh and dry forage yield of pearl millet , crude protein yield , crude fiber yield, ash yield , total digestible nutrient and digestible crude protein yield(Bouton *et al*., 1979 ; Wani *et al*., 1988; Mahmoud *et al.,* 1994; Amal Helmy, 2003; Abdel-Galil *et al*., 2006 and Hoda Ibrahim *et al.*, 2009). Baker and Klopper (1990) concluded that application of bacterial mixtures would be close to simulate the natural soil biological system than using a single inoculum.

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 sidrophores (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 millet, it could be concluded that combination between PGPRs and N_2 – fixer bacteria inoculants combined with 75% of its recommended nitrogen rate 100% N (120 kg $Nfed^{-1}$) increased growth, forage yield and quality traits of pearl millet and save about 25% of nitrogen fertilizer with decreasing hazard environmental effects that may be caused by mineral N-fertilizer.

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إنتاجية و جودة دخن العلف وتأثرها بالتسميد النيتروجينى والحيوى تحت ظروف الوادى الجديد

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اقيمت تجربتان حقليتان بمحطة البحوث الزراعية بالوادى الجديد خلال الموسمين الزراعيين الصيفيين ٢٠١٢ و٢٠١٣ لدراسة تأثير اربع مستويات من التسميد النيتروجينى (صفر , ٦٠, ٩٠ و١٢٠ كجم نيتروجين /فدان) و التفاعل بين التسميد الحيوى(بلقاح السيانوبكتريا أوالازوسبيريللام أو كلاهما معاً) ومستويان من التسميد النيتروجينى (٦٠ و ٩٠ كجم نيتروجين /فدان) على إنتاجية وجودة محصول دخن العلف (صنف شندويل-١) وكذلك التقييم الاقتصادى للمعاملات المدروسه. وكان التصميم التجريبى المستخدم هو القطاعات الكاملة العشوائية فى ثلاثة مكررات.

وقد أظهرت نتائج التحليل التجميعى للموسمين ان زيادة النيترجين المعدنى ادى الى زيادة معنوية فى انتاجية وجودة محصول العلف وكانت اعلى القيم عندما اضيف المعدل الموصى بة لنبات الدخن وهو ١٢٠كجم نيترجين /فدان.

كما اثبتت النتائج ان نباتات الدخن الملقحة بلقاح السيانوبكتريا + الازوسبيريللم +٩٠كجم نيتروجين /فدان أدى الى زيادة معنوية فى ارتفاع النبات (٢,٥%) وعدد الافرع / نبات (١١%) وقطر الساق (١٠%) محصول العلف الأخضر (١٥%) ومحصول العلف الجاف (٨ %) ومحصول الاليا ف (٧,٨%) ومحصول الرماد (٢٠,٧ %) ومحصول المواد الكلية المهضومة (٧,٥%) ومحصول البروتين المهضوم (٠,٤٠ %) مقارنة بالنباتات غير الملقحة والمسمدة بالمعدل الموصى به من التسميد النيتروجينى (١٢٠كجم نيترجين /فدان) . كما وجد ان إضافة ١٢٠كجم نيتروجين /فدان اعطت محصول بروتين مشابه لما تم الحصول عليه من اضافة معاملة السيانوبكتريا مع الازوسبيريللم + ٧٥ % من معدل النيتروجين الموصى به.

كما أوضحت النتائج ان معاملة التسميد الحيوى المزدوج (السيانوبكتريا+ الازوسبيريللم) مع إضافة ٧٥ % من التسميد النيتروجينى الموصى به (١٢٠كجم /فدان) هى الأعلى فى عائد الجنيه المستثمر ويقدر بنحو ٣,٧٠ جنيه اى ان كل جنيه يتم إنفاقه فى زراعة محصول الدخن لهذه المعاملة يعود على المزارع بالجنيه الذى تم انفاقه فى الزراعة مضافاً اليه صافى العائد الذى يقدر بنحو ٢,٧٠ جنيه وتعتبر هذه المعاملة افضل المعاملات المدروسه.

من النتائج المذكورة سابقاً يمكن التوصيه بإضافة التسميد الحيوى المزدوج (السيانوبكتريا+ الازوسبيريللم) مع إضافة ٧٥ % من التسميد النيتروجينى الموصى به (١٢٠كجم / فدان) تعطى أعلى إنتاجية من دخن العلف وأعلى صافى عائد للمزارع تحت ظروف الوادى الجديد.

قام بتحكيم البحث

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Table 6. Estimated net return L.E.fed.$^{-1}$ of forage millet crop treated with cyanobacteria and / or *Azospirillum* sp under different nitrogen levels over the two seasons of 2012-2013.

Treatments / Cost of production inputs	Zero % N	50 % N	75 % N	100 % N	cyanobacteria + 50%N	*Azospirillum* sp. + 50%N	cyano.+*Azo.* sp. + 50%N	cyanobacteria + 75%N	*Azospirillum* sp. + 75%N	cyano.+ *Azo.* sp. + 75%N
land preparation										
Tillage	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
Planting	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00
Seeds	220.00	220.00	220.00	220.00	220.00	220.00	220.00	220.00	220.00	220.00
Irrigation	680.00	680.00	680.00	680.00	680.00	680.00	680.00	680.00	680.00	680.00
Mineral fertilization										
Ammonium nitrate (33.5% N)	-	245.00	367.50	490.00	245.00	245.00	245.00	367.50	367.50	367.50
superphosphate (15.5% P_2O_5)	165.00	165.00	165.00	165.00	165.00	165.00	165.00	165.00	165.00	165.00
potassium sulphate (48% K_2O)	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
Biofertilization	-	-	-	-	25.00	25.00	25.00	25.00	25.00	25.00
Hoeing	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00
Harvesting	600.00	600.00	600.00	600.00	600.00	600.00	600.00	600.00	600.00	600.00
Total variable cost	**2445.00**	**2690.00**	**2812.50**	**2935.00**	**2715.00**	**2715.00**	**2715.00**	**2837.50**	**2837.50**	**2837.50**
Yield ton fed^{-1}	23.92	31.10	36.02	60.60	35.55	42.32	56.37	44.42	46.28	69.93
Price ton^{-1}	150.00	150.00	150.00	150.00	150.00	150.00	150.00	150.00	150.00	150.00
Total revenue	**3588.00**	**4665.00**	**5403.00**	**9090.00**	**5332.50**	**6348.00**	**8455.50**	**6663.00**	**6942.00**	**10489.50**
Net return	**1143.00**	**1975.00**	**2590.50**	**6155.00**	**2617.50**	**3633.00**	**5740.50**	**3825.50**	**4104.50**	**7652.00**
Return of invested L.E.	**1.47**	**1.73**	**1.92**	**3.10**	**1.96**	**2.34**	**3.11**	**2.35**	**2.45**	**3.70**
Net return of invested L.E.	**0.47**	**0.73**	**0.92**	**2.10**	**0.96**	**1.34**	**2.11**	**1.35**	**1.45**	**2.70**

Net return (L.E.fed.$^{-1}$) = Total revenue - Total variable cost

Return of invested L.E. = $\frac{\text{Total revenue}}{\text{Total variable cost}}$

Net return of invested L.E. = Return of invested L.E - 1