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EFFECT OF N_2-FIXING AND PHOSPHATE DISSOLVING BACTERIA ON YIELD AND CHEMICAL COMPOSITIONS OF FORAGE PEARL MILLET (*Pennisetum glaucum*)

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ABSTRACT

The experiments were conducted at, the Forage Research Department, FCRI, Giza station, (ARC), during summer seasons 2001 and 2002 to evaluate the effect of different strains of bacteria as biofertilizers on forage pearl millet. Inoculation treatments used in this investigation were combined with 50% of N-recommended dose (60 kg N/fed).

The obtained results proved that the use of biofertilizer especially with *Azotobacter* spp. + *Azospirillum* spp. and *Bacillus megatherium* + *B. polymyxa* combined with 60 kg N/fed affected strongly the plant growth through the positive effect on fresh and dry forage yields (ton /fed) at all cuts and seasonal yield. The same trend was obtained with plant height, number of tillers, roots dry weight and mineral content especially P and K as well as organic compounds, protein, fiber and ash yields. Pearl millet inoculated with *Azotobacter* spp. + *Azospirillum* spp. gave mean values comparable with those obtained when using the high dose of N-fertilization (120 kg N/fed). Thus, it saves about 50% of the required nitrogen which reduces costs and environmental pollution of mineral nitrogen fertilizer.

INTRODUCTION

Forage grasses represent one of the major sources of animal feed in the world. In Egypt, especially in summer, pearl millet is an important forage crop beside sorghum, they occupy about 15.000 feddans. Pearl millet has an excellent growing habit, quick regrowth after harvesting and high nutritive value. Nitrogen plays an important role in increasing forage production with better nutritive value. The cost of nitrogen fertilizers is very expensive; it becomes imperative to substitute nitrogen by some other cheaper sources, which may partially meet the nitrogen required by the crop. A useful method to reduce the input of chemical fertilizers in agriculture and to control the soil and water pollution may be represented by the use of asymbiotic nitrogen fixing microorganisms as well as phosphate dissolving bacteria as biological fertilizers

After the discovery of a very active association between *Azospirillium* spp. and the root system of various graminceous plants (Dobereiner and Day, 1976), the use of these bacteria as biofertilizer fixing atmospheric nitrogen and producing phytohormones such as acetic acid, gibberellins, cytokinins, etc. (Baschan and Levanony, 1990; Okon and Labandera-Gonzalez, 1994) has attracted the attention of agronomists.

Application of nitrogen-fixers bacteria (*Azotobacter*, *Azospirillum*, *Bacillus*, *Klebsiella*, and *Pseudomonas*) as biofertilizers with pearl millet; wheat, maize and sorghum showed significant increases in dry matter, associated with increased harvest of nitrogen per unit area (Smith *et al.*, 1984; Saleh *et al.*, 2000 and Saubidet *et al.*, 2002). In addition, inoculation could minimize N-fertilizer requirments up to 35%. Many workers found that N-utilization in inoculated plants becomes more efficient (Okon and Labandera-Conzalez, 1994). Inoculation of wheat and sorghum by *Azotobacter* and *Azospirillum* increased the uptake of N, P, K, Fe, Zn, Mn and Cu (Amara and Dahdoh, 1997 and Lippman *et al.*, 1995).

Phosphorus (P) is one of the major plant growth-limiting nutrients, although it is abundant in soils in both inorganic and organic forms. Phosphate solubilizing microorganisms (PSMs) are ubiquitous in soils and could play an important role in supplying P to the plants in a more environmentally friendly and sustainable manner (Gyaneshwar *et al.*, 2002).

Biofertilization with phosphate dissolving bacteria and Plant Growth Promoting Rhizobacteria (PGPR) were applied with many crops (sorghum, maize and radish;, it apparently promotes plant growth mainly by stimulating root development, which improves N, P, K, micro elements and water uptake (Antuon *et al.*, 1998). On the other hand, rhizobia are capable to colonize the roots of non-legumes and produce siderophores as well as hydrogen cyanide (HCN), (Antoun *et al.*, 1998). They also exhibit antagonistic effects against many pathogenic fungi.

The objectives of this study are (1): Minimize N-fertilizer requirement. (2): To evaluate the role of biofertilizers on improvement productivity and quality of forage pearl millet. (3): To evaluate the functional groups of microorganisms to be used as biofertilizer adapted for pearl millet as forage.

MATERIALS AND METHODS

Two field experiments were executed at Forage Research Department, FCRI, Giza station, (ARC), during summer seasons 2001 and 2002.The soil type of field experiments is presented in Table (1). The experiments were laid out in a complete randomized block design with four replications, each consisted of seven plots. The plot size was 8 m^2 (4.0x2.0 m). To reduce the possibility of cross contamination, a space of 1.5 m between adjacent plots were left empty. Treatments used in this investigation were: 1- 0 kg N/fed , 2- 60 kg N/fed, 3- 120 kg N/fed, 4- 60 kg N/fed + inoc. (*Rhizobium*), 5- 60 kg N/fed + inoc. (*Bradyrhizobium*), 6- 60 kg N/fed + inoc. (*Azotobacter* + *Azospirillum*) and 7- 60 kg N/fed + inoc. (*B. megatherium* + *B. polymyxa*). The preceding crop in the two seasons was Egyptian clover and sowing date was 28^{th} and 30^{th} April in the first and second seasons respectively. Pearl millet var. Shandawil-1 was sown in rows apart 30 cm with a seeding rate of 15 kg/fed.

Nitrogen application was in the form of ammonium sulphate (20.6%) as three equal doses after twenty-one days of sowing for the first application and the rest after each cut. Recommended rates of phosphores, 15.5 % P_2O_5 (150kg/fed) as super phosphate and potassium, 48% K_2O (100kg/fed) as potassium sulphate were applied just after land preparation and before planting. Three cuts were obtained for each of the two successive seasons, 50, 85, and 120 days after planting.

Table (1): Chemical and physical properties of the used soil

Physical properties		Chemical properties	
Mechanical analysis:		Organic matter%	1.47
Clay%	39.71	Soluble cations (meq l^{-1}):	
Silt%	23.18	Ca^{++}	6.12
Coarse sand%	15.22	Mg^{++}	8.46
Fine sand%	21.89	Na^{+}	7.38
Textural class	clay loamy	K^{+}	0.37
SP%	56.70	Soluble anions (meq l^{-1}):	
pH	7.95	CO_3^{--}	0.00
EC (ds/m)	1.96	HCO_3^{-}	4.90
		Cl^{-}	8.73
		SO_4^{--}	8.70
		Available P (mg/kg soil)	10.00
		Total soluble N (mg/kg soil)	80.00

Bacterial strains and inocula preparation

Fast growing *Rhizobium leguminosarum* bv. *Phaseoli* (127k80c), slow growing *Bradyrhizobium* spp. (lupini) were supplied by Nottingham Univ. UK, while *Azotobacter chroococcum* (ARC AZ.1), *Azospirillum brasilense* (ARC AZP.II), *Bacillus megatherium* var.*phosphaticum* and *Bacillus polymyxa* were provided by ARC, Giza, Egypt. Broth culture of each strain which contained ca.10^{9} cells ml^{-1}, was separately carried on vermiculite-based (1:3 v/w) as a local carrier material. Just before sowing, each separate carrier were mixed according to each treatment and used in a rate of 400g/fed. Arabic gum was applied as adhesive material for seed inoculation treatment. The uninoculated seeds received only bacterial culture medium.

Agronomic parameters

At each cut, the following traits were recorded:

1- Fresh and dry matter yield (ton/ fed)
2- Plant height (mean of 10 plants, cm)
3- Number of tillers plant $^{-1}$(mean of 10 plants)
4- Roots dry weight (mean of 10 plants, gm/plant).

Chemical compositions

Plant shoot were dried, pulverized for crude protein; crude fiber and ash yield as well as potassium and phosphorus components of the two seasons were determined according to AOAC (1990).

Statistical analysis system were carried out using MSTAT ver. 4 (1986) on all variables measures using the ANOVA (analysis of variance) procedure. Combined analysis over seasons was performed and used in the discussion of the obtained results. The differences among averages of the studied traits were judged with the least significant differences (L.S.D) at 5% level of significance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Fresh and dry forage yield

Results of the field experiments revealed significant differences due to inoculation with (*Azotobacter+Azospirillum*) and (*B. megatherium+B. polymyxa*) regarding fresh and dry forage yield which were significantly superior over the uninoculated plants received 60 kgN/fed at all single cuts as well as seasonal yields. The data presented in Table (2) indicated the promising role of (*Azotobacter+Azospirillum*) and (*B. megatherium+B. polymyxa*) in increasing total fresh yield (30.93; 19.17%) and dry forage (40.62; 19.97%) over control treatment. On the other hand, treatments with *Rhizobium* and *Bradyrhizobium* showed an opposite trend and gave lowest values of seasonal fresh and dry forage yields (43.31; 45.27) and (7.74; 7.60) ton/fed., for the two treatments compared to control respectively. Application of *Azotobacter* and *Azospirillum* together with medium dose of N fertilization (60 kg N/fed) produced fresh and dry matter yield comparable to the recommended full-N dose fertilization (120 kg N/fed). In spite of inoculation with *B. megatherium* + *B. polymyxa* had significant effect in increasing total fresh and dry forage yield compared with control treatment but still lower than those treated with *Azotobacter* and *Azospirillum* by 5.03 and 1.52 ton/fed. respectively.

An increase in forage yield with an associative diazotrophes and P-dissolving bacteria can be attributed not only to their N_2-fixing proficiency but also, with their ability to produce plant growth hormones. Such growth hormones mainly IAA, gibberillins and cytokinines could stimulate the plant growth absorption of nutrient, efficiency of nutrients and photo-synthetic products (Dobereiner, 1977 and Tein, *et al.*, 1979). These results are in agreement with those reported by Mahmoud *et al.* (1994), on pearl millet, Desale *et al.* (1999) on sorghum.

Hegazi *et al.* (1996) found that improvement growth of wheat, sorghum and maize could be attributed to N_2-fixation and / or certain growth promoting substances. Kapulink *et al.* (1981) noticed that the yield of sorghum and corn increments due to inoculation ranged from 12.0 to 39.0%. The p-solublization effect seems to play an important role to stimulate pearl millet rhizosphere and enhance plant growth. The positive and significant effect of p-dissolving bacteria was similar to that reported by Antoun *et al.*, 1998 and Rashad *et al.*, 2001).

Plant height

Data presented in Table (3) indicated that inoculation of pearl millet with any of the inoculant bacteria except the *Bradirhizobium* combined with 60 kg N/fed., recorded significant increases in plant height compared to the untreated ones. Plant height increases due to inoculation ranged from 10.38% at first cut up to 30.64% at third cut, depending on the type of inoculant. Under 120 kg N/fed significant response in plant height was recorded in comparison to uninoculated treatment received 60 kg N/fed. No significant differences in plant height were observed between treatments inoculated with (*Azotobacter* + *Azospirillum*) and *Rhizobium* with 60 kg N/fed and treatment received full dose of N fertilization.

Table (2): Fresh and dry matter yield of fodder millet as affected by different treatments of bio- fertilizers and N-fertilizers (combined analysis over two seasons 2001 and 2002).

Treatments	Fresh yield (ton / fed)				Dry forage yield (ton / fed)			
	Cuts				Cuts			
	1st	2nd	3rd	Total	1st	2nd	3rd	Total
0 nitrogen	10.49	16.00	8.33	34.82	1.65	2.57	2.21	6.43
60 kgN/fed	12.27	20.00	10.50	42.77	1.81	3.08	2.42	7.31
120 kgN/fed	18.43	25.10	15.17	58.70	2.76	3.75	3.94	10.45
60 Kg N/fed + *Rhizobium* spp.	12.81	20.10	10.50	43.41	2.04	3.20	2.50	7.74
60 Kg N/fed + *Bradyrhizobium* spp.	12.27	21.17	11.83	45.27	1.72	3.13	2.75	7.60
60 Kg N/fed + *Azotobacter* spp. + *Azospirillum* spp	15.03	27.50	13.47	56.00	2.50	4.28	3.50	10.28
60 Kg N/fed+*Bcaillus megatherium*+*Bacillus polymyxa*	15.00	23.47	12.50	50.97	2.26	3.35	3.16	8.77
LSD 0.05	2.60	3.78	2.51	6.48	0.55	0.57	0.58	1.13

Table (3): **Morphological parameters of fodder millet as affected by different treatments of bio-fertilizers and N- fertilizers (combined analysis over two seasons 2001 and 2002).**

Treatments	Plant height (cm)			Number of tillers per plant		
	Cuts			Cuts		
	1st	2nd	3rd	1st	2nd	3rd
0 nitrogen	76.67	103.30	108.30	6	6	5
60 kgN/fed	80.00	116.70	125.00	8	7	6
120 kgN/fed	96.67	136.70	151.70	13	10	8
60 Kg N/fed + *Rhizobium* spp.	91.67	130.00	153.40	10	7	8
60 Kg N/fed + *Bradyrhizobium* spp.	75.00	116.70	116.00	8	8	6
60 Kg N/fed + *Azotobacter* spp. + *Azospirillum* spp.	95.00	133.30	163.30	11	10	9
60 Kg N/fed + *Bacillus megatherium* + *B. polymyxa*	88.33	128.30	141.70	12	8	7
LSD 0.05	6.58	12.28	14.00	2.13	1.58	1.83

Regarding the influence of the inoculation with different inoculant treatments on plant height, it is demonstrated that pearl millet plants inoculated with *Azotobacter* + *Azospirillum* gave the highest magnitudes, where their height reached 95.00, 133.30 and 163.30 cm in the three successive cuts, respectively. In this respect, Saleh *et al.* (2000) reported that inoculation of maize plants by composite inocula and half dose of N-fertilization (60 kg N/fed) gave significant increases in plant height of 20.20% over the uninoculated plants.

Number of tillers

Number of tillers as shown in Table (3) indicated the presence of significant differences in number of tillers per plant in all three cuts. First cut had a highest number of tillers (13 tillers $plant^{-1}$) compared to second (10 tillers $plant^{-1}$) and third cut (8 tillers $plant^{-1}$). Nitrogen fertilization by 120 kg N/fed. lead to significant increase in number of tillers in first and second cuts while inoculation with *Bradyrhizobium* had insignificant effect. On the contrary, inoculation of pearl millet with *Rhizobium* and ((*Azotobacter* + *Azospirillum*) under 60 kgN/fed., significantly increased number of tillers in second and third cuts while, (*B. megatherium* + *B. polymyxa*) recorded the highest number of tillers with the first one.

Root dry weight

Means of root dry weight varied among the various treatments (Fig. 1). The lowest root dry weight was attributed to uninoculated treatments fertilized with low N fertilization. While, the opposite trend was noticed with all inoculation treatments. Application of *Azotobacter* + *Azospirillum* recorded highest root dry weight (33.14 g/plant) followed by full N-dose treatment (28.15 g/plant). In addition to inoculation with *B. megatherium* + *B. polymyxa* had a positive response on root dry weight reached to 24.84 g/plant. The positive effect of inoculation with PGPR on root dry weight was related to biological nitrogen fixation and production of phytohormons that promote root development and proliferation (Hartmann *et al.*, 1983 and Haahtela *et al.*, 1990).

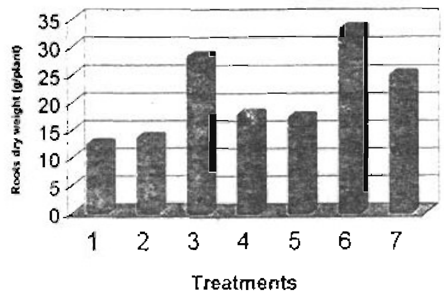

Fig. (1): Response of roots dry weight of pearl millet to inoculation with different bacterial strains

Forage quality (chemical composition)

Data presented in Table (4) indicated that increasing level of nitrogen from 0.0, 60.0 and 120.0 kg N/fed progressively increased crude protein, crude fiber and ash yields in individual cuts as well as in total seasonal yield. Inoculation significantly increased crude protein, crude fiber and ash seasonal yield particularly with treatments (*B. megatherium* + *B. polymyxa*) and (*Azotobacter* + *Azospirillum*) and the increases ranged from 28.59-63.41, 8.90-25.46 and 49.39-89.38% over the uninoculated ones that received the same amount of N-fertilizer respectively. In addition, significant increases with crude protein and ash seasonal yield only were attributed to treatment with *Bradyrhizobium* that reached to 22.95% and 43.42% compared with the control for the two components respectively. On the other hand, insignificant effects were noticed on the same traits by inoculation with *Bradyrhizobium* compared to treatment received (60 kg N/fed). Application of 120 kg N/fed gave crude protein, crude fiber and ash yield similar to that obtained from inoculation with (*Azotobacter* + *Azospirillum*). These results are in agreement with those reported by Sabry *et al.* (2001).

Nitrogen fixers and phosphate dissolving bacteria were reported to increase protein yield in pearl millet (Smith *et al.*, 1976 and Mahmoud *et al.*, 1994). Bashan *et al.* (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 (Pacoveskey *et al.*, 1985 and Kapulnike *et al.*, 1985).

The high percent increases of ash due to inoculation with the same strains showed an indirect clue on possible beneficial mechanism other than N_2-fixation of this particular system. These includes support of plant growth through hormones produced by inoculated organism, increases of root biomass as well as uptake of such nutrient in soil (Kapulnike *et al.*, 1981 and Pacovesky *et al.*, 1985).

Table (4): Crude protein, crude fiber and ash yield ton/fed as affected by different treatments of bio-fertilizers and N- fertilizers of fodder pearl millet (combined analysis over two seasons 2001 and 2002).

Treatments	Crude protein yield (ton/fed)				Crude fiber yield (ton/fed)				Ash yield (ton/fed)			
	Cuts				Cuts				Cuts			
	1st	2nd	3rd	Total	1st	2nd	3rd	Total	1st	2nd	3rd	Total
0 nitrogen	0.109	0.172	0.146	0.427	0.622	0.949	0.826	2.397	0.230	0.333	0.296	0.859
60 kgN/fed	0.172	0.282	0.172	0.626	0.603	1.045	0.881	2.529	0.291	0.425	0.357	1.073
120 kgN/fed	0.278	0.374	0.348	1.000	0.812	1.192	1.323	3.327	0.495	0.607	0.617	1.719
60 kgN/fed + *Rhizobium* spp.	0.224	0.352	0.251	0.827	0.729	1.105	0.957	2.791	0.351	0.542	0.431	1.324
60 kgN/fed + *Bradyrhizobium* spp.	0.124	0.225	0.176	0.525	0.605	1.072	1.044	2.721	0.283	0.529	0.420	1.232
60 kgN/fed + *Azotobacter* spp. + *Azospirillum* spp.	0.282	0.411	0.330	1.023	0.731	1.299	1.143	3.173	0.559	0.821	0.652	2.032
60 kgN/fed + *Bacillus megatherium* + *Bacillus polymyxa*	0.208	0.307	0.289	0.805	0.689	1.016	1.048	2.753	0.446	0.617	0.540	1.603
LSD 0.05	0.046	0.063	0.039	0.119	0.191	0.203	0.198	0.368	0.068	0.098	0.086	0.196

Table (5): Phosphorus and potassium kg/fed as affected by different treatments of bio-fertilizers and N-fertilizers of fodder pearl millet (combined analysis over two seasons 2001 and 2002).

Treatments	Phosphorus (kg/fed)				Potassium (kg/fed)			
	Cuts				Cuts			
	1st	2nd	3rd	Total	1st	2nd	3rd	Total
0 nitrogen	8.35	14.32	12.27	34.94	48.50	75.12	61.88	185.50
60 kgN/fed	10.87	16.33	12.20	39.40	57.29	96.17	72.50	225.96
120 kgN/fed	13.52	19.64	20.22	53.38	81.24	105.00	102.30	288.54
60 kgN/fed + *Rhizobium* spp.	7.98	13.55	13.84	35.37	69.15	105.60	77.48	252.23
60 kgN/fed + *Bradyrhizobium* spp.	8.47	15.40	14.37	38.24	53.32	93.23	76.91	223.46
60 kgN/fed + *Azotobacter* spp. + *Azospirillum* spp.	14.48	23.22	18.10	55.80	73.69	124.20	94.48	292.37
60 kgN/fed + *Bacillus megatherium* + *Bacillus polymyxa*	11.16	21.48	18.45	51.09	63.86	95.00	78.17	237.03
LSD 0.05	3.04	3.18	3.65	6.28	16.20	17.30	15.87	43.78

It is clear from the data presented in Table (5) that second cut had the highest value of P and K yield (23.22; 124.20 kg/fed, respectively) compared with the first and third cuts. According to the data presented in the same table, it appears that using 0.0 and 60 kg N/fed., only or with *Rhizobium* and *Bradyrhizobium* resulted in lower values of P and K yield. While, inoculation of forage pearl millet with (*Azotobacter* + *Azospirillum*) did significantly affect P and K yield. Increases in total P and K yield due to inoculation with these strains were 29.39 and 41.62%; for the two elements respectively compared to uninoculated treatment received 60 kg N/fed. However, (*B. megatherium* + *B. polymyx)* showed a positive effect only with P which reached to 51.09 kg/fed. In this respect, no significant differences were noticed between application of 120 kg N/fed and inoculation with (*Azotobacter* + *Azospirillum*) at first and third cuts but the opposite trend was with the second cut in P and K yields.

The increase in both phosphorus and potassium due to the addition of diazotrophs and phosphate dissolving bacteria as a biofertilizers may be partially explained according to the findings of Sundara Rao (1974) who showed that some microorganisms tend to reduce the soil pH, since they produce organic acids, which may have a possible role in solublizing phosphate and other minerals. Our data are similar with several workers who reported that inoculation of plant with phosphate solublizing microorganisms frequently stimulates plant growth by increasing phosphorus and potassium uptake (Chabot *et al.*, 1993 and Kucey *et al.*, 1989).

CONCLUSION

In general, it could be concluded that, application of biofertilizers combined with the medium dose of N-fertilizer i.e 60 kg/N/fed for forage pearl millet seems to be more beneficial from the economical point of view and pollutions as well. Reasonably, higher green and dry forage yield as well as chemical compositions could be obtained with application of inocula contains at least nitrogen fixers and phosphate dissolving bacteria.

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تأثير البكتريا المثبتة للأزوت الجوى والمحللة للفوسفات على المحصول والتركيب الكيماوى فى دخن العلف

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أجري هذا البحث بقسم بحوث محاصيل العلف التابع لمعهد المحاصيل الحقلية – مركز البحوث الزراعية في محطة بحوث الجيزة خلال الموسمين الصيفيين ٢٠٠١ – ٢٠٠٢ وذلك لتقييم تأثير التلقيح ببعض السلالات البكتيرية المختلفة و المستخدمة كمخصبات حيوية على المحصول و الجودة فى دخن العلف (صنف شندويل -١) وكانت كل معاملات التلقيح المستخدمة فى هذه الدراسة يضاف إليها ٦٠ كجم نتروجين تعادل نصف الكمية الموصى بها فى تسميد الدخن وهى ١٢٠ كجم نتروجين للفدان.

وقد أوضحت النتائج المتحصل عليها أن استخدام السلالات البكتيرية كمخصبات حيوية وخاصة الأزوتوباكتر + الأزوسبيريللم وأيضا الباسيلس ميجاثيريم + باسيلس بوليميكسا المضاف إليها ٦٠ كجم نيتروجين للفدان كان له تأثير قوى على نمو النباتات وذلك من خلال التأثير الأيجابى على محصول العلف الأخضر والجاف في كل الحشات وأيضا المحصول الموسمى (طن/فدان). أيضا نفس الاتجاه تم الحصول عليه فى كل من ارتفاع النباتات، عدد الأفرع، وزن الجذور وأيضا العناصر المعدنية وخاصة الفوسفور والبوتاسيوم وكذلك محصول البروتين و الألياف والرماد.

وقد لوحظ أن الدخن الملقح بسلالات الأزوتوباكتر + الأزوسبيريللم كانت قيم المتوسطات له مشابهة لتلك التى تم الحصول عليها باستخدام جرعة تسميد عالية من النيتروجين (١٢٠ كجم نيتروجين للفدان). ومما سبق يتضح أن استخدام المخصبات الحيوية مضاف إليها نصف جرعة التسميد النيتروجينى الموصى بها يؤدى إلى خفض التكاليف وكذلك خفض التلوث البيئي الناتج عن الإفراط في استخدام التسميد النيتروجينى المعدنى.