

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Effect of Inorganic Nitrogenous Fertilizer on Productivity of Recently Reclaimed Saline Sodic Soils with and Without Biofertilizer

S.M. Mehdi, M. Sarfraz, G. Shabbir and G. Abbas
Soil Salinity Research Institute, Pindi Bhattian, Pakistan

Abstract: Saline sodic soils after reclamation become infertile due to leaching of most of the nutrients along with salts from the rooting medium. Microbes can play a vital role in the productivity improvement of such soils. In this study a saline sodic field having EC_e 6.5 dS m^{-1} , pH, 9.1 and gypsum requirement (GR) 3.5 tons $acre^{-1}$ was reclaimed by applying gypsum at the rate of 100% GR. Rice and wheat crops were transplanted/sown for three consecutive years. Inorganic nitrogenous fertilizer was used with and without biofertilizers i.e., Biopower (*Azospirillum*) for rice and diazotroph inoculums for wheat. Nitrogen was applied at the rate of 0, 75% of recommended dose (RD), RD, 125% of RD and 150% of RD. Recommended dose of P without K was applied to all the plots. Biopower significantly improved Paddy and straw yield of rice over inorganic nitrogenous fertilizer. In case of wheat diazotroph inoculum improved grain and straw yield significantly over inorganic nitrogenous fertilizer. Among N fertilizer rates, RD + 25% additional N fertilizer was found to be the best dose for rice and wheat production in recently reclaimed soils. Nitrogen concentration and its uptake by paddy, grain and straw were also increased by biopower and diazotroph inoculum over inorganic nitrogenous fertilizer. Among N fertilizer rates, RD + 25% additional N fertilizer was found to be the best dose for nitrogen concentration and its uptake by paddy, grain and straw. Total soil N, available P and extractable K were increased while salinity/sodicity parameters were decreased with the passage of time. The productivity of the soil was improved more by biofertilizers over inorganic N fertilizers.

Key words: Saline sodic soils, biofertilizers, productivity, rice and wheat yield

INTRODUCTION

In the current agriculture, nitrogen is a limiting nutrient for growth and yield of crops. This element (N_2) is found in the gaseous form in atmospheric air and plants and animals do not get to use it in this form for their metabolism (Dobereiner, 1997). The plants get nitrogen, mainly from the application of nitrogen fertilizers, industrially synthesized from the atmospheric dinitrogen (N_2). This element, becomes also available for the plants by the Biological Nitrogen Fixation (BNF), realized by some bacteria denominated diazotroph, which possesses an enzymatic apparatus capable to break the triple bond between two nitrogen atoms from the atmospheric nitrogen, forming ammonia that is similar to the industrial process (Dobereiner and Baldani, 1998; Okon and Vanderleyden, 1998; Victoria *et al.*, 1992).

Nitrogen that is one of the essential elements is absorbed in great quantities by the plants, needing to be available at high concentration in the soil (Dalla Santa,

2004). Among the agricultural systems that contribute to the recycle of lost nitrogen to the atmosphere, the most important are the symbiosis of bacteria's with leguminous plants although, other less specific associations with cereals and various Graminae are standing out (Dobereiner, 1997).

The knowledge of nitrogen biological fixation in non-leguminous plants, mainly in Graminae, among them several cereals, became one of the largest challenges (Boddey and Dobereiner, 1994; Dobereiner, 1997). Microorganisms capable of fixing nitrogen in free life, in spite of important in number, in general contribute a little with fixed nitrogen. The effects of those bacteria's are mainly in the promotion of plants root growth, as they produce growth-promoting substances. The genera *Azospirillum*, *Herbaspirillum*, *Azotobacter* and *Acetobacter* show generalized occurrence in economically important cultures such as corn, rice, sorghum and sugar cane, like this being with frequency, in experiments seeking the agronomic utilization as biofertilizers

(Dobereiner, 1997; Reinhold and Hurek, 1988; Sundaram *et al.*, 1988). The bacteria of the *Azospirillum* genera presents application potential in agricultural systems, around 70% of the experiments up to 30% in productivity (Bashan and Holguin, 1997; Fani *et al.*, 1995; Dalla Santa, 2004).

The promotion of plants growth inoculated with *Azospirillum* has been obtained in field conditions and greenhouse experiments, resulting in significant changes in several characteristics of the plants. The inoculation can cause increase in the dry weight and in the accumulation of total nitrogen of the plant, in the grain yields and in the weight of grains, in the germination rate of the seeds and in changes in the duration of the plants growth stages (Boddey and Dobereiner, 1988; Sumner, 1990; Fages, 1994; Fallik and Okon, 1996; Pandey *et al.*, 1998).

The *Azospirillum* inoculation responses in non-leguminous plants are still difficult to estimate. The results of inconsistency in inoculation experiments is related with the inoculation techniques, inoculation rate, low survival of inoculated strains, physical and chemical characterization of the soil, Physiological state of the bacteria, improper strain, plant genotype, presence of high number of native microorganisms and pesticide influence (Bashan *et al.*, 1995; Fages, 1994). Keeping all this in view a study was planned with the objective to compare the effect of single application of inorganic nitrogenous fertilizer and combined application of inorganic nitrogenous fertilizer and biofertilizers on productivity of recently reclaimed saline sodic soil.

MATERIALS AND METHODS

A field experiment was conducted at Haveli Karim Dad, tehsil Pindi Bhattian, Distt. Hafizabad during the year 2000 to 2003 to see the effect of bio-fertilizers and inorganic fertilizer alone on the rice and wheat in recently reclaimed soils. A barren saline-sodic field having EC_e 6.5 dS m⁻¹, pH_s 9.1 and gypsum requirement (GR) 3.5 tons acre⁻¹ was reclaimed by applying gypsum at the rate of 100% G.R i.e., 3.5 tons acre⁻¹. The field was flooded with good quality of water for one month to complete the reclamation and after that rice variety Shaheen Basmati was transplanted in the field. In bio-fertilizer treated plots, the seedlings were dipped for one and half hour before transplanting in slurry of Bio-Power inoculum of NIBGE, which was prepared by mixing inoculum in 10% sucrose solution.

The following treatment combinations were applied to rice crop.

Treatment	Set-A (N-P-K)	Set-B (Bio-Power + N-P-K)
T ₁	0-0-0	Bio-Power + 0-0-0
T ₂	75% RD N-110-60	Bio-Power + 75% RD N-110-60
T ₃	Recommended Dose.	Bio-Power + Recommended Dose
T ₄	125% RD N-110-60	Bio-Power + 125% RD N-110-60
T ₅	150% RD N-110-60	Bio-Power + 150% RD N-110-60

RD = Recommended Dose

The system of lay out was split plot with bio-fertilizer in main plot and rate of N application in sub-plots. There were three replications. Zinc Sulphate (ZnSO₄) was also applied at the rate of 12.5 kg ha⁻¹ (33% pure). All cultural practices were applied when required. The rice crop was harvested at maturity and yield data were recorded. Paddy and straw samples were collected for N analysis. After rice crop, in the same layout, wheat crop was sown (Fifteen days after rice harvest) and instead of Bio-Power, diazotroph inoculum (mixture of *Azotobacter* and *Azospirillum* cultures) of Bacteriology Section AARI, Faisalabad was used. Other treatments were same as for rice. All the cultural practices were applied when required. Crop was harvested at maturity, grain and straw yield data were recorded and also samples were collected for total N analysis. The same experiment continued for three consecutive years in the same layout. After the harvest of each crop, the soil samples were collected for chemical (EC_e, pH_s, SAR) and nutritional (total N, available P and extractable K) analysis. The analyses were done according to the methods given in Hand Book No. 60 (US Salinity Lab. Staff, 1954) except texture by Moodie *et al.*, (1959), total N in soil and plant samples by Jackson (1962), Available P in soil by Watanabe and Olsen (1965). All the data were statistically analyzed by using methods of Steel and Torrie (1980).

RESULTS AND DISCUSSION

The average of three years data showed that paddy and straw yields (Table 1) were increased significantly by bio-fertilizer+ inorganic nitrogenous fertilizers over nitrogenous fertilizer alone. The effect of fertilizer application rates remained significant on both paddy and straw of rice. The yield was increased with the increasing rates of N application and optimum rate determined was recommended dose with 25% additional of recommended N while recommended dose with 50% additional of recommended N remained nonsignificant with 25% additional of recommended N. The interaction between chemical fertilizer and bio-fertilizer was also significant in case of both paddy and straw of rice. Maximum paddy and straw yields were recorded in the combination of biofertilizer + RD N fertilizer plus 25% additional of recommended N.

In case of wheat, the three years pooled data indicated that grain and straw yields were also improved significantly by the application of bio-fertilizer + nitrogenous fertilizer over nitrogenous fertilizers alone (Table 2). Inorganic N application rates increased the grain and straw yield of wheat during the study. Yield was increased with the increasing rates of N application and optimum dose for grain and straw was found recommended dose with 25% additional dose of recommended N. The interaction between chemical fertilizer and bio-fertilizer was also significant in case of both grain and straw of wheat. Maximum grain and straw yields were recorded in the combination of biofertilizer + RD. N fertilizer plus 25% additional of recommended N. The effect of bio-fertilizer on paddy and straw of rice, grain and straw of wheat over chemical fertilizer might be due to the reason, when salt affected soils are reclaimed by the addition of amendments followed by subsequent leaching results in leaching of salts and deficiency of the nutrients in these soils. Moreover, there is no biological

activity in the soils, that start after reclamation and the application of bio-fertilizer enhances the biological activity and improved the fertility status of the recently reclaimed soils. This process is further increased by the application of inorganic fertilizer that resulted in increasing of yield. Similar results were reported by Reinhold and Hurek (1988), Sundaram *et al.* (1988), Fani *et al.* (1995), Dobereiner (1997), Bashan and Holguin (1997) and Dalla Santa *et al.* (2004). The increase in paddy/grain and straw of rice and wheat in control plots with the passage of time might be due to the improvement in the fertility status of the soil due to the application of Biopower and Diazotroph inoculum and organic residues of crop plants (Roots) increased organic matter content of soil resulting in improvement of yield. Similar explanation was given by Dobermann and Fairhurst (2000).

The average of three years data showed that nitrogen concentration in paddy and straw of rice was affected significantly by the application of bio-fertilizer + inorganic nitrogenous fertilizer over inorganic N fertilizers (Table 3).

Table 1: Comparative effects of inorganic nitrogenous fertilizer and inorganic nitrogenous and bio-fertilizer mixture on paddy and straw yield of rice (t ha⁻¹)

Treatments	Paddy			Straw		
	N fertilizer	Bio-fertilizer +N fertilizer	Mean	N fertilizer	Bio-fertilizer +N fertilizer	Mean
T ₁ Control	1.430e	1.520e	1.475D	3.39f	3.60f	3.495D
T ₂ 75% (N) R.D+P	2.200d	2.370d	2.285C	5.74e	6.43d	6.085C
T ₃ (N) RD + P	2.680c	2.910b	2.795B	6.76cd	7.44b	7.100B
T ₄ 125%(N) RD +P	3.050ab	3.180a	3.115A	7.18bc	8.30a	7.740A
T ₅ 150%(N) RD +P	3.060ab	3.160a	3.110A	7.11bc	8.30a	7.705A
Mean	2.484B	2.628A	-	6.036	6.814	-

Each figure is average of three years. RD = Recommended Dose. Figures having the same letter(s) are non significant at 5% level of probability

Table 2: Comparative effects of inorganic nitrogenous fertilizer and inorganic nitrogenous and bio-fertilizer mixture on grain and straw yield of wheat (t. ha⁻¹)

Treatments	Grain			Straw		
	N fertilizer	Bio-fertilizer +N fertilizer	Mean	N fertilizer	Bio-fertilizer +N fertilizer	Mean
T ₁ Control	1.03e	1.09e	1.06D	1.37d	1.49d	1.43D
T ₂ 75% (N) R.D+P	2.10d	2.22c	2.16C	2.97c	3.12c	3.045C
T ₃ (N) RD + P	2.36b	2.45ab	2.405B	3.46b	3.60ab	3.53B
T ₄ 125%(N) RD +P	2.45ab	2.52a	2.485A	3.69ab	3.76a	3.725A
T ₅ 150%(N) RD +P	2.40b	2.45ab	2.425AB	3.61ab	3.67ab	3.64AB
Mean	2.068B	2.146A	-	3.02B	3.128A	-

Each figure is average of three years. RD = Recommended Dose. Figures having the same letter(s) are non significant at 5% level of probability

Table 3: Comparative effects of inorganic nitrogenous fertilizer and inorganic nitrogenous and bio-fertilizer mixture on Nitrogen concentration in paddy and straw (%)

Treatments	Paddy			Straw		
	N fertilizer	Bio-fertilizer +N fertilizer	Mean	N fertilizer	Bio-fertilizer +N fertilizer	Mean
+N fertilizer	Mean	N fertilizer	Bio-fertilizer			
+N fertilizer	Mean					
T ₁ Control	1.06f	1.11e	1.085D	0.35g	0.38f	0.365D
T ₂ 75% (N) R.D+ P	1.19d	1.25c	1.22C	0.42e	0.48d	0.45C
T ₃ (N) RD + P	1.24c	1.30b	1.27B	0.49c	0.54b	0.515B
T ₄ 125%(N) RD +P	1.30b	1.31ab	1.305A	0.56a	0.55ab	0.555A
T ₅ 150%(N) RD +P	1.31ab	1.32a	1.315A	0.56a	0.56a	0.56A
Mean	1.22B	1.258A	-	0.476B	0.502A	-

Each figure is average of three years. RD = Recommended Dose. Figures having the same letter(s) are non significant at 5% level of probability

The increasing rates of N application increased N concentration in paddy and straw significantly and optimum rate determined was recommended dose with 25% additional N of recommended dose and the higher rates remained non-significant. The interaction between bio-fertilizer and inorganic N fertilizer remained significant and maximum nitrogen concentration in paddy and straw was recorded in the combination of biofertilizer + RD. N fertilizer plus 50% additional of recommended N.

In case of wheat, the mean of three years data indicated that bio-fertilizer + inorganic nitrogenous fertilizers significantly increased nitrogen concentration in grain and straw over chemical fertilizer (Table 4). Like rice, the increasing rates of N application significantly increased the N concentration in grain and straw of wheat. The optimum rate of N application both in grain and straw of wheat was found recommended N with 25% additional of recommended N. The interaction between bio-fertilizer and inorganic nitrogenous fertilizer remained significant in case of both N concentration in grain and straw of wheat during whole the course of study. Maximum nitrogen concentration in grain and straw was recorded in the combination of biofertilizer + RD. N fertilizer plus 50% additional of recommended N. The results indicated that the effect of bio-fertilizer + inorganic N fertilizer over chemical fertilizers was significant in increasing the N concentration of grain and straw of wheat and the concentration was continuously increased with the passage of time. The effect of bio-fertilizer over nitrogenous fertilizer both in paddy and straw of rice and

grain and straw of wheat was found significant that might be due the reason that there was a microbial activity initiated after the reclamation. So, the bio-fertilizer played the vital role in increasing the N concentration in paddy and straw of rice and grain and straw of wheat. The microbial activity became more effective in both the plots in which bio-fertilizers + inorganic N fertilizers were applied. So, response to bio-fertilizers was recorded. The increasing rates of N application increased the N concentration in paddy and straw of rice and in grain and straw of wheat because the soil was deficient in total N during all the three years. So, response was recorded during the whole course of study up to a certain level i.e. recommended dose with 25% additional of recommended N and higher rates remained at par. Similar findings were reported by Boddey and Dobereiner (1988), Sumner (1990), Fages (1994), Bashan *et al.* (1995), Fallik and Okon (1996) and Pandey *et al.* (1998).

The three years average data of N-uptake by paddy and straw of rice showed that this was significantly affected by application of bio-fertilizer + inorganic N fertilizer over nitrogenous fertilizers (Table 5). The increasing rate of N application increased the N-uptake both in paddy and straw of rice during the course of study and optimum dose was found to be recommended dose with 25% additional N of recommended dose in case of paddy and recommended dose with 50% additional N of recommended dose in case of straw of rice and higher rate in case of paddy remained nonsignificant with recommended dose plus additional 25% N of

Table 4: Comparative effects of inorganic nitrogenous fertilizer and inorganic nitrogenous and bio-fertilizer mixture on Nitrogen concentration in wheat grain and straw (%)

Treatments	Grain			Straw		
	N fertilizer	Bio-fertilizer +N fertilizer	Mean	N fertilizer	Bio-fertilizer +N fertilizer	Mean
T ₁ Control	1.37e	1.39e	1.38D	0.51g	0.56f	0.535D
T ₂ 75% (N) R.D+P	1.50d	1.53d	1.515C	0.61e	0.67d	0.640C
T ₃ (N) RD + P	1.60c	1.69b	1.645B	0.73c	0.79b	0.760B
T ₄ 125%(N) RD +P	1.71ab	1.72ab	1.715A	0.80ab	0.82ab	0.810A
T ₅ 150%(N) RD +P	1.72ab	1.73a	1.725A	0.82ab	0.83a	0.825A
Mean	1.58B	1.612A		0.694B	0.734A	

Each figure is average of three years. RD = Recommended dose, Figures having the same letter(s) are non significant at 5% level of probability.

Table 5: Comparative effects of inorganic nitrogenous fertilizer and inorganic nitrogenous and bio-fertilizer mixture on Nitrogen Uptake by paddy and straw (kg ha⁻¹)

Treatments	Paddy			Straw		
	N fertilizer	Bio-fertilizer +N fertilizer	Mean	N fertilizer	Bio-fertilizer +N fertilizer	Mean
T ₁ Control	11.12f	12.03f	11.575D	4.83g	5.62g	5.225E
T ₂ 75% (N) R.D+P	20.10e	22.00e	21.05C	11.10f	14.06e	12.58D
T ₃ (N) RD + P	26.13d	28.78c	27.455B	16.76d	20.46c	18.61C
T ₄ 125%(N) RD +P	30.54bc	32.20ab	31.37A	21.80c	25.40b	23.60B
T ₅ 150%(N) RD +P	31.95ab	33.46a	32.705A	24.24b	27.95a	26.095A
Mean	23.968B	25.694A		15.746B	18.698A	

Each figure is average of three years. RD = Recommended dose. Figures having the same letter(s) are non significant at 5% level of probability

recommended dose. The interaction between bio-fertilizer and inorganic fertilizer remained significant in case of both N uptakes by paddy and straw of rice. Maximum nitrogen uptake in paddy and straw was recorded in the combination of biofertilizer + RD. N fertilizer plus 50% additional of recommended N.

The three years pooled data of N-uptake by grain and straw of wheat was found significant and more N uptake was observed by the application of bio-fertilizer + inorganic nitrogenous fertilizer over chemical fertilizers (Table 6). The increasing rates of N application significantly affected the N-uptake by grain and straw during the course of study and optimum dose was found to be the recommended dose plus 25% additional N of recommended dose and higher rate was non-significant. The interaction between bio-fertilizer + inorganic N fertilizers and inorganic fertilizer also remained significant in case of both grain and straw of wheat during whole the

course of study. The effect of bio-fertilizer over chemical fertilizer on N-uptake in case of paddy and straw was significant that might be due to the reason that microbial activity started after reclamation of soil which resulted in more availability of N to plants for uptake than chemical fertilizer so more N uptake was observed in bio-fertilizer treated plots over chemical fertilizers treated plots. Similarly in case of wheat more nitrogen was taken up by plants in biofertilizers treated plots over chemical fertilizers treated plots. The reason might be that diazotroph also played a vital role in making more N available to wheat plants resulting in more uptake of N. Similar results were reported by Boddey and Dobereiner (1988), Sumner (1990), Fages (1994), Fallik and Okon (1996) and Pandey *et al.* (1998).

The post-harvest soil analysis indicated that chemical and bio-fertilizers decreased the chemical characteristics of soil i.e., EC_e, pH_e and SAR continuously

Table 6: Comparative effects of inorganic nitrogenous fertilizer and inorganic nitrogenous and bio-fertilizer mixture on Nitrogen uptake by wheat grain and straw (kg ha⁻¹)

Treatments	Grain			Straw		
	N fertilizer	Bio-fertilizer +N fertilizer	Mean	N fertilizer	Bio-fertilizer +N fertilizer	Mean
T ₁ Control	11.42f	12.21f	11.815D	5.17d	6.19d	5.68D
T ₂ 75% (N) RD+P	25.01e	27.55d	26.28C	13.72c	15.89c	14.805C
T ₃ (N) RD + P	31.70c	33.69bc	32.695B	19.46b	21.85ab	20.655B
T ₄ 125%(N) RD +P	35.26ab	36.49a	35.875A	22.52a	24.45a	23.485A
T ₅ 150%(N) RD +P	35.17ab	36.17ab	35.67A	22.80a	23.55a	23.175A
Mean	27.712B	29.222A		16.734B	18.386A	

Each figure is average of three years. RD = Recommended dose. Figures having the same letter(s) are non significant at 5% level of probability

Table 7: Post rice harvest soil analysis 2000

Treatments	EC _e (dS m ⁻¹)	pH _e	SAR (m mol L ⁻¹) ^{1/2}	Total N (%)	Available P (mg kg ⁻¹ soil)	Extractable K (mg kg ⁻¹ soil)
N fertilizer						
T ₁ Control	3.82	8.41	22.8	0.036	5.80	78
T ₂ 75% (N) RD +P	3.78	8.39	22.1	0.041	6.10	79
T ₃ (N) RD +P	3.76	8.38	22.0	0.046	6.00	81
T ₄ 125%(N) RD +P	3.58	8.31	19.6	0.049	6.24	82
T ₅ 150%(N) RD +P	3.54	8.35	19.2	0.052	6.25	80
N fertilizer + bio-fertilizer						
T ₁ Control	3.85	8.43	22.4	0.037	5.91	77
T ₂ 75% (N) RD+P	3.69	8.36	22.2	0.043	6.02	78
T ₃ (N) RD + P	3.74	8.33	19.6	0.048	6.35	80
T ₄ 125%(N) RD +P	3.57	8.31	19.2	0.051	6.37	82
T ₅ 150%(N) RD+P	3.48	8.30	19.0	0.056	6.29	82

Table 8: Post wheat harvest analyses of the soil (2002-03)

Treatments	EC _e (dS m ⁻¹)	pH _e	SAR (m mol l ⁻¹) ^{1/2}	Total N(%)	Available P (mg kg ⁻¹ soil)	Extractable K (mg kg ⁻¹ soil)
N fertilizer						
T ₁ Control	3.20	8.15	13.59	0.019	5.01	90
T ₂ 75% (N) RD +P	3.30	8.16	14.16	0.056	7.32	96
T ₃ (N) RD +P	3.21	8.16	14.12	0.058	7.46	100
T ₄ 125%(N) RD +P	3.19	8.15	14.10	0.061	7.54	104
T ₅ 150%(N) RD +P	3.16	8.15	13.78	0.063	7.55	105
N fertilizer + bio-fertilizer						
T ₁ Control	2.71	8.21	14.68	0.023	5.19	89.0
T ₂ 75% (N) RD+P	2.65	8.20	14.02	0.058	7.24	98.0
T ₃ (N) RD + P	2.60	8.20	12.68	0.058	7.51	104.0
T ₄ 125%(N) RD +P	2.82	8.20	13.72	0.064	7.42	108.0
T ₅ 150%(N) RD+P	2.79	8.18	14.62	0.065	7.57	110.0

with the passage of time from first year to third year and increased the fertility status of soil i.e., N, P and K of soil (Table 7 and 8). The decrease in chemical characteristics was more in case of bio-fertilizer than chemical fertilizer. Whereas improvement in fertility status was more in bio-fertilizer treated plots than the inorganic fertilizer during all the three years. The reason might be that there was more population of plants in bio-fertilizer over chemical fertilizer (yield), which resulted in more decrease of EC_e, pH_s and SAR due to their reclamative effects. Hence these parameters were decreased. More population in bio-fertilizer treated plots over chemical fertilizer produced more root bio-mass resulting in the more production of OM in soil and on their decomposition fertility status of soil was improved in case of bio-fertilizer treated plots over the inorganic fertilizer treated plots. Similar results were reported by Dobermann and Fairhurst (2000).

REFERENCES

- Bashan, Y., E. Puente, N.N. Rodriguez-Mendoza, G. Holguin, G. Toledo, R. Ferrera-Cerrato and S. Pedrin, 1995. Soil Parameters Which Affect the Survival of *Azospirillum brasilense*. In: *Azospirillum and Related Microorganisms*. Fendrik, I., M., Del Gallo, J. Vanderleyden and M. Zamaroczy (Eds.), pp: 441-450. Germany: Springer, Verlag.
- Bashan, Y. and G. Holguin, 1997. *Azospirillum*-plant relationships: Environmental and physiological advances (1990-1996). Can. J. Microbiol., 43: 103-121.
- Boddey, R.M. and J. Dobereiner, 1988. Nitrogen fixation associated with grasses and cereals: Recent results and perspectives for future research. Plant Soil, 108: 53-65.
- Boddey, R.M. and J. Dobereiner, 1994. Biological Nitrogen Fixation Associated with Graminaceous Plants. In: Okon, Y. (Ed.). *Azospirillum Plant Associations*. USA: CRC-Press, pp: 119-130.
- Dalla Santa, O.R., R.F. Hernandez, L. Gergina, M. Alvarez, P.R. Junior and C.R. Soccol, 2004. *Azospirillum* sp. Inoculation in wheat, barley and oats seed green house experiment. Braz. Arch. Biol. Technol., 47: 843-850.
- Dobereiner, J., 1997. Biological nitrogen fixation in tropics: Social and economic contribution. Soil Biol. Biochem., 29: 771-774.
- Dobereiner, J. and V.L.D. Baldani, 1998. New biocombustiveis technologies. Biotechnol. Sci. Dev. Bras., 1: 16-17.
- Dobermann and T. Fairhurst, 2000. Nutrient Disorders and Nutrient Management. Int. Rice Res. Inst. Philippines. Oxford Graph Printers Pvt. Ltd., pp: 191.
- Fages, J., 1994. *Azospirillum* Inoculants and Field Experiments, In: *Azospirillum Plant Associations*. Okon, Y. (Ed.), pp: 88-105. USA: CRC-Press.
- Fallik, E. and Y. Okon, 1996. The response of maize (*Zea mays*) to *Azospirillum* inoculation in various types of soils in the field. World J. Microb. Biotechnol., 12: 511-515.
- Fani, R., C. Bandi, M. Bazzicalupo, G. Damiani, F. Di Cello, S. Fancelli, E. Gallori, L. Gerace, A. Grifoni, P. Lio and E. Mori, 1995. Phylogenetic Studies of the Genus *Azospirillum*. In: *Azospirillum and Related Microorganisms*. Fendrik, I., M. Del Gallo, J. Vanderleyden and M. Zamaroczy (Eds.), pp: 59-75. Germany: Springer Verlag.
- Jackson, M. L., 1962. Soil Chemical Analysis Prentice Hall, Englewood Cliffs, New York, USA.
- Moodie, C.D., H.W. Smith and R.A. Mc Creery, 1959. Laboratory manual for soil fertility. State College Washington Mimeograph, pp: 31-39.
- Okon, Y. and J. Vanderleyden, 1998. Root-associated *Azospirillum* species can stimulate plants. Features, 63: 366-370.
- Pandey, A., E. Sharma and L.M. S. Palni, 1998. Influence of bacterial inoculation on maize in upland farming systems of the Sikkim himalaya. Soil Biol. Biochem., 30: 379-384.
- Reinhold, B. and T. Hurek, 1988. Location of diazotrophs in the root interior with special attention to the *Kallar grass* association. Plant Soil, 110: 259-268.
- Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. Mc Graw Hill Book Co. Inc., New York, USA.
- Sumner, M.E., 1990. Crop Responses to *Azospirillum* Inoculation. In: *Advances in Soil Science*. Stewart, B.A. (Ed.), pp: 52-123. New York: Springer-Verlag.
- Sundaram, S., A. Arunakumari and R.V. Klucas, 1988. Characterization of *Azospirilla* isolated from seeds and roots of turf grass. Can. J. Microbiol., 34: 212-217.
- U.S. Salinity Lab. Staff, 1954. Diagnosis and improvement of saline and alkali soils. Agri. Handbook No. 60, USDA, Washington, D.C., USA.
- Victoria, R.L., M.C. Piccolo and A.A.T. Vargas, 1992. Microbiology of the ground Campinas, Brazilian science of the ground. The Cycle of Nitrogen. pp: 105-117.
- Watanabe, F.S. and S.L. Olsen, 1965. Test of an Ascorbic acid method for determining P in water and NaHCO₃ extract from soil. Soil Sci. Soc. Am. Proc., 29: 577-578.