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Studies on *Azospirillum* isolated from the soils of Thiruvarur Dt., Tamilnadu, India

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ABSTRACT

Totally 10 different paddy field soils were collected from in and around Thiruvarur district, Tamilnadu and their physico-chemical properties and population density of Azospirillum were analyzed. Among them, 5 samples were sandy clay loamy soil, while 3 samples were loamy soil and 2 samples were sandy loam. The pH (7.2-8.2), bulk density (1.11g/^{cm3} -1.60g/cm^3), water holding capacity (33.41% - 18.31%), electrical conductivity (0.89 - 0.24), organic carbon (0.97% - 0.57%), total nitrogen (1.72% - 0.75%), phosphorus content (0.185% - 0.125%), potassium (1.85% - 1.18%) also available micronutrients like Zn (2.03% - 1.16%), Cu (3.68% - 1.27%), Fe (9.16% - 7.40%), Mn (5.21% - 3.10%), B (0.590% - 0.390%), available nitrogen (191.0kg/acre - 110.5kg/acre), phosphorous (9.6kg/acre - 4.5kg/acre), potassium (295.0kg/acre - 190.5kg/acre) were in all sampling station. The population density of Azospirillum was maximum observed in sandy loam soil at Kudavasal. The minimum population density was observed in loamy soil at Thiruthuraipoondi.

Key words: *Azospirillum* spp, Physico-chemical properties and Population density.

INTRODUCTION

Soil microorganisms, like *Azospirillum* spp., *Azotobacter* Sp. and *Enterobacter* Sp. have shown to encourage plant growth, by promoting the outbreak of secondary roots. *Azospirillum* have been isolated from the rhizosphere and roots of a variety of plants including cereals and grasses. Inoculation with indigenous *Azospirillum* is an important procedure when studying their inherent capacity to benefit crops. In some cases, indigenous strains can perform better than introduced strains in promoting the growth of crops due to their superior adaptability to the environment. *Azospirillum* species are commonly found in soils and in association with roots of plants namely rice, maize, wheat and vegetables. Rhizosphere colonization by *Azospirillum* species has been shown to stimulate the growth of a variety of plant species. The success of the *Azospirillum* plant interaction depends on the survival and persistence of these bacteria in soil and the effective colonization of the rhizosphere. Chemotaxis is one of the several properties which may contribute to survival, rhizosphere colonization and the initiation of mutualistic interactions by *Azospirillim* species (Lopez-de-victoria, 1989).

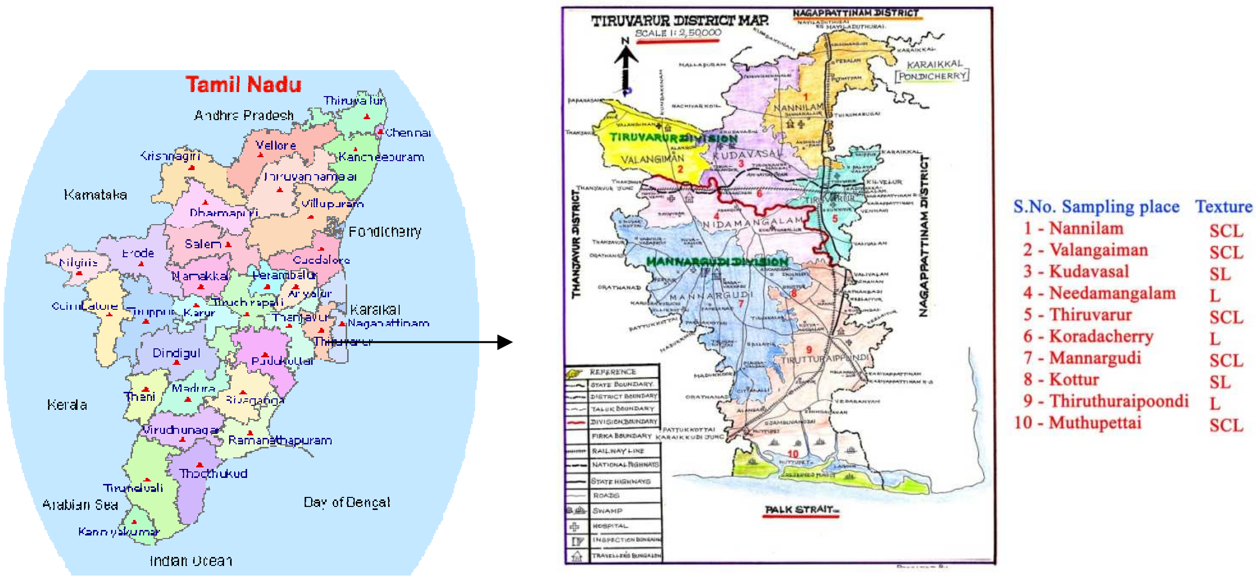
Apart from being a general plant colonizer (Bashan *et al.*, 2004), *Azospirillum* is remarkably versatile. *Azospirillum* is not only able to fix atmospheric N (Dobereiner and Day 1976), but also to mineralize nutrients from the soil, to sequester, Fe, to survive to marsh environmental conditions, and can help plants minimize the negative effects of abiotic stresses. Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil physical properties, both of which rely on soil biological process and soil biodiversity. An understanding of microbial diversity perspectives in agricultural context is important and useful to arrive of measures that can act indicators of soil quality and plant productivity.

MATERIALS AND METHODS

Description of the study area

The present study focused on the area in Thiruvarur district (Fig-1). The study area is situated in Tamilnadu state (lat.10 °20' - 11 °07' N- S and Long. 79 °15' - 79 ° 45' E-W) with the significant features of evergreen forests and also it was a less explored ecosystem for the investigation of *Azospirillum* population.

Fig-1 Map showing the study stations



For the enumeration of *Azospirillum,* soil samples were collected by aseptic manner at a depth of 5-10 cm according to the V – shaped method, at ten different locations in and around the Tiruvarur district. From each site, five samples were collected and pooled together and considered as one sample. The soil samples were brought to the laboratory and kept in the refrigerator for further process.

Analysis of physico - chemical parameters of the soil

After removing the debris, the soil samples were suspended in distilled water (1:2 w/v) and allowed to settle down the sand particles. The pH of the suspension was determined using pH meter (Systronics, India). Electrical conductivity of the soil was determined in the filtrate of the water extract using Conductivity Bridge as described by Jackson (1973), Cation exchange capacity (CEC) of the soil was determined by using 1 N ammonium acetate solution as described by Jackson (1973).

Nutrient analysis

Organic carbon (OC) content was determined by adopting chromic acid wet digestion method as standard procedure of Walkley and Black (1934), available nitrogen was estimated by alkaline permanganate method (Subbiah and Asija, 1956) and available Phosphorus by Brayl method (Bray and Kutz, 1945). Available potassium was extracted from soil with neutral 1N ammonium acetate (1:5) and the potassium content in the extract was determined by using flame photometer (Standfold and English, 1949). Calcium (Neutral 1N NH_4 OAC extractable 1:5) was extracted with neutral 1N ammonium acetate and the available calcium in the extract was determined by versenate method (Jackson, 1973). Available micronutrients such as Zn, Cu, Mn were determined in the diethyl triamine pentaacetic acid extract of soil using Perkin-Elmer Model 2280 Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978). Other nutrients such as magnesium, sodium and available iron were also analyzed (Muthuvel and Udayasoorian, 1999).

Isolation of *Azospirillum*
The soil samples were collected from 10 agronomical divisions of Thiruvarur district, Tamilnadu, South India for isolation of *Azospirillum.* From the collected soil samples, 1 g was taken and serially diluted using sterile distilled water up to 10^{-8} dilutions. One ml of diluted sample from 10^{-6} to 10^{-8} dilutions was taken and 0.1ml of aliquot was inoculated in test tube containing Nfb (Nitrogen free bromothymol) semisolid media. All the tubes were incubated at 32°C for 48 h and observed the growth by the formation of pellicles. The pellicles were streaked on Nfb solid media and incubated at 32°C for 24 h.

Morphologically divergent *Azospirillum* colonies (white, yellow and pink) were picked from the plates and streaked on basal minimal salt agar medium and incubated at 32ºC for 24 h. after attained sufficient growth, all the isolates were preserved and used for further investigation. The stock cultures were sub cultured in fresh nutrient agar slants once in a month and maintained at refrigerator condition. The isolates were also streaked separately on Basal Minimal Salt and Potato agar media separately and incubated at 32º C for 48 h.

Enumeration of *Azospirillum* isolates
For enumeration of *Azospirillum* cells, Nfb solid medium was used. After 48 h of incubation the colonies in the NFB plates were counted by using Quebec colony counter.

Population density is expressed in terms of colony forming unit (CFU) per gram of soil with dilution factor

$$\text{Number of cells / ml} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

Statistical analysis
Pearson's correlation co efficient analysis was made to assess the relationship between physico-chemical parameters and total population density of *Azospirillum* isolates. The data were computed and analyzed using Statistical Package for Social Sciences (SPSS) software.

RESULTS AND DISCUSSION

Plants play an important role in selecting and enriching the types of bacteria by the constituents of their root exudates. Thus, depending on the nature and concentrations of organic constituents of exudates, and the corresponding ability of the bacteria to utilize these as source of energy, the bacterial community develops in the rhizosphere. Bacteria living in the soil are called free living as they do not depend on root exudates for their survival. Rhizosphere bacterial communities have an efficient system for uptake and catabolism of organic compounds present in root exudates (Barraquio *et al.*, 2000)

The rhizosphere is the narrow zone of soil, surrounding the root that is under the immediate influence of the root system. This zone is rich in nutrients when compared with bulk soil, due to the accumulation of a variety of organic compounds released from roots by exudation, secretion and deposition (Curl and Truelove, 1986). Because, these organic compounds can be used as carbon and energy sources of microorganisms for their growth and activity particularly, intense in rhizosphere. In the present study, totally 10 *Azospirillum* isolates were screened from 10 different paddy field soil samples (10^5 dilution) collected from in and around Thiruvarur district. Kanimozhi and Panneerselvam (2011) reported that totally 30 *Azospirillum* isolates were screened from 30 different paddy field soil samples collected from in and around Thanjavur district. Cristyakova and Kalininskaya (1984) reported that bacteria of the genus *Azospirillum* were widespread in the soils of various regions and usually occur in the rhizosphere of vascular plants. Their presence in the rhizosphere soil has been observed in the paddy field, where their cell numbers did not exceed 2×10^5 cells/g of soil.

Physico-chemical properties of soil
The results of physico-chemical properties of soil samples from 10 different locations of Thiruvarur district were summarized in table 1. Out of 10 soil samples 5 samples were sandy clay loamy soil, while 3 samples were loamy soils and 2 samples were sandy loam. The maximum pH (8.1) of the soil was recorded at Muthuppetai and minimum pH (7.2) was recorded at Kudavasal. The bulk density of the soil was maximum 1.60g/cm^3 recorded at Needamangalam and minimum was 1.11g cm^3 recorded at Kottur. The maximum (33.41%) water holding capacity

(WHC) of the soil was recorded at Thiruthuraipoondi and minimum 18.31% was recorded at Kudavasal. The electrical conductivity of soil was maximum (0.89) recorded in Kudavasal and minimum was (0.24) in Mannargudi.

Table 1. Physico – chemical properties of soil samples from Thiruvarur Dt.

S.No.	Sampling places	Texture	pH	Bulk density (g/cm^3)	Water holding capacity (%)	Electrical conductivity (dsm^{-1})	Organic Carbon %
1	Nannilam	SCL	7.6	1.360	21.250	0.54	0.76
2	Valangaiman	SCL	7.5	1.520	25.160	0.62	0.81
3	Kudavasal	SL	7.2	1.120	18.310	0.89	0.57
4	Needamangalam	L	7.3	1.600	29.150	0.72	0.62
5	Thiruvarur	SCL	7.8	1.350	31.750	0.27	0.92
6	Koradacherry	L	7.4	1.175	24.560	0.36	0.79
7	Mannargudi	SCL	7.7	1.450	32.150	0.24	0.85
8	Kottur	SL	7.5	1.110	24.760	0.69	0.97
9	Thiruthuraipoondi	L	7.6	1.540	33.410	0.42	0.75
10	Muthupettai	SCL	8.1	1.460	30.640	0.77	0.95

L=Loamy; SL=Sandy loam; SCL=Sandy Clay Loam

Nutrients

The organic carbon content of the soil maximum as 0.97% recorded at Kottur and minimum was 0.57% at Kudavasal (Table 1). The total nitrogen maximum as 1.72% was recorded at Kudavasal soil and minimum was 0.75% at Koradacheri. The maximum (0.185%) phosphorus content of the soil was observed at Kottur and minimum 0.125% was recorded at Muthupettai soil. The maximum (1.85%) potassium of the soil was observed in Needamangalam and minimum 1.18% in Valangiman soil. The available micronutrients like Zn of the soil were recorded maximum as 2.03% at Muthupettai and minimum as 1.16% at Valangaiman. The Cu content of the soil was maximum as 3.68% at Mannargudi and minimum 1.27% was in Kudavasal soil. The Fe content of the soil was maximum (9.16%) at Thiruthuraipoondi and minimum (7.40%) at Kudavasal soil. The Mn content of the soil maximum as 5.21% was recorded in Thiruthuraipoondi and minimum as 3.10% at Nannilam soil. The maximum content of the B (0.590%) was recorded in Thiruvarur and minimum 0.390% in Nannilam soil (Table 2).

Table 2. Micronutrients of soil sample from Thiruvarur Dt.

S.No.	Sampling places	Total Nutrients (%)			Available Micro Nutrients (%)				
		N	P	K	Zn	Cu	Fe	Mn	B
1	Nannilam	1.25	0.142	1.37	1.36	1.60	8.40	3.10	0.390
2	Valangaiman	0.96	0.136	1.18	1.16	2.90	8.10	4.18	0.460
3	Kudavasal	1.72	0.161	1.56	1.48	1.27	7.40	3.50	0.480
4	Needamangalam	1.36	0.172	1.85	1.78	3.10	8.60	4.20	0.540
5	Thiruvarur	0.84	0.134	1.44	1.24	1.94	7.90	3.80	0.590
6	Koradacherry	0.75	0.165	1.28	1.56	2.80	9.10	4.35	0.580
7	Mannargudi	0.91	0.157	1.64	1.29	3.68	7.47	3.49	0.470
8	Kottur	1.21	0.185	1.49	1.68	2.92	8.27	4.68	0.520
9	Thiruthuraipoondi	0.86	0.144	1.72	1.96	3.27	9.16	5.21	0.527
10	Muthupettai	0.94	0.125	1.32	2.03	1.90	8.85	4.47	0.471

N=Nitrogen; P=Phosphorous; Zn=Zinc; Cu=Copper; Fe=Iron; Mn=Manganese; B=Boron

Table 3. Macronutrients of soil sample from Thiruvrur Dt.

S.No.	Sampling places	Available Nutrients (Kg/acre)		
		N	P	K
1	Nannilam	132.0	7.0	220
2	Valangaiman	125.6	6.1	190
3	Kudavasal	129.3	4.5	240
4	Needamangalam	142.4	4.7	265
5	Thiruvarur	191.0	6.3	225
6	Koradacherry	110.5	5.7	270
7	Mannargudi	138.4	4.9	212
8	Kottur	145.8	9.6	291
9	Thiruthuraipoondi	118.6	6.9	272
10	Muthupettai	122.3	5.4	295

N=Nitrogen; P=Phosphorus; K=Potassium

The maximum (191.0kg/acre) available nitrogen was observed at Thiruvarur and minimum (110.5kg/acre) was recorded at Koradacheri. The phosphorous of soil was maximum (9.6kg/acre) in Kottur soil and minimum (4.5kg/acre) was recorded at Valangaiman soil. The maximum potassium (295.0kg/acre) content of the soil was observed at Muthupettai and minimum (190.5kg/acre) was recorded at Valangaiman soil (Table 3).

Azospirillum spp. are members of the α – subclass of proteobacteria. *Azospirilla* have worldwide distribution and occur in large numbers (up to 10^{-7}/g) in rhizosphere soils and associated with the roots, stems and leaves of a large variety of plants. Members of the genus *Azospirillum* fix nitrogen under microaerophillic conditions and frequently associated with root and rhizosphere, these are known as associated diazotrophs. Sen (1929) made one of the earliest suggestions that the nitrogen content of cereal crops could be met by the activity of associated nitrogen fixing bacteria namely *Azospirillum.* This organism came into focus with the work of Dobereiner from Brazil (Doberenier *et al.,* 1976), followed by reports from India (Lakshmi-Kumari *et al.,* 1976; Kavimandan *et al.,* 1978; Tilak and Murthy, 1981). After establishing in the rhizosphere Azospirilla, usually but not always, promote the growth of plants (Okon, 1985; Tilak and Subba Rao, 1987; Bashan and Holguin, 1997b). Despite their N_2 fixing capability, the increase in yield is mainly attributed to improved root development due to the production of growth promoting substances and consequently increased rates of water and mineral uptake (Dewan and Subba Rao, 1979; Okon and Kapulnik, 1986; Fallik *et al.,* 1994). Azospirilla proliferated in the rhizosphere of numerous plant speices and the genus *Azospirillum* now contains seven species *A.brasillense* (Tarrand *et al.,* 1978), *A.lipoferum* (Tarrand *et al.,* 1978) *A.amazonense* (Magalhaes *et al.,* 1983), *A.halopraferens* (Reinhold *et al.,* 1987), *A.irakense* (Khammas and Kaiser, 1991), *A.doberelinerae* and *A.largimobile* (Eckert *et al.,* 2001).

Table 4. Population density of *Azospirillum* isolates from Thiruvarur Dt.

S.No.	Sampling places	No. of population x10^{-5} dilution CFU/g of soil
1	Nannilam	79
2	Valangaiman	84
3	Kudavasal	190
4	Needamangalam	98
5	Thiruvarur	112
6	Koradacherry	85
7	Mannargudi	79
8	Kottur	128
9	Thiruthuraipoondi	76
10	Muthupettai	92

Diversity of *Azospirillum*

For the isolation of *Azospirillum* Spp., Nfb semisolid medium was used. After 24 h incubation, the Nfb semi-solid medium showed white coloured pellicle. Appearance of pellicle formation on Nfb semi solid medium indicated successful isolation of *Azospirillum.* The pellicles were transferred in to Nfb plates. After 48 h white, merged colonies were observed on the medium. Typical white or pink, often wrinkled colonies were picked out and transferred into Nfb semi-solid medium. Kanimozhi and Panneerselvam (2011) reported highest population density of *Azospirillum* was observed in sandy loam soil. In the present study total number of 10 morphologically distinct *Azospirillum* isolates were isolated and tabulated. For enumeration of population density, the number of colonies on the plates was counted in the range of 76-190 colonies. The highest population density was observed in sandy loam soil at Kudavasal. The lowest population density was observed in loamy soil at Thiruthuraipoondi (Table 4; Fig.2).

Correlation coefficient analysis

The correlation coefficient analysis revealed that the significant positive correlation between total nitrogen and total potassium ($r = 0.338$; $P<0.04$) and total potassium and copper ($r = 0.356$) whereas the significant negative correlation was obtained between total nitrogen and available nitrogen ($r = 0.02$; $P<0.01$) and zinc and manganese ($r = 0.688$; $P<0.01$)(Table 5)

Fig.-2 Population density X 105 of *Azospirillum* isolated from 10 different soils of Thiruvarur District

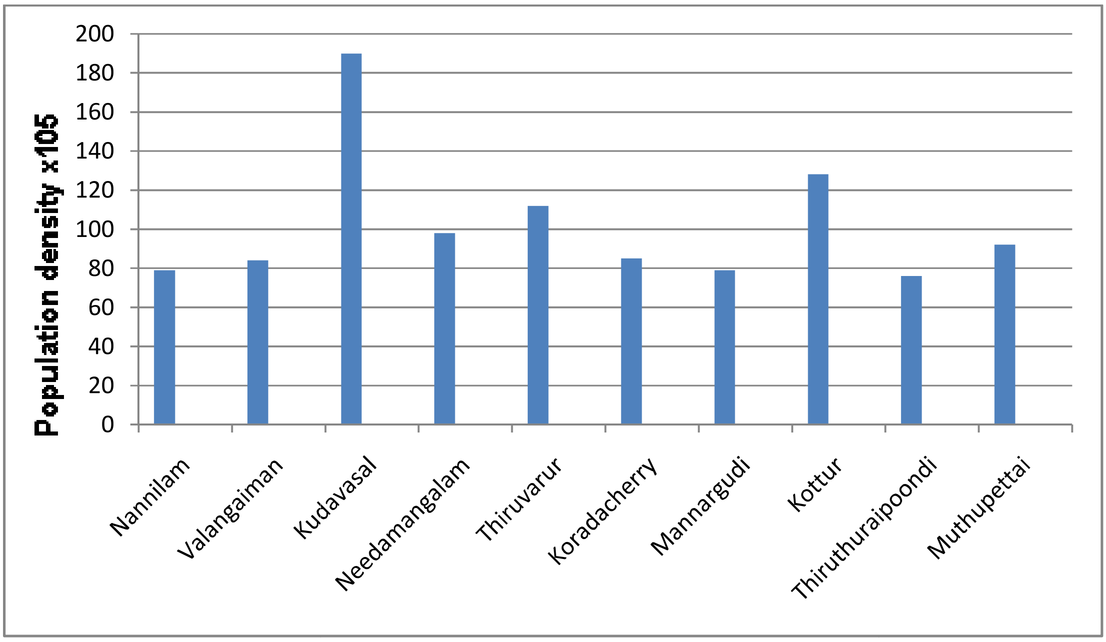


Table 5. Correlation coefficient values for various chemical parameters and total population density recorded from Thiruvarur District (n = 10).

	TN	TP	TK	Azn	ACu	AFe	AMn	AN	AP	AK	PD
TN	1										
TP	.402	1									
TK	.338*	.438	1								
Azn	.011	.088	.366	1							
ACu	-.470	.346	.356	.087	1						
AFe	-.445	-.087	-.061	.679*	.204	1					
AMn	-.388	.104	.128	.688*	.468	.684*	1				
AN	-.002*	-.053	.162	-.388	-.156	-.461	-.277	1			
AP	-.147	.241	-.186	.076	.108	.226	.368	.154	1		
AK	-.001	.321	.231	.896**	.029	.617	.656*	-.234	.270	1	
PD	.753*	.344	.128	-.053	-.550	-.544	-.213	.205	-.090	.104	1

TN – Total Nitrogen *AZn – Available Zinc* *AN – Available Nitrogen*
TP – Total Phosphorus *ACu – Available Copper* *AP – Available Phosphorus*
TK –Total Potassium *AFe – Available Iron* *AK – Available Potassium*
AMn – Available manganese PD – Population Density
0.04% significant level* * 0.01% significant level*

The availability of selective media for isolating diazotrophs belonging to the genus *Azospirillum* and the case of its detection by characteristic features of sub-surface white pellicle formation in semi-solid agar medium has helped to isolate from the rhizosphere and root surface (Doberenier *et al.*, 1976; Hegazi *et al.*, 1979; Baldani *et al.*, 1986; Ladha *et al.*, 1987; Sundaram *et al.*, 1988). Several isolates were obtained on semi solid nitrogen- free media from roots of Kallar grass (Bilal and Malik, 1987; Zafer *et al.*, 1987; Malik *et al.*, 1991). These isolates formed a fine sub-surface white pellicle in nitrogen free malate medium with in 24 h, which gradually moved to the surface (Krieg and Dobereiner, 1984).

More meaningful results were obtained when *A.brasilense* and *A.lipoferum* were inoculated into the soil in *in vitro* condition to measure their population dynamics. In a comparision between the levels of *A.brasilense* in the rhizosphere and bulk soil of a heavy –textured tropical soil from Martique (French West India), *A.brasilense* had a preference mainly for the macro-aggregates of the soil where it sustained a high population level of over 10^6 cells g^{-1} of soil fraction and to a lesser extent for the fine clay particles. Yet, these numbers represented only 0.18% of the total bacterial counts of these fractions (Kabir *et al.*, 1995).

Soil microorganisms play an important role in biogeochemical process which determine plant productivity, successful functioning of introduced microbial bioinoculants and their influence on soil health. Exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behavior in soil habitats (Hill, 2000).

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REFERENCES

[1] Bray, R.H. and Kutz, L.T., **1945.** *Soil Sci.,* **59**: 39-45.
[2] Bilal, R. and Malik, K.A., **1987**. *J. appl. Bacterial.*, **62**: 289-294.
[3] Barraquio, W.L., Segubre, E.M., Gonzalez, M.S., Verma, S.C., James, E.K., Ladha, J.K. and Tripathi, A.K., **2000.** In the Quest for Nitrogen fixation in Rice. IRRI, Los Banos, Philippines., pp: 93 – 118.
[4] Baldani, V.L.D., Alvarez de, B., Baldani, J.I. and Dobereiner, J., **1986**. *Plant Soil*., **90**: 35 – 46.
[5] Bashan, Y., Holguin, G. and de-Bashan, L.E., **2004**. *Can. J. Microbiol.,* **50**: 521 – 577.
[6] Bashan, Y. and Holguin, G., **1997b**. Short and medium term avenues for *Azospirillum* inoculation. In: Ogoshi A, Kobayashi K., Homma Y., Kodama, F., Kondo, N., Akino, S. (eds) plant growth promoting rhizobacteria, present status and future prospects. Proceedings of the fourth international workshop of plant growth promoting rhizobacteria. Nakanishi, Sapporo, Japan, 130 – 149.
[7] Cristyakova, I.K and Kalininskaya,TA., **1984.** *Ser. Biol*., **1**: 149 – 153.
[8] Curl, E.A and Truelove, B. 1986. The rhizosphere spinger- verlag, berlin.
[9] Dewan, G.I. and Subba Rao, N.S., **1979**. *Plant Soil.,* **53**: 295 – 302.
[10] Dobereiner, J., Marriel, J.E. and Nevy, M., **1976.** *Can. J. Microbiol.,* **22**: 1464 – 1473.
[11] Dobereiner, J. and Day, J.M., **1976.** Associative symbiosis and free-living systems. In: Newton, W.E., Nyman, C.J., (eds.) Proceedings of the 1st International symposium on Nitrogen fixation. Washington state University. Press. Pullman., 518 – 538.
[12] Eckert, B., Weber O.B., Kirchhuf G., Halbriffer A., Stoffels, M. and Haartman, A., **2001.** *Int J. Syst. Evol. Microbiol.,* **51**: 17 – 26.
[13] Fallik, E., Sarig, S. and Okon, Y., **1994**. Morphology and physiology of plant roots associated with *Azospirillum*. In: *Azospirillum* Plant Associations (ed. Okun, Y). CRC Press, Raton., 77-84.
[14] Hegazi, N.A., Amer, H.A. and Monib, M., **1979.** *Soil Biol. Biochem.,* **11**: 437-438.
[15] Hill , G.T., **2000.** *Appl. Soil Microbiol.*, **15**: 25-36.
[16] Jackson, M.L., **1973**. Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi.
[17] Kabir, M., Faure, D., Heulin, T., Achouak. W., and Bally, R**., 1995**. *Eur. J. Soil. Biol*., **32**: 157-163.
[18] Kanimozhi, K and Panneerselvam, A., **2011.** *Archieves of Applied Science Research,* **3** (2):525-536
[19] Krieg, V.R. and Dobereiner, J., **1984.** Genus *Azospirillum.* In: Bergey's Manual of systematic Bacteriology, Williams & Wilkins, Baltimore. **1**: 99.
[20] Khammas, K.M. and Kaiser, P**., 1991**. *Plant. Soil*., **137**: 75 – 79.
[21] Kavimandan, S.K., Subba Rao, N.S. and Mohrir, A.V**., 1978**. *Curr. Sci*., **47**: 96.
[22] Lopez-de-Victoria, G., **1989.** Chemotactic behaviour of deep subsurface bacteria toward carbohydrates, amino acids and a chlorinated alkene. M.Sc., Thesis University of Pureto Rica. Rio Piedras.
[23] Lindsay, W.C. and Norvell, A., **1978**. *Proc. Soil sci. soc. Am.,* **42**: 421 – 428.
[24] Lakshmi – Kumari, M.l., Kavimandan, S.K. and Subba Rao, M.S., **1976**. *Indian. J. Exp. Biol.,* **14**: 638 – 639.
[25] Ladha, J.K., Tirol-padre, A., Punzalan, G.C. and Watanabe, I**., 1987.** *Soil science and plant nutrition*., **33**: 187-200.
[26] Malik, K.A., Bilal, R., Rasul, G., Mahmood, K. and Sajjad, M., **1991**. *Plant soil*., **137**: 67-74.
[27] Magalhaes, F.M., Baldani, J.I., Souto, S.M., Kuykendall, J.R. and Dobereiner, J., **1983**. *J. Ann. Acad. Braz. Cienc.*, **55**: 417 – 430.
[28] Muthuvel, P. and Udayasoorian, C., **1999.** Soil, plant, water are agrochemical analysis. Tamilnadu. Agricultural Unviersity, Coimbatore, India.
[29] Okon, Y**., 1985**. *Trends Biotechnol*., **3**: 223 – 228.
[30] Okon, Y. and Kapulnik, Y., **1986.** *Plant Soil*., **90**: 3 -16.
[31] Reinhold, B., Hurek, T. and Fendrik, I., **1987.** *Appl. Environ, Microbiol*., **53**: 889-891.
[32] Subbaiah, B.V. and Asija, G.L., **1956**. *Curr. Sci.,* **25**: 258 – 260.

[33] Standfold, S. and English, L., **1949**. *Agron. J.*, **41**: 446 – 447.
[34] Sen, J., **1929**. *Agric.J. India*., **24**: 967 – 980.
[35] Sundaram, S., Arunkumari, A. and Klucas, R.V., **1988.** *Can. J. Microbiol.*, **34**: 212-217.
[36] Tarrand, J.J., Krieg, N.R. and Dobereiner, J., **1978**. *Can. J. microbial.*, **24**: 967 – 980.
[37] Tilak, K.V.B.R. and Murthy, B.N., **1981**. *Curr. Sci*., **50**: 496 – 498.
[38] Tilak, K.V.B.R. and Subba Rao, N.S., **1987.** *Biol. Fertil. Soils*., **4**: 97 – 102.
[39] Walkey, A. and Black, I.A., **1934**. *Soil Sci*., **37**: 29 – 38.
[40] Zafer, Y., Malik, K.A. and Niemann, E.G**., 1987**. *J. Appl. Microbiol.*, **3**: 45-56.