original research paper

Acta Agriculturae Serbica, Vol. XVI, 32 (2011) 97-122

Acta Agriculturae Serbica FACULTY OF AGRONOMY • ČAČAK •

Effect of different agricultural practices on population dynamics of *Azotobacter chroococcum* and on its nitrogen fixing potentiality in Trans – Ganga and Trans-Yamuna plains of India

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Abstract: A study was conducted for two consecutive years 2003-04 and 2004-05 at Allahabad, one of the most fertile regions of Indo-Gangetic plane on wheat crop. The aim was to assess the effect of different agricultural practices on the population dynamics and nitrogen fixing potentiality of free living aerobic nitrogen fixing bacteria *Azotobacter chroococcum.* Heavy input of fertilizers, mechanical tillage, frequent irrigation, monocropping have resulted in decrease in population of *Azotobacter chroococcum* and its nitrogen fixing potentiality. The fields in which there was judicious use of chemical fertilizers along with crop rotation, use of FYM, low tillage practice shows better population of the *Azotobacter chroococcum* as well as nitrogen fixing potentiality. Crop rotation and use of the FYM have exerted better effect on the growth and development of *Azotobacter chroococcum* as well as its nitrogen fixing potentiality.

Keywords: Intensification, *Azotobacter chroococcum*, Nitrogen fixing potentiality, trans Ganga, trans Yamuna

Introduction

Application of modern means of agriculture has been increased tremendously during the past few decades. This heavy input of NPK based

Received: 10 October 2010 / *Accepted*: 25 November 2011

fertilizer, pesticides, HYV, modern means of irrigation and mechanical tillage have increased productivity to a great extent. Indo- Gangetic plane especially the doab region is highly intensified. However, increased agricultural intensity has also resulted in an increasing pressure on biodiversity and this is likely to continue in the coming decades if not managed properly (Tilman *et al.,* 2001; Petit *et al.,* 2001).

Cultivation has a major effect on soil fertility and productivity. Within 50 years of tilling, 40-70% of the original store of soil organic matter (carbon and nitrogen) is lost (Parton and Rasmussen, 1994). Most marked effect of agronomic practices on microbial biomass was observed with the fertilizer treatment. The application of fertilizers decrease biomass of soil (Mc Andrew and Mahli, 1992; Djukic, Mandic, 2000). Different sources of nitrogen such as ammonia, nitrate or urea have been known to suppress the nitrogenase activity, which is the key enzyme for biological nitrogen fixation in natural ecosystem (Postgate, 1982; Merrick, 1992, Egner *et al*.,1999). Even low concentrations of combined nitrogen and ammonium or nitrate lead to repression of nitrogenase genes (Merrick, 1992; Egener *et al.,* 1999) and to an inactivation of nitrogenase activity (Nordlund, 2000; Martin and Reinhold-Hurek, 2002). Long term experiment on continuous rice- wheat system has resulted in yield decline due to deterioration of soil physical condition and soil fertility (Sharma and Jain, 1997). The deteriorating soil physical conditions have been credited to tillage for rice-wheat system (Meelu *et al.,* 1979; Bajpai and Tripathi, 2000; Tripathi *et al.,* 2003; Narayan and Kehri, 2008).

The objective of the present study was to evaluate the effect of different degrees of agricultural intensification on the population dynamics of free living aerobic nitrogen fixing bacteria *Azotobacter chroococcum* and to study the nitrogen fixing potentiality under different agricultural practices.

Material and methods

Site selection

For investigating the impact of agricultural intensification on population and diversity of *Azotobacter chroococcum,* a number of agricultural fields with varying degrees of intensification in terms of water resources, chemical and mechanical inputs and crop diversification were identified in and around Allahabad. In all, two sites were selected namely Site I and Site II, for the purpose.

Wheat fields were selected for the present investigation in a rice-wheat cropping pattern, which is the most dominant pattern in Indo-Gangetic plains. Selections of the fields were mainly based on in-depth interviews with farmers about the fields, cropping pattern, fertilization, chemical and mechanical inputs *etc.* On the basis of these inputs, the fields were categorized into highly intensified, moderately intensified and low intensified fields. Farmers who are practicing monocropping (wheat/rice) with high yielding varieties, heavy fertilization (200-360 kg /ha urea; 160-240 kg/ha diammonium phosphate (DAP);

140 kg/ha nitrogen, phosphorus, potassium (NPK), heavy irrigation and heavy mechanical tillage, there fields were categorized as highly intensified. Those who are practicing monocropping (wheat + mustard /rice), with high yielding varieties with moderate fertilization (120-200 kg/ha urea; 80-140 kg/ha diammonium phosphate (DAP), moderate irrigation and mechanical tillage after hiring only once or twice were categorized as moderately intensified fields. Whereas those who are practicing crop rotation (wheat + mustard + pea /legumes/rice) with local varieties, very low dose of fertilizers (0-60 kg/ha urea), mold board plough with FYM were categorized as low intensified fields. From each site three sub-sites were selected on the basis of the socio-economic status and land holdings of the farmers. Further from each sub-site three fields with high, moderate and low intensification were selected.

From all these fields' soil samples were collected randomly from cropped areas of the fields leaving surface soil up to a depth of 5 cm and brought aseptically in the laboratory. The samples of each field were composited and mixed thoroughly. A working sample was drawn from the composite sample and processed for estimating the population of *Azotobacter* in the non-rhizosphere and rhizosphere soil. Samples of root and non-rhizosphere soil were collected when the crops were at their seedling, vegetative, flowering and fruiting stages.

Isolation of *Azotobacter*

Soil dilution and plate count method of Timonin (1940) was used for estimating population of *Azotobacter* in the rhizosphere and non-rhizosphere of different samples. 100-1000 dilution, which gave counts between 10-20 colonies per Petri plate were employed. The population of *Azotobacter / g* oven dry soil was computed on basis of weight of the oven dried soil and the number of colonies appeared on Petri plates.

For identification and purification of *Azotobacter chroococcum*, methodology given by Aquilanti *et al.* in 2004 was followed. The different isolates isolated by the above method were streaked on the Jensen's agar medium. Then, the purified isolates of *Azotobacter chroococcum* were again streaked on silica gel plates. The gram positive and gram negative isolates were differentiated by the KOH test described by Ryu (1938) and Gram staining. Isolates were then screened for growth ability, colony morphology, pigment production and acid production on N-free medium (Turner and Gibson, 1980) containing sucrose as a sole carbon source and blue bromothymole as pH indicator. Production of acid was indicated by a change in colour of the medium from blue or dark green to a definite yellow.

Molecular analysis was done by method given by Aquilanti *et al.* (2004). Bacteria were grown for 2-3 days on Silica gel plate. Crude template DNA was prepared by suspending two or three colonies in 500µl of sterilized double distilled water collected by centrifugation, resuspended in 100 µl of water, added with 100 µl of 10 mM Tris- HCl pH 8.2 and vortexed. Finally, 13 µl of 1 mg/ml proteinase K were added. After incubation at 55^0C for 2 h, cells undergone to lyses were pelleted by centrifugation and 2 µl of the supernant fluid were used for the PCR amplification.

The amplification of the 16S rRNA gene amplification via PCR was performed in a 50 µl reaction mixture containing 1 U of Taq polymerase, 10 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 200 µM of dNTPs and 2 µM of each universal primer 27f and 1495r. After incubation at 95^0C for 4 minute, the reaction mixture was cycled through the following temperature profile, initial denaturation at 95^0C for 4 minute followed by 35 cycles at 94^0C for 1 min, 55^0C for 1 min and 72^0C for two min, each. The PCR session was terminated at 72^0C for 15 min, following by cooling at 4^0C. DNA amplification was checked by electrophoresis of 8 µl of each PCR product in a 1.5% (w/v) agarose gel, in TBE buffer (0.09M Tris base, 0.09M sodium borate, 2.5mM EDTA, pH 8.3) for 1h at 3.2 V/cm. A 12.5 µl aliquot of the PCR product was digested in a final volume of 25 µl for 8 h at 37^0C with 5 U of restriction enzyme (Hha I). DNA digests were separated by 2.5 % (w/v) agarose gel electrophoresis in TBE buffer for 4 h at 3.2 V/cm. Gels were stained in ethidium bromide for 15 min and thereafter washed for 5 min. DNA fragments were visualised at 312 nm with a UV-transilluminator Image Master. Gels electronic image was captured by LISCAP software. Molecular identification of the soil isolates was done by comparing their restriction profiles with the reference strains available (Aquilanti *et al.* 2004).

Evaluation of Nitrogen Fixing Potentiality

After the purification on silica gel, the isolates of *A. chroococcum* were grown in Browns liquid medium for 5 days. On the 5th day, 1 ml of each culture was transferred to a sterilized 250 ml conical flask containing 50 ml of Browns liquid medium. Each isolate was allowed to grow in five such flasks in incubator at proper temperature and aeration. After 14th day the contents of each flask were transferred to 100 ml Micro Kjeldahl Flask and were allowed to dry on water bath. The residue was dried in an oven at 40-50°C and 0.2 g of dry matter was digested in concentrated sulphuric acid in the presence of 0.1 g of the catalyst (catalyst was prepared by mixing 1.0 g copper sulphate, 8.0 g sodium sulphate and 1.0 g selenium dioxide). Digestion was performed at low heat until frothing stopped and fumes of sulphuric acid freely evolved. After 10 minutes, the temperature was increased and digestion was continued for at least 30 minutes until digest became apple green. Finally nitrogen fixing potentiality was determined by the elemental analyzer (Vario, made in Germany) in terms of %N fixed/ 10 mg dry weight of bacteria.

Chemical Analysis of Soils

Collected soil samples were air dried and sieved. Soil pH and electrical conductivity was measured using glass electrode after dilution of soil with distilled water (1:1 V/V) (Rhodes, 1982). Soil extract was prepared and organic carbon was estimated by Titration Method (Nelson and Sommers, 1982), phosphorus was estimated by Olsen's method (Watanabe and Olsen, 1965); potassium was estimated by flame photometer method (Jackson, 1973) and nitrogen was estimated by the method given by Bremmer (1982). (Table 1)

Meteorological Data

Mean monthly temperature (maximum and minimum), rainfall and mean monthly relative humidity (maximum and minimum) during the period of survey (Jan, 2003 – Dec, 2005) were procured from Meteorological Centre, Pune, India (Table 2).

Statistical analysis

All the data obtained from different agricultural fields were statistically analyzed using two-way ANOVA with Microsoft Excel 2003 to calculate the MSS. Critical difference (at P=0.05) was calculated by the method given by Panse and Sukhatme (1985).

Results

The population of *A. chroococcum* in the non-rhizospheric soil showed some fluctuations during the survey period. However, these fluctuations may be safely attributed to the variations in the environment and abiotic characters of the soil during different seasons (Table 1 and 2). The results further show that in all the fields, the population of *A. chroococcum* in the rhizosphere region was higher than that in the non-rhizosphere region at all the stages of plant growth. A comparison of the population shows that the rhizosphere effect varied with the sites, agricultural intensification and stages of the plant growth. However, maximum effect was exerted at the flowering stage. *A. chroococcum* was present in all the rhizospheric soils of wheat collected from the fields of all the four sites showing different degrees of intensification at different stages of plant growth (Table 3-8).

During survey the range of population in the fields of high intensification at two sites during different stages of plant growth in the rhizosphere of wheat ranged from 0.4 to 3.8 x 10^4 /g oven dry soil and in non-rhizosphere it ranged from 0.2 to 1.3 x 10^4 /g oven dry soil. The range of rhizosphere population in the fields of moderate intensification ranged from 0.8 to 4.0 x 10^4 /g oven dry soil and in non-rhizosphere it ranged from 0.3 to 1.5 x 10^4 /g oven dry soil. The range of population in the fields of low intensification at different sites during different stages of plant growth in the rhizosphere of wheat ranged from 1.5 to 4.8 x 10^4 /g oven dry soil and in non-rhizosphere it ranged from 0.6 to 1.9 x 10^4 /g oven dry soil. In all the cases minimum average population was present in the fields of Site I, while maximum average population was present in the fields of Site II (Table 3-8).

The range of population of *A. chroococcum* in the fields of Site I at different degrees of intensification in the rhizosphere soils of wheat ranged from 0.5 to 2.9 x 10^4 /g oven dry soil and the maximum population was always recorded in the low intensified fields. The non-rhizospheric population ranged from 0.2 to 1.1 x 10^4 /g oven dry soil, highest being in the low intensified fields

and lowest in the highly intensified fields (Table 3-5). The range of population of *A. chroococcum* in the fields of Site II at different degrees of intensification in the rhizosphere soils of wheat ranged from 1.7 to 4.8 x 10^4 /g oven dry soil and the maximum population was always recorded in the low intensified fields. The non-rhizospheric population ranged from 0.6 to 1.9 x 10^4 /g oven dry soil and was always highest in the low intensified fields and lowest in highly intensified fields (Table 6-8).

Tab. 1. Average physicochemical characteristics of soils of wheat fields of Site I and II for years 2003-04 and 2004-05

		Site I					Site II				
Sub Sites/Degree of Intensification		pH	OC (%)	P_2O_5 (Kg/ha)	K_2O (Kg/ha)	N_2 (%)	pH	OC (%)	P_2O_5 (Kg/ha)	K_2O (Kg/ha)	N_2 (%)
1	High	6.00	0.46	95	242	0.986	6.10	0.57	24	199	0.958
2	Moderate	6.60	0.73	73	219	0.769	6.90	0.71	16	242	0.759
3	Low	7.00	0.95	40	128	0.579	7.20	0.95	8	175	0.541
1	High	6.12	0.54	98	359	0.966	6.40	0.54	22	302	0.861
2	Moderate	7.30	0.84	60	188	0.799	7.10	0.79	12	195	0.745
3	Low	7.00	0.92	30	110	0.558	7.60	0.98	19	107	0.535
1	High	5.98	0.62	90	282	0.987	6.50	0.61	29	325	0.847
2	Moderate	6.66	0.70	50	112	0.678	7.10	0.75	10	252	0.774
3	Low	7.00	0.98	21	99	0.445	7.70	1.04	18	125	0.421

Tab. 2. Temperature, rainfall and relative humidity of Allahabad and adjoining areas (for year 2003, 2004 and 2005)

Months	Year 2003					Year 2004					Year 2005				
	Temperature (^{0}C)		Rainfall (cm)	Relative Humidity		Temperature (^{0}C)		Rainfall (cm)	Relative Humidity		Temperature (^{0}C)		Rainfall (cm)	Relative Humidity	
	Max.	Min		Max	Min	Max.	Min		Max	Min	Max	Min		Max	Min
JAN	17.87	6.87	0.58	98.83	60.77	19.67	9.35	00	86.06	51.77	23.63	10.10	3.5	94.43	42.96
FEB	26.0	14.25	1.47	92.92	47.21	27.68	12.41	0.17	90.31	35.65	27.03	12.75	1.0	88.67	37.42
MAR	32.81	17.65	0.19	87.32	29.22	36.67	19.12	00	95	7.00	34.4	18.36	5.4	80.03	26.6
APR	40.43	23.9	0.06	64.23	16.96	39.83	23.26	00	92	7.00	39.36	21.6	2.1	47.96	9.5
MAY	41.32	26.90	0.03	53.16	15.64	41.80	27.22	6.0	91	7.00	41.90	25.96	1.6	55.35	14.74
JUN	40.76	29.03	1.96	69.23	9.13	36.26	26.03	6.63	79.06	79.06	42.33	29.83	11.11	52.03	24.0
JUL	40.76	29.03	1.96	69.23	36.73	34.32	26.35	3.39	88.19	55.83	33.16	25.74	9.03	91.74	60.12
AUG	33.29	26.93	5.87	94.03	67.09	33.03	25.70	7.37	92.19	64.93	33.96	26.29	3.08	89.70	61.64
SEP	31.96	26.03	14.83	94.90	71.33	33.36	23.96	1.7	87.7	52.03	33.06	25.66	0.012	91.86	62.8
OCT	31.90	22.12	0.22	92.38	47.58	32.19	18.51	00	88.38	37.96	32.45	22.61	00	89.80	50.45
NOV	29.96	14.16	00.00	90.16	29.1	30.19	11.93	00	92.3	30.33	29.3	11.9	00	84.46	26.33
DEC	24.51	10.06	0.07	98.5	46.01	25.12	9.61	00	95.90	42.22	24.5	9.8	00	93.78	43.12

Tab. 3. Population of *Azotobacter chroococcum* in the rhizosphere (R) and nonrhizosphere (NR) of wheat at different stages of plant growth, cultivated under varying degrees of agricultural intensification at Site I, (Population in terms of $1x10^4$/g oven dry soil, year 2003-2004 and 2004-05)

	Degree of Intensification/ Stages of Plant Growth	Seedling		Vegetative		Flowering		Fruiting	
		R	NR	R	NR	R	NR	R	NR
2003-04									
1	DAP 240 Kg/ha + Urea 280 Kg/ha + NPK 100 Kg/ha + Wheat + Bullock tillage + Tube well Irrigation 3 Times + Monoculture	0.5	0.2	0.9	0.4	1.5	0.5	1.1	0.4
2	DAP 140 Kg/ha + Urea 140 Kg/ha + Wheat + Mustard + Mechanical tillage + Tube well Irrigation 3 Times + Monoculture	1.1	0.4	1.4	0.5	2.1	0.7	1.7	0.6
3	Urea 60 Kg /ha+ FYM + Wheat + Mustard + Pea +Tube well Irrigation 3 Times + Mechanical tillage+ Crop Rotation	1.7	0.7	2.1	0.8	2.8	1.0	2.5	0.9
2004-05									
1	DAP 240 Kg/ha + Urea 280 Kg/ha + NPK 100 Kg/ha + Wheat + Bullock tillage + Tube well Irrigation 3 Times + Monoculture	0.4	0.2	0.7	0.4	1.4	0.5	1.1	0.4
2	DAP 140 Kg/ha + Urea 140 Kg/ha + Wheat + Mustard + Mechanical tillage + Tube well Irrigation 3 Times + Monoculture	0.8	0.3	1.1	0.5	1.7	0.6	1.4	0.5
3	Urea 60 Kg /ha+ FYM + Wheat + Mustard + Pea +Tube well Irrigation 3 Times + Mechanical tillage+ Crop Rotation	1.5	0.6	2.0	0.8	2.7	1.0	2.1	0.8
	Minimum difference required for significance (C. D.) at 5% level:	0.18	0.07	0.89	0.29	0.38	0.24	0.31	0.31

Tab. 4. Population of *Azotobacter chroococcum* in the rhizosphere (R) and nonrhizosphere (NR) of wheat at different stages of plant growth, cultivated under varying degrees of agricultural intensification at Site I, (Population in terms of $1x10^4$/g oven dry soil, year 2003-2004 and 2004-05)

Degree of Intensification/ Stages of Plant Growth		Seedling		Vegetative		Flowering		Fruiting	
		R	NR	R	NR	R	NR	R	NR
2003-04									
1	DAP 220 Kg/ha + Urea 300 Kg/ha + NPK 120 Kg /ha+ Wheat + Mechanical tillage + Tube well Irrigation 3 Times + Monoculture	0.7	0.3	1.1	0.5	1.6	0.6	1.3	0.5
2	DAP 160 Kg/ha + Urea 160 Kg/ha + Wheat + Mustard + Mechanical tillage + Tube well Irrigation 3 Times + Monoculture	1.1	0.5	1.5	0.6	2.4	0.8	2.1	0.7
3	Urea 60 Kg/ha + FYM + Wheat + Mustard + Pea +Tube well Irrigation 3 Times + Mechanical tillage+ Crop Rotation	1.7	0.8	2.3	0.8	3.1	1.0	2.7	0.9
2004-05									
1	DAP 220 Kg/ha + Urea 300 Kg/ha + NPK 120 Kg /ha+ Wheat + Mechanical tillage + Tube well Irrigation 3 Times + Monoculture	0.5	0.2	0.9	0.4	1.5	0.5	1.2	0.4
2	DAP 160 Kg/ha + Urea 160 Kg/ha + Wheat + Mustard + Mechanical tillage + Tube well Irrigation 3 Times + Monoculture	1.0	0.4	1.3	0.5	2.2	0.7	1.8	0.6
3	Urea 60 Kg/ha + FYM + Wheat + Mustard + Pea +Tube well Irrigation 3 Times + Mechanical tillage+ Crop Rotation	1.6	0.7	2.1	0.8	2.9	1.0	2.6	0.9
	Minimum difference required for significance (C. D.) at 5% level:	0.29	0.37	0.07	0.35	0.40	0.33	0.49	0.29

Tab. 5. Population of *Azotobacter chroococcum* in the rhizosphere (R) and nonrhizosphere (NR) of wheat at different stages of plant growth, cultivated under varying degrees of agricultural intensification at Site I, (Population in terms of $1x10^4$ /g oven dry soil, year 2003-2004 and 2004-05)

	Degree of Intensification/ Stages of Plant Growth	Seedling		Vegetative		Flowering		Fruiting	
		R	NR	R	NR	R	NR	R	NR
2003-04									
1	DAP 240 Kg/ha + Urea 280 Kg/ha + NPK 120 Kg/ha+ Wheat + Mechanical tillage + Tube well Irrigation 3 Times+ Monoculture	0.9	0.4	1.2	0.5	2.0	0.7	1.7	0.6
2	DAP 140Kg/ha + Urea 35 Kg/ha + Wheat + Mustard + Mechanical tillage + Tube well Irrigation 3 Times + Monoculture	1.3	0.6	1.9	0.7	2.3	0.8	2.0	0.7
3	Urea 60 Kg/ha + FYM + Wheat + Mustard + Pea + Tube well Irrigation 3 Times + Mechanical tillage+ Crop Rotation	1.8	0.8	2.1	0.9	2.8	1.1	2.4	1.0
2004-05									
1	DAP 240 Kg/ha + Urea 280Kg/ha + NPK 120 Kg/ha+ Wheat + Mechanical tillage + Tube well Irrigation 3 Times+ Monoculture	0.7	0.3	1.1	0.5	1.8	0.6	1.4	0.5
2	DAP 140Kg/ha + Urea 35 Kg/ha + Wheat + Mustard + Mechanical tillage + Tube well Irrigation 3 Times + Monoculture	1.2	0.5	1.6	0.6	2.2	0.8	1.7	0.6
3	Urea 60 Kg/ha + FYM + Wheat + Mustard + Pea + Tube well Irrigation 3 Times + Mechanical tillage+ Crop Rotation	1.6	0.7	1.9	0.8	2.7	1.1	2.1	0.9
	Minimum difference required for significance (C. D.) at 5% level:	0.09	0.14	0.28	0.28	0.29	0.22	0.29	0.22

Tab. 6. Population of *Azotobacter chroococcum* in the rhizosphere (R) and nonrhizosphere (NR) of wheat at different stages of plant growth, cultivated under varying degrees of agricultural intensification at Site II, (Population in terms of $1x10^4$/g oven dry soil, year 2003-2004 and 2004-05).

	Degree of Intensification/ Stages of Plant Growth	Seedling		Vegetative		Flowering		Fruiting	
		R	NR	R	NR	R	NR	R	NR
2003-04									
1	DAP 200 Kg /ha + Urea 160 Kg/ha + Mechanical tillage + Tube well irrigation 3 Times + Wheat Only + Monoculture	2.6	0.9	3.0	1.1	3.3	1.3	3.1	1.2
2	DAP 100 Kg/ha + Urea 80 Kg/ha + Mechanical tillage + Tube well Irrigation 3 Times + Wheat + Mustard + Monoculture	3.2	1.2	3.5	1.3	4.0	1.5	3.7	1.4
3	FYM + Bullock tillage + Wheat + Mustard+ Pea + Canal Irrigation 3 Times+ Crop rotation	3.8	1.5	4.1	1.7	4.8	1.8	4.5	1.6
2004-05									
1	DAP 200 Kg /ha + Urea 160 Kg/ha + Mechanical tillage + Tube well irrigation 3 Times + Wheat Only + Monoculture	2.4	0.8	2.9	1.0	3.1	1.2	3.0	1.1
2	DAP 100 Kg/ha + Urea 80 Kg/ha + Mechanical tillage + Tube well Irrigation 3 Times + Wheat + Mustard + Monoculture	3.0	1.1	3.3	1.2	3.9	1.4	3.7	1.3
3	FYM + Bullock tillage + Wheat + Mustard+ Pea + Canal Irrigation 3 Times+ Crop rotation	3.7	1.5	4.0	1.6	4.8	1.9	4.4	1.6
	Minimum difference required for significance (C. D.) at 5% level:	0.07	0.07	0.09	0.31	0.39	0.52	0.29	00

Tab. 7. Population of *Azotobacter chroococcum* in the rhizosphere (R) and nonrhizosphere (NR) of wheat at different stages of plant growth, cultivated under varying degrees of agricultural intensification at Site II, (Population in terms of $1x10^4$/g oven dry soil, year 2003-2004 and 2004-05)

	Degree of Intensification/ Stages of Plant Growth	Seedling		Vegetative		Flowering		Fruiting	
		R	NR	R	NR	R	NR	R	NR
2003-04									
1	DAP 200 Kg/ha + Urea 160 Kg/ha + Mechanical tillage + Tube well Irrigation 3 Times + Wheat Only + Monoculture	2.4	0.8	2.8	1.0	3.2	1.2	3.0	1.1
2	DAP 120 Kg/ha + Urea 120 Kg/ha + Tube well Irrigation 3 Times + Mechanical tillage + No Previous Crop + Wheat + Mustard	2.7	1.0	3.3	1.2	4.0	1.4	3.5	1.3
3	FYM + Bullock tillage + Canal Irrigation 3 Times + Wheat + Mustard+ Pea + Crop Rotation	3.5	1.3	3.9	1.5	4.7	1.7	4.4	1.6
2004-05									
1	DAP 200 Kg/ha + Urea 160 Kg/ha + Mechanical tillage + Tube well Irrigation 3 Times + Wheat Only + Monoculture	2.1	0.7	2.6	0.9	3.0	1.1	3.0	1.0
2	DAP 120 Kg/ha + Urea 120 Kg/ha + Tube well Irrigation 3 Times + Mechanical tillage + No Previous Crop + Wheat + Mustard	2.6	0.9	3.1	1.1	3.8	1.3	3.4	1.2
3	FYM + Bullock tillage + Canal Irrigation 3 Times + Wheat + Mustard+ Pea + Crop Rotation	3.2	1.2	3.6	1.4	4.5	1.7	4.1	1.5
	Minimum difference required for significance (C. D.) at 5% level:	0.32	0.07	0.37	0.07	0.16	0.19	0.07	0.29

Tab. 8. Population of *Azotobacter chroococcum* in the rhizosphere (R) and nonrhizosphere (NR)of wheat at different stages of plant growth, cultivated under varying degrees of agricultural intensification at Ghoorpur, (Population in terms of $1x10^4$ /g oven dry soil, year 2003-2004 and 2004-05).

	Degree of Intensification/ Stages of Plant Growth	Seedling		Vegetative		Flowering		Fruiting	
		R	NR	R	NR	R	NR	R	NR
2003-04									
1	DAP 200 Kg/ha + Urea 160 Kg/ha + Wheat Only + Mechanical tillage + Tube well Irrigation 3 Times + Monoculture	2.0	0.7	2.3	0.8	3.2	1.1	2.8	1.0
2	DAP 120 Kg /ha + Urea 80 Kg/ha + Wheat + Mustard + Mechanical tillage + Tube well Irrigation 3 Times+ Monoculture	2.5	0.9	2.9	1.1	3.7	1.3	3.4	1.2
3	FYM + Bullock tillage + Canal Irrigation 3 Times + Wheat + Mustard + Pea + Crop Rotation	3.6	1.2	3.9	1.3	4.6	1.6	4.2	1.4
2004-05									
1	DAP 200 Kg/ha + Urea 160 Kg/h a+ Wheat Only + Mechanical tillage + Tube well Irrigation 3 Times + Monoculture	1.7	0.6	2.1	0.7	3.2	0.9	2.6	0..8
2	DAP 120 Kg /ha + Urea 80 Kg/ha + Wheat + Mustard + Mechanical tillage + Tube well Irrigation 3 Times+ Monoculture	2.3	0.8	2.8	1.0	3.5	1.2	3.2	1.1
3	FYM + Bullock tillage + Canal Irrigation 3 Times + Wheat + Mustard + Pea + Crop Rotation	3.7	1.2	4.0	1.4	4.7	1.7	4.3	1.5
	Minimum difference required for significance (C. D.) at 5% level:	0.53	0.04	0.07	00	0.14	0.07	0.09	0.09

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Population vs Chemical Inputs

Organic Carbon: Population of *Azotobacter chroococcum* varied with the organic carbon content of soil in both sites. Comparative data of the population of *A. chroococcum* with reference to % organic carbon content in the highly intensified fields of both sites ranged from 0.4 to 3.3 x10^4 /g oven dry soil in rhizosphere while the non-rhizospheric population ranged from 0.2 to 1.3 x 10^4 /g oven dry soil in the highly intensified fields. Similarly in the moderately intensified fields of both sites ranged from 0.8 to 4.0 x 10^4 /g oven dry soil while non-rhizospheric population ranged from 0.3 to 1.5 x 10^4 /g oven dry soil in the moderately intensified fields and in the low intensified fields of both sites ranged from 1.5 to 4.8 x 10^4 /g oven dry soil while non-rhizospheric population ranged from 0.6 to 1.9 /g oven dry soil (Fig. 1).

Nitrogen Content: Nitrogen is one of the most important factors affecting the population size of *Azotobacter chroococcum.* Population of *A. chroococcum* varied with the nitrogen content of soil in both sites. Comparative data of the population of *A. chroococcum* with reference to nitrogen in the low intensified fields of both sites ranged from 1.5 to 4.8 x 10^4 /g oven dry soil while non-rhizospheric population ranged from 0.6 to 1.9 /g /g oven dry soil while in the moderately intensified fields of both sites ranged from 0.8 to 4.0 x 10^4 /g oven dry soil while non-rhizospheric population ranged from 0.3 to 1.5 x 10^4 /g oven dry soil and in the highly intensified fields of both sites ranged from 0.4 to 3.3 x10^4 /g oven dry soil while non-rhizospheric population ranged from 0.2 to 1.3 x 10^4 /g oven dry soil (Fig. 1).

Phosphate: Population of *Azotobacter chroococcum* varied with the soil phosphate at both sites. Comparative data of the population of *A. chroococcum* with reference to phosphate in the low intensified fields of both sites ranged from 1.5 to 4.8 x 10^4 /g oven dry soil while non-rhizospheric population ranged from 0.6 to 1.9 /g /g oven dry soil while in the moderately intensified fields of both sites ranged from 0.8 to 4.0 x 10^4 /g oven dry soil while non-rhizospheric population ranged from 0.3 to 1.5 x 10^4 /g oven dry soil and in the highly intensified fields of both sites ranged from 0.4 to 3.3 x10^4 /g oven dry soil while non-rhizospheric population ranged from 0.2 to 1.3 x 10^4 /g oven dry soil (Fig. 1).

Potassium: Comparative data of the population of *A. chroococcum* with reference to potassium in the low intensified fields of both sites ranged from 1.5 to 4.8 x 10^4 /g oven dry soil while non-rhizospheric population ranged from 0.6 to 1.9 /g /g oven dry soil while in the moderately intensified fields of both sites ranged from 0.8 to 4.0 x 10^4 /g oven dry soil while non-rhizospheric population ranged from 0.3 to 1.5 x 10^4 /g oven dry soil and in the highly intensified fields of both sites ranged from 0.4 to 3.3 x10^4 /g oven dry soil while non-rhizospheric population ranged from 0.2 to 1.3 x 10^4 /g oven dry soil (Fig. 1).

Data show that irrespective of the degree of intensification, highest population of *A. chroococcum* was recorded in the fields of Site II and lowest at Site I. This may be attributed to the changes in the chemical properties of the soil with reference to the land use practices. High organic carbon content and low N and P content in the fields of Site II may be because of organic/FYM amendments, application of lower doses of inorganic fertilizers, mold-board tillage and crop rotation. On the other hand, lowest population at Site I may be because of

application of highest doses of inorganic fertilizers without any organic/FYM amendments, very high mechanical tillage and monoculturing.

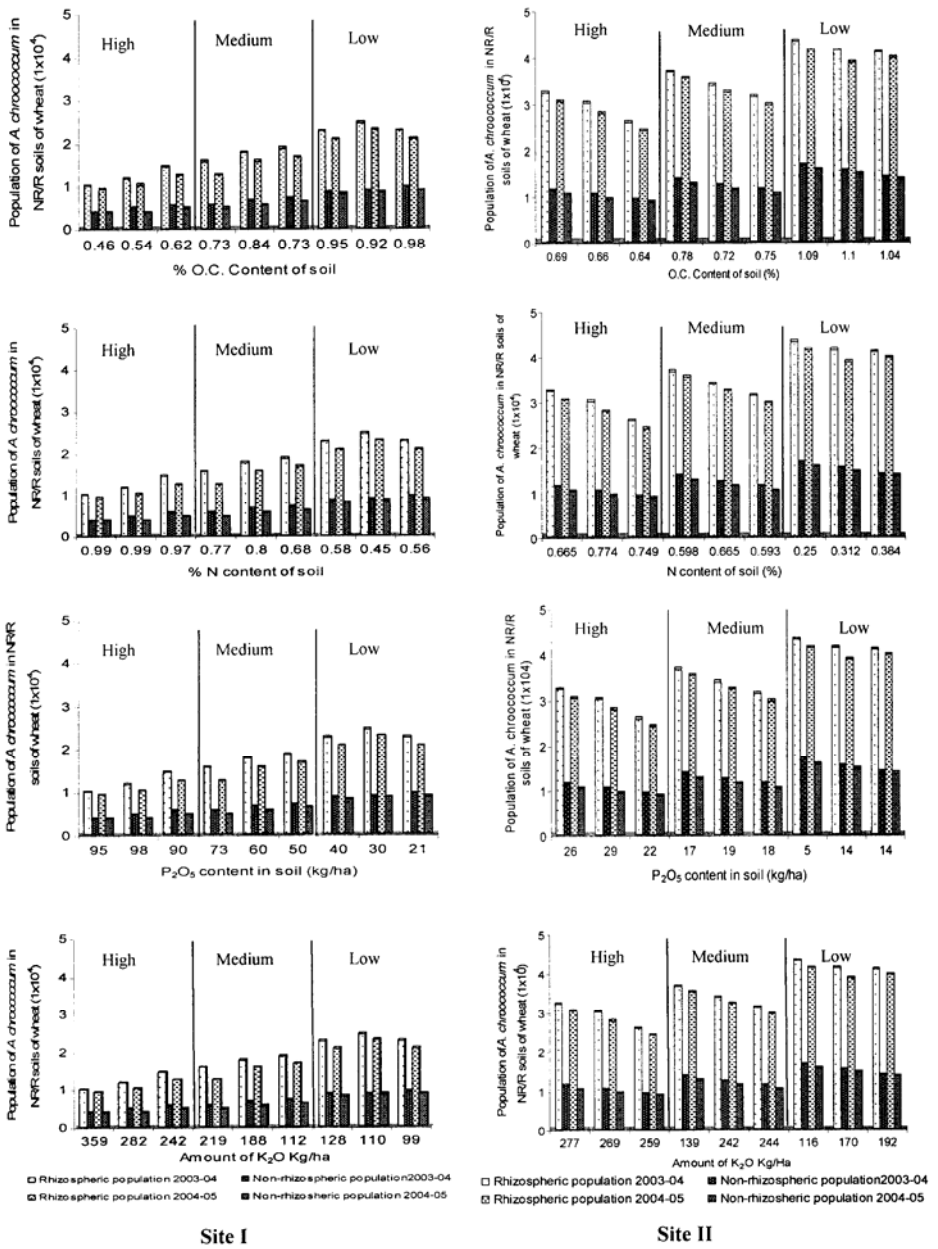

Fig. 1.The effects of various inorganic fertilizers on population size of *Azotobacter chroococcum*

Agricultural Intensification vs Nitrogen Fixing Potentiality

Nitrogen fixing potentiality of *A. chroococcum* is affected by various environmental as well as externally supplied chemical and mechanical inputs.

Tab. 9. Nitrogen fixing potentiality of *Azotobacter chroococcum* in wheat fields under different degrees of agricultural intensification at Site I and Site II (% N_2 fixed /10 mg dry weight of bacteria)

Subsite /Degree of Intensification		Site I								Site II							
		S		V		Fl		Fr		S		V		Fl		Fr	
		R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR
I	High Intensification	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	3.5	3.5	3.6	3.5	3.8	3.6	3.6	3.6
	Moderate Intensification	3.1	3.0	3.1	3.0	3.2	3.0	3.1	3.0	4.2	4.2	4.3	4.2	4.3	4.2	4.3	4.2
	Low Intensification	3.4	3.3	3.4	3.3	3.5	3.3	3.5	3.3	4.4	4.3	4.4	4.3	4.5	4.3	4.5	4.3
II	High Intensification	3.0	3.0	3.1	3.2	3.2	3.0	3.1	3.2	3.5	3.5	3.6	3.5	3.7	3.6	3.6	3.6
	Moderate Intensification	3.6	3.5	3.6	3.5	3.7	3.5	3.7	3.5	4.2	4.2	4.3	4.2	4.3	4.2	4.3	4.2
	Low Intensification	3.8	3.7	3.8	3.7	3.8	3.8	3.8	3.7	4.4	4.3	4.4	4.3	4.5	4.3	4.5	4.3
III	High Intensification	2.9	2.8	2.9	2.8	2.9	2.8	2.9	2.8	3.4	3.3	3.4	3.4	3.5	3.3	3.4	3.3
	Moderate Intensification	3.3	3.2	3.3	3.2	3.3	3.2	3.3	3.2	4.1	4.1	4.1	4.1	4.2	4.1	4.2	4.1
	Low Intensification	4.0	3.9	4.0	3.9	4.1	4.0	4.1	3.9	4.6	4.4	4.7	4.6	4.8	4.6	4.6	4.5

S= Seedling stage of Wheat. V= Vegetative Stage of Wheat. Fl= Flowering stage of Wheat. Fr= Fruiting stage of Wheat

Data collected on the nitrogen fixing potentiality of different isolates of A. *chroococcum* varied from site to site and field to field according to their chemical and mechanical inputs. Comparative data of nitrogen fixing potentiality of *A. chroococcum* collected from the highly intensified fields of both sites ranged from 2.8 to 3.8 /10 mg dry weight of bacteria. The nitrogen fixing potentiality of *A. chroococcum* collected from the moderately intensified fields of both sites ranged from 3.0 to 4.3 /10 mg dry weight of bacteria. Similarly in low intensified fields of both sites nitrogen fixing potentiality ranged from 3.3 to 4.8 /10 mg dry weight of bacteria. Maximum nitrogen fixing potentiality of *A. chroococcum* was recorded in the low intensified fields (Table 9).

Disscussion

Data collected from the high, moderate and low intensified fields of Allahabad and adjoining areas show that the population of *Azotobacter chroococcum* in the non-rhizosphere soil of a particular field remains more or less constant throughout the cropping period. Since the physical and chemical properties of the soil are known to affect the microflora in it (Biederbeck *et al.,* 1984; Goyal *et al.,* 1992; Kanazawa *et al.,* 1988), minor fluctuations in the population of *A. chroococcum* in the non-rhizosphere soil recorded during the survey period. These fluctuations may also be attributed to the change in the environment and soil characteristics such as organic content, pH, moisture and other nutrients (Tables 1).

Data shows that irrespective of the sites and degrees of agricultural intensification, the population of *A. chroococcum* was relatively higher in the rhizosphere soil than in non-rhizosphere (Table 2-13). This may be because of the activities of the roots, which may bring change in pH, moisture level as well as CO_2 and O_2 concentrations of the soil in their vicinity. In addition, they may also bring a change in the nutrient level of the soil by absorbing certain forms and releasing others in form of root exudates and sloughed off dead cells (Bertin *et al.,* 2003; Uren, 2000). Root exudation includes the secretion of ions, free oxygen, water, enzymes, mucilage and diverse array of carbon containing primary and secondary metabolites (Bertin *et al.,* 2003; Uren, 2000; Govedarica *et al*. 1999). The root exudates contain low-molecular weight compounds such as amino acids, organic acids, sugars, phenolics, polysaccharides and proteins (Bertin *et al.,* 2003; Uren, 2000). This may bring a change in its microbial populations. It may be concluded from the results that in wheat the microenvironment and nutrient status in the rhizospheric soil were better than non-rhizospheric soil. Investigations reporting similar observations substantiated with reasons have been extensively reviewed by Grayston *et al.* (1998), Bais *et al.* (2004).

It is also clear from the results of the present study that population of *A. chroococcum* recorded in the non-rhizosphere and rhizosphere varied with the stages of plant growth. Rhizosphere effect was caused at all stages but it varied with the age of plants (Table 3-8). The number of rhizosphere microorganisms increases with the age of plant normally reaching its maximum at stages of greatest vegetative growth or up to flowering and thereafter shows a gradual reduction (Khan and Prakash, 1982; Brady and Weil, 1999; Brimecombe *et al.,* 2001).

Population of *Azotobacter chroococcum* vs Agricultural Intensification

Data show that the maximum population in non-rhizospheric region was recorded in the low intensified fields of both sites, irrespective of the degrees of intensification. These are sites where farmers are incorporating FYM in the fields, following crop rotation and mixed cropping and applying optimum to meager doses of fertilizers in comparison to the other sites.

Manures are rich in NPK, Ca, Mg, Na and other trace elements (Eck and Stewart, 1995). Several works have shown that manure application characteristically results in increased biomass, activity and abundance of soil microbes (Collins *et al.,* 1992; Goyal *et al.,* 1992). FYM treated plots sustained crop productivity and microbial population at higher level (Mahajan *et al.,* 2007, Narayan and Kehri, 2008). Maximum counts of *Azotobacter* were observed in treatments where FYM was applied in addition to mineral fertilizers.

Crop rotation is known to influence beneficially many chemical properties of the soil including organic C content, N supply and transformation, pH and amount and availability of P, K, Ca and Mg (Stevenson and Van Kessel, 1996; Aref and Wander, 1998; Arshad *et al.,* 1998; Narayan and Kehri, 2008). Crop rotations with legumes are especially more beneficial than other crops (Stevenson and Van Kessel, 1996; Arshad *et al.,* 1998). Minimum population was observed in the non-rhizosphere soils of highly intensified fields. This may be due to indiscriminate use of chemical fertilizers, heavy mechanical tillage and continuous cultivation of rice and wheat (Narayan and Kehri, 2008).

Several works have shown that heavy use of chemical fertilizers limits the population of soil microbes (Ladd *et al.,* 1994; Smolander *et al.,* 1994; Omay *et al.,* 1997; Narayan and Kehri, 2008). Effect of nitrogenous fertilizers on the population of *A. chroococcum* was studied in several works (Emmimnath and Rangaswami, 1971; Mandic *et al.,* 2005; Mahajan *et al.,* 2007). According to them, heavy use of nitrogenous fertilizers not only affects the activity of the microbes but also the population of the diazotrophs. A depressive effect on the population of *Azotobacter* was recorded by these scientists. It was also found that effect of nitrogenous fertilizers was more effective in the non-rhizosphere than in rhizosphere (Mandic *et al.,* 2005; Mahajan *et al.*2007; Narayan and Kehri, 2008). Mahajan *et al.* (2007) after 33 years of continuous application of mineral fertilizers recorded decrease in the population of *A. chroococcum*. Higher range of *Azotobacter* counts was recorded in the fields treated with mineral fertilizers and FYM and lower in the fields treated with heavy application of mineral fertilizers.

Phosphorus has stimulatory effect on *Azotobacter* number in rhizosphere and non-rhizosphere soil (Roy and Mandal, 1968) but P fertilizers have indirect effect. P fertilizers often contain a significant amount of cadmium, mercury and lead (Mc Laughlin *et al.,* 2000) and long term chronic toxicity due to gradually accumulating metals appears to be far more dangerous to these microorganisms (Ceiller *et al.,* 1998).

Heavy tillage practices when applied indiscriminately in dry land cultivation frequently have disastrous effect. Tillage breakdowns soil structure, soil compaction, reduces infiltration, increases evasion and reduces soil organic

matter by subsequent degradation of previously protected organic matter (Elliott, 1986; de Luca and Keeney, 1994; Reicosky *et al.,* 1997). Several works have shown that tillage reduces microbial biomass and number when applied indiscriminately and frequently (Govedarica *et al*., 2001; Calderon *et al.,* 2001; de Luca and Kenney, 1994; Alvarez *et al.,* 1995).

Low percentage of organic carbon content in highly intensified fields is also one of the factors for low population of *A. chroococcum* (Figure 8, 9). Soils generally low in organic content can limit overall microbial activity and specific biological processes such as non-symbiotic nitrogen fixation. Therefore, significant nitrogen fixers' population can occur near decomposing crop residues and FYM (Roper, 1985; Gupta and Roper, 1993; Mahajan *et al.,* 2007) and in rhizosphere (Dobereiner *et al.,* 1972; Li and Macarae, 1991).

Monoculture is another reason for low population size. Continuous monoculture systems deteriorate soil physical condition and soil fertility (Sharma and Jain, 1997). Monoculture leads to change in microbial community composition attaching to the crops (Power and Flecker, 1996). It is generally agreed that large scale monoculture farming with mineral fertilizing has shown a decrease in the quantity of microbes in the soil and has also caused decrease in the diversity of microorganisms in agricultural land (Sharma and Jain, 1997).

Non-rhizospheric data analysis of the two consecutive years shows that there was decrease in population of *A. chroococcum* during second year in highly and moderately intensified fields, while in fields where sustainable use of the input was practiced rhizosphere population in all sites was always higher than the non-rhizospheric soil due to the rhizosphere effect. The rhizospheric population showed an increasing trend from seedling to flowering stage of plant growth and then a decline was recorded. This trend was observed in almost all the findings of other investigators (Timonin, 1940; Mall, 1975; Srivastava and Dayal, 1980; Verma, 1981; Khan and Prakash, 1982). Maximum rhizospheric population was recorded in the fields of Ghoorpur while minimum population was recorded at Soraon.

Nitrogen Fixing Potentiality vs Agricultural Intensification

Azotobacter chroococcum is known for its nitrogen fixing potentiality with the cereal crops. Nitrogen fixing potentiality of *A. chroococcum* is affected by the various environmental and chemical factors. It strongly depends on favourable moisture conditions, oxygen and organic food source. Isolates of *A. chroococcum* collected from the rhizospheric and non-rhizospheric soils of the same field show nearly similar nitrogen fixing potentiality. However, it varies from site to site and even in sub-sites according to their chemical and mechanical inputs. The maximum nitrogen fixing potentiality was observed by the isolates collected from the low intensified fields, which have less chemical and mechanical inputs and higher amount of organic carbon. On the other hand, minimum nitrogen fixing potentiality was observed in the isolates collected from highly intensified fields with heavy input of chemical fertilizers, monocropping and heavy mechanical tillage. Site wise analysis of data shows that maximum nitrogen fixing potentiality was recorded in the low intensified fields while

minimum nitrogen fixing potentiality was recorded in the high intensified fields. The high input of chemical fertilizers especially nitrogenous fertilizers are known to suppress the nitrogen fixing potentiality of *A. chroococcum.* Nitrogen applied in different forms such as ammonium, nitrate and urea suppresses the nitrogenase synthesis (Postgate, 1982; Merrick, 1992; Egener *et al.,* 1999). Furthermore, an immediate inhibition of nitrogenase activity in whole organisms, in response to exogenously supplied ammonium ions has been described to occur in *Azotobacter* (Laane *et al.,* 1980; Cejudo *et al.,* 1984). It has been shown by several authors that symbiotic and asymbiotic nitrogen fixation declines with increasing soluble inorganic nitrogen (Peoples *et al.,* 1995; Limmer and Drake, 1998). Even low concentrations of combined nitrogen, ammonium or nitrate lead to repression of nitrogenase genes (Merrick, 1992; Egener *et al.,* 1999) and to an inactivation of nitrogenase activity (Nordland, 2000; Martin and Reinhold-Hurek, 2002) in most of the bacteria.

The effect of phosphorus compounds on nitrogen fixation is not well understood. Becking (1961) reported that after certain limit, if the concentration of phosphorus compounds increases, the assimilation of nitrogen stops altogether, while Salmeron *et al.* (1990) investigated the effect of available phosphate on the nitrogen fixation and correlated them positively *i.e.* more nitrogenase activity is detected as the solubilization of phosphate increases.

Other agricultural practices such as tillage, irrigation, monoculture, mixed cropping, plant species and root exudates *etc*. also have indirect effects on the nitrogen fixing potentiality of *A. chroococcum*. Crop rotation mixed cropping and high concentration of organic carbon can influence nitrogen fixation by providing better food and nutrition to the bacteria. These inputs provide better environment for the growth and development of the *A. chroococcum*.

Conclusions

It is clear from the results of the present study that population of *A. chroococcum* recorded in the non-rhizosphere and rhizosphere varied with the stages of plant growth, chemical and mechanical inputs. Population of bacteria was maximum in the low intensified fields and minimum in highly intensified fields.

Acknowledgements

The authors are thankful to the Head, Department of Botany, University of Allahabad, Allahabad, India, for providing the library and laboratory facilities and to CSIR, New Delhi for providing financial assistance to Mr. Raghvendra Pratap Narayan during his D. Phil.

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Acta Agriculturae Serbica, Vol. XVI, 32 (2011) 97-122

UTICAJ RAZLIČITIH POLJOPRIVREDNIH MERA NA DINAMIKU POPULACIJE *Azotobacter chroococcum* I NJEN POTENCIJAL AZOTOFIKSACIJE U TRANS-GANGSKIM I TRANS-JAMUNSKIM RAVNICAMA INDIJE

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Ispitivanje je izvršeno tokom dve uzastopne godine 2003-2004 i 2004-05 u Alahabadu, jednom od najplodnijih regiona Indo-gangskih ravni za proizvodnju pšenice. Cilj je bio ispitati uticaj različitih poljoprivrednih mera na dinamiku populacije i potencijal azotofiksacije slobodnih aerobnih azotofiksirajućih bakterija *Azotobacter chroococcum.* Velike količine unetih đubriva, mehanička obrada tla, često navodnjavanje i monokultura doveli su do pada brojnosti *Azotobacter chroococcum* i njenog potencijala azotofiksacije. Veća brojnost populacije *Azotobacter chroococcum* i bolji potencijal azotofiksacije utvrđen je na poljima na kojima je vršena racionalna primena hemijskih đubriva, uz upotrebu plodosmene, stajskog đubriva i niskog stepena obrade. Plodosmena i primena stajskog đubriva izvršili su bolji uticaj na rast i razvoj *Azotobacter chroococcum* kao i na njen potencijal azotofiksacije.

Ključne reči: intenzifikacija, *Azotobacter chroococcum,* potencijal azotofiksacije, trans-Gang, trans-Jamuna