

Original Research Article

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Potential Impact of Long Term Use of Integrated Nutrient Management Practices on the Population of *Azotobacter* and *Azospirillum* spp. and urease activity to Sorghum-Wheat Sequence in Vertisol

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ABSTRACT

The cultivated soils have heterogeneous environments in which the soil conditions affecting microbial population, its growth and diversity fluctuate very widely in space and time and that regulate nutrients availability and crop productivity. In present study, the effect of long term (32 years) use of mineral fertilizer with and without organic manures were evaluated for monitoring the population of the nitrogen fixing *Azotobacter* and *Azospirillum* spp. community structure, activity of urease, status of available nitrogen-their depletion and buildup in irrigated Vertisols under sorghum –wheat cropping sequence. The All India Co-ordinated Research Project of ICAR have initiated the long term fertilizer field experiment during *Kharif* 1984-85 at Main Center of Integrated Farming Systems Research Project of Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra). This experiment consists of 12 treatment combinations of only inorganics fertilizers, INM: NPK integrated with organics viz., FYM, Wheat Cut Straw and green manure, farmers practice and unfertilized control with 4 replications in RBD experimental design. The results of the long term experiment revealed that the significant the highest increase in population of *Azotobacter* (31.67×10^6 cfu g^{-1} soil) and *Azospirillum* (22.60×10^6 cfu g^{-1} soil) were observed in the INM treatment combination of organic manure (FYM)with chemical fertilizer i.e. 50% NPK through chemical + 50% N through FYM to *Kharif* and 100% RDF to in *Rabi* and the same treatment recorded highest urease activity ($42.09 \text{ mg NH}_4^+ - \text{N } 100 \text{ g}^{-1}$ soil) over rest of treatments combinations. The occurrence of the dense community of *Azotobacter* and *Azospirillum* in the INM was probably due to higher availability of substrate as carbon which help in increasing microbial population in soil.

Keywords

Azotobacter and *Azospirillum* spp. population, Urease activity, Available N, INM, Long-term fertilization

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Introduction

After the successful first green revolution soil nutrient management strategies were mainly dependent on the only use of inorganic

chemical fertilizers, but its injudicious uses caused a serious threat not only to soil health but also to human health and environment quality. Fertilization is the most common management input for productivity of

agricultural soils. Organic and inorganic fertilizers are primarily used to increase crop productivity, and in the short-term fertilizer experiments, they do not show a significant effect on microbial community; however, in long-term fertilizer experiments, they can affect the function, community structure and population of soil microorganisms. Biological nitrogen fixation contributes about 60% of the total nitrogen fixed on Earth, on the contrary, commercial fertilizers contribute 25%. Long term fertilization usually not only strongly favors the accumulation of bacterial residues but also increases soil microbial biomass, which is a key soil factor that regulates and maintain soil health.

Among the requirement of crops for their productivity after water, nitrogen is most often limiting and a unique and major plant nutrient because unlike the other essential nutrient elements, plant can use it either in the cation form (NH_4^+) or in the anion form (NO_3^-). Only a small part of total soil nitrogen occurs in these forms at any one time. The addition of organic matter in the form of manures greatly influences the transformation and availability of nitrogen (N) and several other essential plant nutrients through its impact on the chemical and microbiological properties of soil. Of these the role of organic manures in supplying plant nutrients, particularly N is most prominent. Crop response to fertilization by N is inextricably linked to its biogeochemical cycling among its numerous biotic and abiotic forms in soil. Since microbes carry out the most significant N cycle processes, they have a major impact on the abundance of all forms of soil N (Rai, 2013).

Urease activities in soils have received a lot of attention since it was first reported by Rotini (1935), a process considered vital in the regulation of N supply to plants after urea fertilization. Urea is one of the most

important chemical nitrogen fertilizers; urea hydrolysis in soil is an enzymatic decomposition process by enzyme urease.

Many long-term experiments conducted in India showed increasing yields and accumulation of soil organic carbon and biological properties due to combined application of fertilizers and manures (Manna *et al.* 2005, 2007, Mandal *et al.*, 2007). The hypothesis of the present study was that the effect of long term use of integrated nutrient management practices on the population of *Azotobacter* and *Azospirillum spp.* to Sorghum-Wheat cropping system in Vertisol under semi-arid climatic condition of central India.

Materials and Methods

Experimental site and treatments

The long term (32 years) fertilization experiment was initiated in *Kharif* 1984-85 at Main Center of Integrated Farming Systems Research Project of Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra) by the All India Co-ordinated Research Project (AICRP) of ICAR. The experimental field is located at $19^{\circ}47'N$ latitude, $74^{\circ}18'E$ longitude and at an elevation of about 495 m above mean sea level. Agro climatically the experimental site is semi-arid with hot and dry summer and cool to mild winter.

The soil of the experimental field has a clay loam texture (sand 22.3%, silt 26.4%, and clay 51.3%), *Typic Haplustert* with pH 8.2, organic carbon 0.64 g kg^{-1} , electrical conductivity 0.27 dSm^{-1} and bulk density 1.32 Mg m^{-3} of soil. The soil available N, P and K were 153, 14.2, 705 kg ha^{-1} , respectively. The DTPA-extractable iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were 12.95, 22.10, 0.87 and 3.27 mg kg^{-1} , respectively.

Sorghum cultivar CSH-9 and wheat HD-2189 were grown in sequence during *Kharif* and *Rabi* season respectively at the same site. The experiment consisted of twelve treatments, each replicated four times in randomized block design (RBD) having individual plot size of 12.6m x 8.10m. The fertilization treatment details for *Kharif* and *Rabi* are given in Table 1.

Dhaincha (*Sesbania aculeata*) was raised during late summer in a separate field as green manuring crop and incorporated after 55 days growth period in respective treatment plots. Calculated amount of organics viz., well decomposed FYM and crop residue of wheat straw were uniformly spread and incorporated into the soil with the help of power tiller before sowing of sorghum in respective treatments. The N content (quantity applied) of FYM, CR and GM used in the experiments over the years at 50% N were 0.50–0.62% (12.00 to 9.67 t ha⁻¹), 0.43 to 0.50% (13.95–12.00 t ha⁻¹) and 1.7–2.4% (3.52–2.5 t ha⁻¹), respectively.

The recommended dose for sorghum and wheat was 120:60:40 kg N: P₂O₅:K₂O ha⁻¹. All P₂O₅ and K₂O and a half dose of N were drilled at sowing. The remaining N was top-dressed at 30 days in sorghum and 21 days after sowing in wheat. The sources of N fertilizer were urea (46%N), P fertilizer were single superphosphate (16 % P₂O₅) and for K fertilizer muriate of potash (60 % K₂O).

Soil sampling and analysis

After completion of 32 years of experiment for the proposed study, total 48 samples were taken at 0-15 cm depth, processed in laboratory and used for chemical properties and for biological parameters stored in deep fridge. These stored samples were further used for microbial population count of *Azotobacter* and *Azospirillum* spp.

(Halvorsun and Zeiglar, 1993), urease activity (Tabatabai and Bremner, 1972), available N (Subbaih and Asija, 1956). The data Statistical analyzed by using methods of Panse and Sukhatme 1985) are presented in Table 2 with respect of microbial population, urease activities and soil available nitrogen.

Results and Discussion

Azotobacter population

The *Azotobacter* population was significantly varied between 15.75 - 31.67 x 10⁶cfu g⁻¹ soil among the all treatments. As compared to the population of *Azospirillum*, the all 12 treatments recorded the higher population of *Azotobacter*. It was also observed that the lowest population (15.75 x 10⁶ cfu g⁻¹ soil) was observed in the unfertilized control plot (T₁) and significantly the highest population was observed in the treatment received 50 % RDF + 50 % N-FYM in *Kharif* and 100 % RDF in *Rabi* (T₆). The treatment (T₆) also recorded 50.26 per cent more *Azotobacter* population over the control. The average *Azotobacter* population due to application of only chemical fertilizers treatments (T₂ to T₅) was 19.24 x 10⁶ cfu g⁻¹ soil, which was observed to be increased to 28.75 x 10⁶cfu g⁻¹ soil in the treatments (T₆ to T₁₁) involving integrated use of chemical fertilizers and organic manures. It was also noticed to be low *Azotobacter* population (17.57 x 10⁶cfu g⁻¹ soil) in the farmers practice treatment (T₁₂).

Azotobacter is a heterotrophic, aerobic nitrogen fixation bacterium that lives freely. Fertilization with NPK can increase *Azotobacter*, but if it is done with inorganic fertilizers continuously, it reduces the population (Rao, 1994). The increase in *Azotobacter* population in INM (FYM + NPK) treatments was probably due to higher availability of substrate as carbon from supplied FYM, intensive rooting activity and

better soil water status and also nutrient available through chemical fertilizers in INM treatments. The similar observations were reported by Mahajan *et al.*, (2007) and Nerula *et al.*, (2002). In the present study, there was significantly higher number of *Azotobacter* in the treatment with FYM compared to the other treatments. These results are in accordance with the results published by Mikanova *et al.*, (2009), who showed that nitrogen fertilization in organic form (FYM) increased the counts of *Azotobacter* spp. Kubat *et al.*, (1997) also documented increasing counts of free-living nitrogen-fixing bacteria in a long term experiment in Ruzyne with FYM + NPK treatment as compared to the control.

Azospirillum population

The results indicated that the significant variations of *Azospirillum* population (10.4 to 22.6×10^6 cfu g^{-1} soil) were recorded in all treatments combination. The *Azospirillum* population was observed significantly the highest (22.6×10^6 cfu g^{-1} soil) in the treatment which received 50 % RDF + 50 % N-FYM in *Kharif* and 100 % RDF in *Rabi* (T_6), whereas, the lowest *Azospirillum* population (10.4×10^4 g^{-1} cfu soil) was observed in absolute control (T_1). However, the significant increase in the *Azospirillum* population in treatment T_6 was 53.98 per cent over the control.

The average *Azospirillum* population recorded from the treatments of chemical fertilizers (T_2 to T_5) was 15.75×10^6 cfu g^{-1} soil which was lower as compare to average population 19.64×10^6 cfu g^{-1} soil in the treatments involving organics in conjunction with chemical fertilizers (T_6 to T_{11}). The *Azospirillum* population (16.1×10^6 cfu g^{-1} soil) was observed to be low in the farmers practice treatment (T_{12}). In general, the study showed that continuous application of NPK fertilizer

in combination with FYM, WCS and GM increased the *Azospirillum* population in soil over control and only 100 per cent RDF treatment (T_5). The similar results were also reported by the Jayathilake *et al.*, (2006) and Chang *et al.*, (2007).

The present study indicated that the application of manure increased the population of *Azospirillum*, whereas, application of only NPK fertilizer alone did not affect significantly the population of *Azospirillum*. Manure application along with NPK fertilizers can increase the content of ammonium in the soil to obtain a high *Azospirillum* population as compared with the only NPK fertilizer treatment and unfertilized control. These results proved that manure with NPK fertilizers can increase the population of bacteria in the soil. It is expected to increase soil fertility and crop productivity. The present results are in the close confirmatory with the earlier findings of Mujiyati and Supriyadi (2008) and Gurusurthy (2014).

Urease activity

The urease activity in experimental soil was ranged from 24.24 to 42.09 mg NH_4^+ - N 100 g^{-1} soil with the highest urease activity (42.09 mg NH_4^+ - N 100 g^{-1} soil) reported in treatment T_6 (50 % RDF + 50 % N-FYM in *Kharif* and 100 % RDF in *Rabi*) and was significantly superior over all other treatments with 42.09 mg NH_4^+ - N 100 g^{-1} soil value of urease activity. The results revealed that conjoint use of organic manures and mineral fertilizers (T_6 to T_{11}) favored the higher urease activity (37.26 mg NH_4^+ - N 100 g^{-1} soil) as compared to treatments receiving only chemical fertilizer (T_2 to T_5) which was 31.41 noticed as mg NH_4^+ - N 100 g^{-1} soil, in farmers practice (T_{12}) as 30.19 mg NH_4^+ - N 100 g^{-1} and the least in control (T_1) (24.24 mg NH_4^+ - N 100 g^{-1} soil).

It was observed that in present study increase in fertilizer level from suboptimum to optimum level significantly increased urease enzyme activities and found to be maximum in 100 per cent recommended dose of fertilizers within the only inorganic fertilizers treatments. Integrated use of mineral fertilizers with FYM (T_6) showed 20.38, 28.27 with 42.40 per cent increase in soil urease activity as compared to 100 per cent RDF, farmers practice and unfertilized control treatments; respectively.

Soil urease mainly originates from SOM, microbes and plants roots secretion that catalyze hydrolysis of urea fertilizers applied to soil or C-N compounds that are supplied through organics into NH_3 with hydrolysis reaction on C-N bonds in substrate or substrate complex (urea and urea like N compounds in soils) supplied through SOM or various applied in INM treatments. In fact, the enzyme activity in the soil is very much governed by the concentration of substrate present and enzyme in the soil.

The rate of urea hydrolysis by soil urease increases with increase in substrate (C-N bounded N complexes) concentration until the quantity of urea added is saturated and its activity becomes constant (Bremner and Mulvaney, 1978). The differences in urease activity between WCS, GM and FYM at the same level of recommended dose fertilizers was very much governed by their N content and C: N ratios. Similar results were also reported by Rai and Yadav (2011). The treatments with chemical fertilizers, farmers practice and absolute control recorded lower urease activities than all the INM treatments which could be attributed to lack of sufficient substrate on concentration of enzyme i.e. organic carbon which act as an energy source for prolife rating the microbial population (Kanchikerimatha and Singh, 2001) that governs the concentration of urease and so

also concentration of substrate like C-N complexes from various treatments. Thus, the balanced nutrition to crops under integrated use of chemical fertilizers with FYM responsible for better proliferation of roots and microbial population as basis for urease concentration and also availability of substrate of urease were responsible for maximum activity of urease. These results are corroborated with the earlier findings of Manna *et al.*, (2005a), Mandal *et al.*, (2007), Rao and Pathak (1996) and Bhattacharyya *et al.*, (2011).

Soil available nitrogen

The soil available N content was varied from 125.20 to 197.05 $kg\ ha^{-1}$ at surface layer. The treatment T_6 (50 % RDF + 50 % N-FYM in *Kharif* and 100 % RDF in *Rabi*) recorded the significantly the highest N content (197.05 and 177.37 $kg\ ha^{-1}$), however, the treatment (T_{10}) 50 % RDF + 50 % N-GM in *Kharif* and 100 % RDF in *Rabi* was significantly at par with T_6 .

The significant per cent increase in available N content in soil were 36.44 and 23.00 per cent over the control and farmers practice; respectively. The average increase in available N content in INM treatments (T_6 to T_{11}) recoded the higher (189.51 $kg\ ha^{-1}$) than the average value (175.49) in only inorganic (T_2 to T_5) fertilizers treatments, farmers practice (151.72 $kg\ ha^{-1}$) and control (125.20 $kg\ ha^{-1}$) at 0-15 cm soil depth. From the present investigation it was clearly observed that the integrated use of organics with balanced inorganic fertilizers recorded higher available N content than imbalanced fertilizers. The lower available nitrogen in control and farmers practices was a result of low available nitrogen with continuous cropping without or inadequate fertilization over a long period (32 years) of time (Fig. 1).

Table.1 Different treatment combinations in the long-term experiment

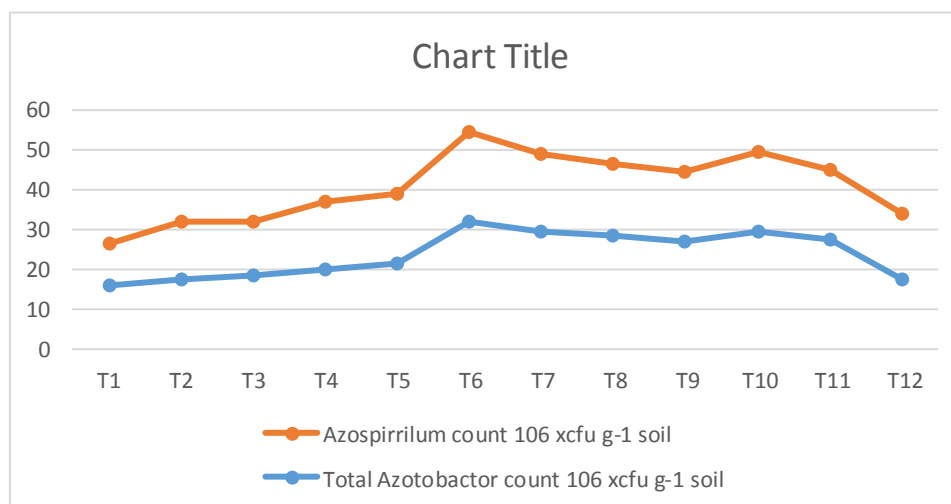
Treatments	Kharif Sorghum	Rabi Wheat
T ₁	No fertilizer, no organic matter (control)	No fertilizer, no organic matter (control)
T ₂	50% recommended NPK through fertilizers	50% recommended NPK through fertilizers
T ₃	50 % recommended NPK through fertilizers	100% recommended NPK through fertilizers
T ₄	75% recommended NPK through fertilizers	75 % recommended NPK through fertilizers
T ₅	100% recommended NPK through fertilizers	100% recommended NPK through fertilizers
T ₆	50% recommended NPK through fertilizers + 50% N through FYM	100% recommended NPK through fertilizers
T ₇	75% recommended NPK through fertilizers + 25% N through FYM	75% recommended NPK through fertilizers
T ₈	50% recommended NPK through fertilizers + 50% N through WCS	100% recommended NPK through fertilizers
T ₉	75% recommended NPK through fertilizers + 25 % N through WCS	75% recommended NPK through fertilizers
T ₁₀	50% recommended NPK through fertilizers + 50% N through GM	100% recommended NPK through fertilizers
T ₁₁	75 % recommended NPK through fertilizers + 25 % N through GM	75% recommended NPK through fertilizers
T ₁₂	Farmer's conventional practice	Farmer's conventional practice

FYM-Farm Yard Manure, WCS- Wheat Cut Straw and GM- Green Manuring

Table.2 Effect of 32 years fertilization on *Azotobacter* and *Azospirillum spp.*, urease activity and available nitrogen

Tr. No.	Kharif Sorghum	Rabi wheat	<i>Azotobacter</i> count (cfu x10 ⁶ g ⁻¹ soil)	<i>Azospirillum</i> count (cfu x10 ⁶ g ⁻¹ soil)	Available N (Kg ha ⁻¹)	Urease activity (mg NH ₄ -N 100 g ⁻¹ soil hr ⁻¹)
	Control	Control	15.75	10.4	125.20	24.24
T ₁	50% RDF	50% RDF	17.32	14.4	161.70	29.58
T ₂	50% RDF	100% RDF	18.33	13.3	170.38	30.80
T ₃	75% RDF	75% RDF	19.75	16.9	179.92	31.76
T ₄	100% RDF	100% RDF	21.59	17.2	189.96	33.51
T ₅	50% RDF+50% N-FYM	100% RDF	31.67	22.6	197.05	42.09
T ₆	75% RDF+25% N-FYM	75% RDF	29.17	19.8	183.82	36.49
T ₇	50% RDF+50% N-WCS	100%RDF	28.25	18.1	186.10	39.99
T ₈	75% RDF+25% N-WCS	75%RDF	26.84	17.5	181.23	32.81
T ₉	50% RDF+50% N-GM	100%RDF	29.25	20.3	196.50	41.42
T ₁₀	75% RDF+25% N-GM	75%RDF	27.34	17.8	192.37	30.80
T ₁₁	Farmers practice	Farmers practice	17.57	16.1	151.72	30.19
Mean			23.57	17.03	176.33	33.64
SE(m)±			0.69	0.61	1.43	0.2
CD at 5%			1.96	1.76	4.20	0.59
Initial			-	-	157	-

Fig.1 Effect of long term application of manures and fertilizers to sorghum-wheat sequence on N-fixing bacteria



Increase soil available nitrogen in INM had attributed to its direct addition through organics as FYM, GM and WCS, because the favorable soil conditions viz. organic carbon, porosity, water holding capacity, higher population of N fixing bacteria and urease activity etc. might have helped in the mineralization and reduced nitrogen fixation of soil nitrogen leading to buildup of higher available nitrogen. Thus, from the results obtained, it was seen that the balanced use of NPK fertilizers along with manures was essential for sustaining N status in Vertisol under cereal – cereal cropping system. The available N status although showed increase under INM, it has not been increased much due to the prevailing climatic condition accelerating oxidation of organic matter as well as the nature of nitrogen forms in soil and the form of its losses through volatilization and leaching. In this view, the results of present investigation suggest that the maintenance of soil available N levels is more challenging. This necessitates regular addition of organics for maintenance of soil fertility in the soils of tropical areas. These results are in conformity with the findings of Babhulkar *et al.*, (2000), Tiwari *et al.*, (2002),

Sammy *et al.*, (2003), Gupta *et al.*, (2006), Singh *et al.*, (2007) Urkurkar *et al.*, (2010) and Kharche *et al.*, (2013).

Thus, it is concluded that, the INM practices are proved to be superior in making availability of soil nitrogen on a sustainable basis. Integration of chemical fertilizers by substituting 50 per cent N through FYM was found the most superior in respect of soil available N content as compared to GM or WCS. The highest availability of nitrogen in this treatment may be attributed to greater microbial (*Azotobacter* and *Azospirillum*) activities caused by the higher supply of active carbon through FYM source for conversion of organically bound N to inorganic form that helped in the higher mineralization of soil N leading to the buildup of available N in the soil.

Adaption of long term integrated nutrient management practice: 50% NPK through chemical + 50% N through FYM to Sorghum and 100% RDF through chemical to wheat helps to fulfill the nitrogen requirement of Sorghum-Wheat cropping sequence in Vertisol by saving 50% N through the activity

of *Azotobacter* and *Azospirillum* also reflected in sustaining crop productivity and soil health.

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