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Impact of various nutrient sources on soil microbial population under pearl millet-wheat cropping system

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Abstract

Background: Soil quality, availability of nutrients, biological health of soil and environmental conditions are important criteria for improving crop yield to achieve food security. In North-Western region of India, pearl millet [*Pennisetum glaucum* (L.)] and wheat (*Triticum aestivum* L.) is one of the vital cropping systems. Due to low organic matter content of soil in this region, fertility of soil is very important for obtaining optimum crop production. The quality of soil organic matter and carbon and nitrogen inputs are the overriding controls to obtain sufficient soil microbial biomass for efficient biogeochemical functioning. Various organisms present in the soil play vital roles in biological nitrogen fixation and solubilization of phosphorus leading to enhanced plant growth. The current study was aimed to evaluate the impact of various nutrient sources on the population of nitrogen- fixing (diazotrophs) bacteria and phosphorus solubilizing bacteria (PSB) under pearl millet-wheat cropping system.

Methods: The experiment was conducted at the **p**ermanent plots of integrated nutrient management on cereal based cropping system, which has been started from *Kharif* 1985. The current experiment was conducted for two years (2015-16 to 2016-17) on permanent plots to evaluate the effect of various nutrient sources on the population of diazotrophs and PSB at Agronomy Research Farm, CCS Haryana Agricultural University, Hisar (India). Surface soil samples (0-15 cm) were collected from the different treatments at two stages of plant growth i.e., one sample before sowing of the crop and another sample at the time of flowering of crop. Soil samples were processed and analyzed for population of nitrogen-fixing *Azotobacter* and phosphorus solubilizing bacteria.

Result: During the first year of pearl millet cultivation, the population of *Azotobacter* was 34% higher in T_6 than T_5 at the time of flowering, whereas the population of A*zotobacter* was 28% higher in T_6 during the second year than T_5. Similarly, the population of PSB was found 17% and 15% higher in T_6 than T_5 during first and second year, respectively at the time of flowering in pearl millet crop. The bacterial count analysis in case of wheat crop showed that total bacterial population increased from 19.5×10^7 (cfu/g soil) to 19.6×10^7 (cfu/g soil) during first and second year, respectively at the flowering time. It was observed that the population of *Azotobacter*, PSB and total bacteria was higher in the treatment T_6 among all the treatments. Results suggested that the quality of soil organic matter and inputs of carbon and nitrogen are the important factors, which affect soil microbial biomass and microbial beneficial functions.

Keywords: *Azotobacter*, chemical fertilizers, FYM, green manure, pearl millet, PSB, wheat, wheat straw

Introduction

The dramatic increase in worldwide agricultural production per unit area significantly reduced starvation rates in the developing world and enhanced the global food supply (Lal, 2009) [18]. During recent intensive farming systems, chemical fertilizers (consisting of N, P and K) are applied excessively to provide the nutrients for plants for increasing agricultural productivity. However, injudicious use of these fertilizers has caused pollution problems leading to public health hazards. Moreover, use of chemical fertilizers alone was not efficient in improving the nutrient status of soil (Kang *et al*., 2005) [15] and integrated nutrient management system was found more appropriate to enhance soil fertility and plant growth. During last decades, it has been widely acknowledged that system oriented production research is needed to be strengthened as it is essential for maximizing land production per unit area, by harnessing synergies generated through microbial interactions in soil-crop-weather systems. Cropping systems approach of resource management was demonstrated to improve nutrient use efficiency.

Pearl millet [*Pennisetum glaucum* (L.)]- wheat (*Triticum aestivum* L.) is one of the vital cropping systems of the country, which is spread over arid and semi-arid regions.

This cropping system contributes significantly towards total food grain production in the country. During the last five decades, consumption of chemical fertilizers (NPK) has increased phenomenally from 0.07 mt in 1950-51 to 25.95 mt in 2016-17 (Anonymous, 2017) [1]. With the application of recommended doses of fertilizers, the yield potential of cereal-cereal cropping system has reached to a plateau. Moreover, application of excessive fertilizers has deteriorated soil health and especially organic matter has depleted.

For obtaining optimum crop production the fertility of soil is very important and the absence of organic matter from soil results in unproductive soil. To supply essential plant nutrients in adequate amounts, huge quantity of organic material is needed. Slow release of plant nutrients after decomposition from organic material has been reported to promote crop growth (Goutami *et al*., 2018) [8]. Organic sources when applied to preceding crop, leaves the residual effect and this benefit is harvested by the succeeding crop and system becomes sustainable through integrated use of crop residues, chemical fertilizers, green manure and FYM (farm yard manure) as a source of nutrients.

Effect of different farm management practices (organic and conventional) on the microbial population have been studied and microbial population were found higher in soils of organic farming than conventional farms (Carine *et al*., 2009; Sudhakaran *et al*., 2013; Uddin *et al*., 2016) [3, 24, 25]. Organic food production relies on microbially derived ecosystem functioning and thus offers a model system for ecological intensification of agriculture (Jackson *et al*., 2012) [11]. The microbial population of the soil is enhanced upon receiving nutrients either through chemical fertilizer or organic manure. Besides this, addition of organic manure along with inorganic fertilizer showed a profound increase in the microbial population in comparison to chemical fertilizer used alone. Organic sources of essential plant nutrients were found useful in enhancing crop productivity, soil properties and biological activity (Singh *et al*., 2003) [23]. The decomposition and mineralization of added organic matter acted as a source of the nutrients, thereby creating a favorable environment for proliferation of microbes in the soil (Lal *et al*., 2012) [17]. Thus, distinct organic amendments like manure, leguminous cover crops and composted materials were found to stimulate microbial biomass differently through increased labile organic matter (Kallenbach and Grandy, 2011) [14].

Higher population of beneficial microbes was observed under integrated nutrient management system than sole application of chemical fertilizers. These rhizosphere-inhabiting microbes provide benefits to crop plants through better nutrient availability by way of atmospheric nitrogen (N_2) fixation or solubilization of fixed mineral forms of nutrients (phosphorus and potassium). Considering the above points in view, the present experiment was conducted to study the effect of various nutrient sources on soil microbial population under pearl millet - wheat cropping system.

Material and Methods

Experiment on integrated nutrient management based on cereal cropping system was started during *Kharif* 1985 on permanent plots in the fields of Agronomy Research Farm, CCS Haryana Agricultural University, Hisar (India). The experiment was conducted for two years to evaluate the effect of various nutrient sources on the population of nitrogen-fixing *Azotobacter* species, and phosphorus solubilizing bacteria during 2015-16 to 2016-17. Experimental design consisted of 12 treatments *viz*., T_1 - control (no fertilizer); T_2 - 50% recommended N and P to pearl millet and wheat through fertilizers; T_3 - 50% recommended N and P to pearl millet and 100% recommended N and P to wheat through fertilizers; T_4 - 75% recommended N and P to pearl millet and wheat through fertilizers; T_5 - 100% recommended N and P to pearl millet and wheat through fertilizers; T_6 - 50% recommended N and P through fertilizer + 50% N (farmyard manure) to pearl millet and 100% recommended N and P to wheat through fertilizer; T_7 - 75% recommended N and P through fertilizer + 25% N (farmyard manure) to pearl millet and 75% recommended N and P to wheat through fertilizer; T_8 - 50% recommended N and P through fertilizer + 50% N (wheat straw) to pearl millet and 100% recommended N and P to wheat through fertilizer; T_9 - 75% recommended N and P through fertilizer + 25% N (wheat straw) to pearl millet and 75% recommended N and P to wheat through fertilizer; T_{10} - 50% recommended N and P through fertilizer + 50% N (green manure) to pearl millet and 100% recommended N and P to wheat through fertilizer; T_{11} - 75% recommended N and P through fertilizer + 25% N (greenmanure) to pearl millet and 75% recommended N and P to wheat through fertilizer and T_{12} - farmers' practice was laid out in randomized block deign with three replications. Farm Yard Manure (FYM), *Sesbania* spp. (as a green manure) and wheat straw were applied in the field for supplying 25% and 50% N before plantation of pearl millet.

Soil samples were collected before sowing and at the time of flowering for both pearl millet and wheat crop. Soil was collected from different treatments at 0-15 cm depth during both years. After collection, soil samples were refrigerated at 4^0C for analysis of the microbial population. Viable counts of *Azotobacter* and PSB under different combination of integrated nutrient management treatments, were determined using serial dilution plating method (Lindow *et al*., 1978) [19]. Various dilutions were spread on appropriate medium plates and incubated for 5 days. Number of colonies (colony forming units; cfu) developed on different dilution media plates was recorded and population of each bacterium per gram soil was enumerated. The significance of treatment effect was analyzed using two factorial RBD analysis using OP stat, CCS HAU, Hisar, software.

Results and Discussion

The viable count of *Azotobacter* species and PSB before sowing of crop and at the flowering in pearl millet was calculated. Results revealed higher microbes' counts in integrated nutrient management treatment as compared to chemical fertilizer treatments. The *Azotobacter* population showed significant variations from 3×10^3 to 38×10^3 and 6×10^3 to 82×10^3 cfu/g of the soil before sowing and at the time of flowering in pearl millet during the first year of the experiment, respectively (Table 1). Similarly, the viable count of *Azotobacter* varied significantly in various treatments from 5×10^3 to 96×10^3 cfu/g of soil with the minimum in control (T_1) and maximum in T_6 at the time of flowering during the second year. Sudhakaran *et al*. (2013) [24] also reported that the *Azotobacter* population was higher in an organic farm and the lower population was observed in control treatment. Lower microbial biomass in soils from conventional agroecosystems is often caused by reduced organic carbon content in the soil (Fliebach and Mader, 2000) [5]. Samples from sustainable farm showed 24.7×10^7 cfu g^{-1} microbial population, whereas conventional farm had 26.3×10^7 cfu g^{-1}

population. In another set of experiments, amendment of the field soil with FYM along with 100% NPK increased the population of *Azotobacter* in comparison to treatments without the application of FYM (Jain *et al*. 2003) [13], indicating stimulating effect of FYM on the population of nitrogen-fixing *Azotobacter* species.

The population of total bacteria ranged from 3.5×10^7 (T_1) to 19.1×10^7 (T_6) cfu/g soil (first year) and 3.4×10^7 (T_1) to 19.4×10^7 (T_6) cfu/g (second year) at the time of flowering. A significant increase in bacterial population was observed under the conjoint use of inorganic fertilizers with FYM. Sudhakaran *et al*. (2013) [24] showed that the bacterial population was higher in organic farming and the sustainable farming system contained 24.3×10^7 cfu g^{-1} soil and the control treatment had 23×10^7 cfu g^{-1} soil. The lowest bacterial population was recorded in the conventional farm system. Selvi *et al*. (2004) [21] also observed similar results in which higher bacterial counts were obtained at the end of the crop after addition of FYM along with 100% NPK followed by 150% NPK. Enhancement in the soil microbial activity (Elliott and Lynch, 1994) [4], microbial diversity (Girvan *et al*., 2004; Grayston *et al*., 2004) [7, 9] and densities of bacteria (Bruggen-Van and Semenov, 2000) [2] were reported after Incorporation of organic amendments. Thus, higher levels of fertilizer nitrogen and organic manures were reported to stimulate the bacterial population as well as crop yields.

In another set of experiments, higher population of phosphorus solubilizing bacteria (PSB) i.e., 35×10^3 (before sowing) and 70×10^3 cfu g^{-1} of soil (flowering time) was observed in treatment T_6 during the first and second year, respectively and closely followed by T_5 treatment (Table 2). The population of PSB increased considerably due to the application of organic manures. During the second year of the experiment, the PSB counts varied from 1×10^3 to 38×10^3 cfu g^{-1} soil before sowing of pearl millet and counts ranged from 1×10^3 to 76×10^3 cfu g^{-1} soil at the time of flowering. Lal *et al*. (2012) [17] also reported maximum PSB count i.e., 35×10^2 cfu g^{-1} soil in the treatment, where the combined application of 100% NPK Zn (STR) + 5 t FYM ha^{-1} + PSB + BGA was made.

Analysis of microbial population for another crop wheat showed that the count of *Azotobacter* varied significantly from 6×10^3 cfu g^{-1} (T_1 treatment) to 66×10^3 cfu g^{-1} (T_5 treatment) soil and 4×10^3 cfu g^{-1} (T_1 treatment) to 74×10^3 cfu g^{-1} soil (T_5 treatment) at flowering time during first and second year of the experiment (Table 3). The count of *Azotobacter* (45×10^3 cfu g^{-1} of soil) and total bacteria (10.4×10^7 cfu g^{-1} of soil) were found significantly higher in treatment T_6 (50% RD-N and P + 50% nitrogen through FYM in pearl millet and 100% RD-N and P in wheat) before sowing, which were closely followed by T_5 treatment (100% RD-N and P in pearl millet and wheat) during both years of the study (Table 3). At the time of flowering of wheat, maximum population of *Azotobacter* and total bacteria were reported in treatment T_6 during both the years of the study. Lal *et al.* (2012) [17] concluded that the population of *Azotobacter* varied significantly according to various treatments from 18×10^2 to 48×10^2 cfu g^{-1} of soil with minimum population in control treatment and maximum population was observed treatment containing amendment of 100% STR + 5 t press mud ha^{-1}. Similarly, increase in population of *Azotobacter*, PSB and total bacteria was observed in organic farming practices, which was correlated with availability of more substrate for growth of microorganisms in the fields applied with organic amendments (Jadhav *et al*., 2016) [12]. Gupta *et al*. (2019) [10] also recorded that microbial population increased by $149\% \times 10^4$ cfu g^{-1} of soil where application of 100% NPK + 25 t vermicompost ha^{-1} was made over to the control treatment while 70% increase was recorded in the treatment where only 100% NPK was applied.

Data depicted in Table 4 indicated that maximum counts and establishment of phosphate solubilizing bacteria were found in treatment T_6 (50% RD-N and P through fertilizer + 50% nitrogen through FYM in pearl millet and 100% RD-N and P in wheat through fertilizer) and it was closely followed by treatment T_5 (100% RD-N and P in both crops) before sowing of wheat and at flowering during both the years of the experiment. Among integrated nutrient treatments, the highest population of phosphate solubilizing bacteria was obtained in treatment T_6 and was closely followed by T_{10} while within chemical fertilizer treatments, maximum PSB counts were observed in treatment T_5. Results indicated that addition of organic matter caused a stimulating effect on the population of N_2-fixing *Azotobacter* species, total bacterial population along with phosphate solubilizing bacteria. Similar stimulatory effect on PSB population were reported by Sheoran *et al*. (2019) [22], and their results showed that population of PSB varied from 8×10^4 to 52.4×10^4 cfu g^{-1} of soil under conventional farming system, whereas higher population of PSB was observed under organic farming system containing 13.1×10^4 to 67.8×10^4 cfu g^{-1} soil. The above results corroborate with the findings of Gawai and Pawar (2006) [6] and Kumar *et al*., (2017) [16]. Thus, results indicated that organic manure is a good source of organic matter, which not only supplies carbon food material to the growth of microorganisms but also provides favourable conditions for increasing beneficial activity of desirable soil organisms (Piraveena and Seran, 2011) [20]. As a result, microbial activity is generally enhanced in organic manure treatments applied to the soil.

Table 1: Effect of different integrated nutrient treatments on *Azotobacter* population [cfu -Colony forming unit/g soil] during pearl millet cultivation

Treatments	2015				2016			
	Before sowing		At flowering		Before sowing		At flowering	
	Azotobacter (10^3)	Total bacteria (10^7)	*Azotobacter* (10^3)	Total bacteria (10^7)	*Azotobacter* (10^3)	Total bacteria (10^7)	*Azotobacter* (10^3)	Total bacteria (10^7)
T_1	3	2.5	6	3.5	3	2.4	5	3.4
T_2	5	6	11	11.2	7	6.4	15	11.3
T_3	14	6.5	27	12.3	17	6.7	36	12.4
T_4	16	6.9	31	12.7	20	7	43	12.9
T_5	29	8	61	15	35	8.3	75	15.2
T_6	38	10	82	19.1	45	10.4	96	19.4
T_7	23	7.6	46	14.4	28	7.8	49	14.5
T_8	26	7.8	50	14.6	31	8	65	14.8

T_9	21	7.5	42	13.9	26	7.6	55	14
T_{10}	28	7.9	56	14.7	33	8.1	69	14.9
T_{11}	22	7.5	45	14.1	27	7.7	57	14.3
T_{12}	25	7.7	51	14.5	30	7.9	63	14.6
S.Em±	1.47	0.72	1.91	0.89	1.43	0.72	1.50	1.09
CD at 5%	4.3	2.1	5.6	2.6	4.2	2.1	4.4	3.2

Table 2: Effect of different integrated nutrient treatments on phosphorus solubilizing bacteria (cfu/g soil) during pearl millet crop period

Treatments	PSB population (10^3)			
	2015		2016	
	Before sowing	At flowering	Before sowing	At flowering
T_1	1	1	1	1
T_2	3	5	4	7
T_3	10	18	13	25
T_4	13	24	17	33
T_5	30	60	33	66
T_6	35	70	38	76
T_7	26	50	29	57
T_8	29	56	32	63
T_9	22	42	25	49
T_{10}	32	62	35	69
T_{11}	24	46	27	53
T_{12}	27	52	30	59
S.Em±	1.53	1.87	1.64	2.21
CD at 5%	4.5	5.5	4.8	6.5

Table 3: Effect of different integrated nutrient treatments on *Azotobacter* population (cfu/g soil) during wheat crop period

Treatments	2015-16				2016-17			
	Before sowing		At flowering		Before sowing		At flowering	
	Azotobacter (10^3)	Total bacteria (10^7)	*Azotobacter* (10^3)	Total bacteria (10^7)	*Azotobacter* (10^3)	Total bacteria (10^7)	*Azotobacter* (10^3)	Total bacteria (10^7)
T_1	3	2.4	3	3.3	2	2.3	4	3.2
T_2	7	6.1	15	11.5	9	6.5	19	11.5
T_3	18	6.8	38	12.7	20	7.1	43	12.8
T_4	16	6.4	33	12.4	18	6.5	37	12.5
T_5	31	8.6	66	15.3	35	8.7	74	15.5
T_6	45	10.4	93	19.5	49	10.6	104	19.6
T_7	26	8	54	14.7	29	8.1	61	14.8
T_8	28	8.3	58	15	32	8.4	67	15.1
T_9	23	7.6	48	14.2	27	7.7	57	14.3
T_{10}	30	8.4	62	15.1	34	8.5	71	15.5
T_{11}	25	7.8	52	14.5	28	7.8	59	14.6
T_{12}	27	8.2	56	14.8	31	8.3	65	14.8
S.Em±	1.57	0.99	1.77	1.06	1.64	0.99	1.84	1.09
CD at 5%	4.6	2.9	5.2	3.1	4.8	2.9	5.4	3.2

Table 4: Effect of different nutrient treatments on phosphorus solubilizing bacteria (cfu/g soil) during wheat crop

Treatments	PSB population (10^3)			
	2015-16		2016-17	
	Before sowing	At flowering	Before sowing	At flowering
T_1	1	1	7	9
T_2	3	5	5	9
T_3	15	28	18	36
T_4	11	20	14	28
T_5	31	60	34	69
T_6	36	70	39	79
T_7	27	52	31	62
T_8	30	58	34	68
T_9	23	44	29	58
T_{10}	33	64	36	72
T_{11}	25	48	30	60
T_{12}	28	54	33	66
S.Em±	1.19	1.84	1.29	1.94
CD at 5%	3.5	5.4	3.8	5.7

Conclusions

Although the synthetic chemical fertilizers alone enhance the crop yields but they deteriorate soil fertility and crop productivity. Moreover, addition of chemical fertilizers adversely affects microbial count in the soil. From the results presented in this study, it can be concluded that the integrated use of chemical fertilizers and organic manure sources like FYM, wheat straw, *Sesbania* in pearl millet-wheat cropping system improved the viable counts of *Azotobacter* and PSB during both the years of the experiment. Enhanced microbial population in the soil may improve the soil fertility and crop productivity due to increased availability of utilizable nutrients for the plant growth.

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