



Nutrients Content, Uptake and Soil Biological Properties as Influenced by Various Nutrient Management Practices under Fodder Pearl Millet Cultivation

Rakesh Kumar, Hardev Ram*, R.K. Meena, Sandeep Kumar, Brijesh Kumar, B.R. Praveen, P.S. Hindoriya and Maneesha¹

Agronomy Section, ICAR-National Dairy Research Institute, Karnal-132 001, India

¹Division of Agronomy, ICAR-Indian Agricultural Research Institute, Pusa, New Delhi-110 012, India

* E-mail: devagron@gmail.com

Abstract: The experiment was conducted during *kharif* season of 2019-20 at Agronomy research farm, ICAR-NDRI, Karnal (Haryana) with eight treatments including recommended dose of fertiliser (80:30:30 kg/ha, N: P₂O₅: K₂O, respectively) in combinations with plant growth promoting Rhizobacteria (PGPR) as seed treatment and cow urine foliar spray (10%). The nutrients content and uptake by fodder pearl millet significantly affected with different nutrient management practices and maximum nitrogen (1.57%), phosphorus (0.27%) and potassium (1.66%) contents as well as uptake of nitrogen (177.40 kg ha⁻¹), phosphorus (30.62 kg ha⁻¹) and potassium (188.56 kg ha⁻¹) was recorded with 100% RDF + PGPR + Cow urine foliar spray, which was statistically at par with 100% RDF + PGPR. Maximum microbial biomass carbon (163.47 and 192.44 µg/g dry soil), dehydrogenase activities (13.25 and 23.83 µg TPF/g soil/day), alkaline phosphatase activities (84.55 and 106.96 µg PNP/g soil/hr.), bacterial counts (14.46×10⁶ and 22.09×10⁶ CFU/g soil), actinomycetes counts (26.40×10⁵ and 37.15×10⁵ CFU/g soil) and fungal counts (14.07×10⁴ and 18.85×10⁴ CFU/g soil) at 40 DAS and harvest in 100% RDF + PGPR + cow urine foliar spray, which was statistically at par with 100% RDF + PGPR followed by 75% RDF + PGPR + cow urine foliar spray and 75% RDF + PGPR at 40 DAS, while at harvest it was found at par with 100% RDF + PGPR and both were found significantly higher over rest of the treatments.

Keywords: Cow urine, Fodder, Pearl millet, Quality, Yield

India has the highest number of livestock animals (535.78 million) in the world's, according to 20th livestock census 2019, the population of major livestock animals *viz.*, buffalo, cattle, sheep and goat in India is 109.85, 192.49, 74.26 and 148.88 million, respectively (Anonymous 2020). Fodder demand increase for ever increasing population of livestock and it is essential component for livestock production as it can curtail the cost of feeding because livestock feeding contributes about 65 to 70% of total cost of livestock farming (Kumar et al 2022). Nutrient management is an important aspect to achieve sustainable crop production. Scenario from green revolution era, shows productivity of cereals increased largely with the use of high yielding variety, intensive agronomic practices and indiscriminate use of higher rate of chemical fertilizers with little or without use of organic source of nutrients to plant, that create adverse effect on soil *viz.*, inadequacy in one or more nutrients and deterioration of soil fertility which leads to stagnating or even declining of crop productivity and quality (Shormy et al 2013). Deficiency of nutrients in soils leads to produce mineral deficient fodder (Shukla et al 2015). However, animal production level and health adversely affected due to nutrients deficient or low-quality fodder offered in feeding,

which ultimately influence returns from livestock sector (Surve et al 2011). Judicious use of organic and inorganic sources of nutrients may sustain and enhance fodder quality and soil health. Among the different fodder crops pearl millet (*Pennisetum glaucum*) is the gifted crops of the tropical and sub-tropical regions that provide fodder and stover (dry straw) to millions of livestock animal of poor resources farmer. Among different organic source of nutrients for plant, cow urine and plant growth promoting rhizobacteria (PGPR) are excellent and important for agriculture uses. Cow urine (CU) contains; nitrogen, phosphorus, potassium, sulphur, sodium, manganese, iron, carbolic acid, silicon, chlorine, enzyme and hormones (Saunders 1982). PGPR, is a consortium of microorganism that actively colonize around plant roots and enhances plant growth and yield (Wu et al 2005). PGPR strains belongs to a wide range of genera *viz.*, *Pseudomonas*, *Azospirillum*, *Bacillus*, *Serratia* and *Azotobacter* (Bashan et al 2004). The beneficial effects of PGPR due to their ability to produce various organic compounds *viz.*, auxins, gibberellins, cytokinin, ethylene, organic acids, siderophores, nitrogen fixation, solubilization of insoluble inorganic soil phosphate to available form, sulphur oxidation, extra cellular production of antibiotics,

increases in root permeability, enhancement of essential plant nutrients uptake (Enebak and Carey 2000). Considering above facts in view the present study was proposed to find out a suitable combination of nutrient source to enhance the fodder quality and soil health.

MATERIAL AND METHODS

Experimental site: The experiment was conducted during *kharif* season, 2019-20 at Agronomy Research Farm, ICAR-NDRI, Karnal (Haryana) located at 29°45' North latitude and 76°58' East longitude and at an altitude of 245m above mean sea level. The climate of the area is semi-arid with a mean annual rainfall is 70.7 cm, and about 70-80% of which is received during the months of July-September and rest during winter and spring season. The mean minimum and maximum temperature during this study was 20.49°C and 34.54°C, respectively. The soil of experimental site was clay loam in texture (Piper 1942) with pH 7.35, Electrical conductivity (EC) 0.37 dS/m (Jackson 1967), organic carbon 0.49 percent (Walkley and Black's 1934), available nitrogen 215 kg/ha (Subbiah and Asija 1956), available phosphorus 24.70 kg/ha (Olsen et al 1954), available potassium 285 kg/ha (Jackson 1967), microbial biomass carbon 120.30 µg/g dry soil (Nunan et al 1998), dehydrogenase activities 8.10 µg TPF/g soil/day (Casida et al 1964), alkaline phosphatase activities 63.55 µg PNP/g soil/hour (Tabatabai and Bermner 1969), bacterial counts 8.2×10^6 CFU/g soil (Gordon et al 1973), actinomycetes counts 17.56×10^5 CFU/g soil (Kenknight and Muncie 1939) and fungal counts 8.86×10^4 CFU/g soil (Martin 1950).

Treatments details and input applications: The experiment was laid out in randomized complete block design with eight treatments viz., T₁: Absolute control; T₂: 100% RDF; T₃: 100% RDF + Cow urine foliar spray; T₄: 100% RDF + PGPR; T₅: 100% RDF + PGPR + cow urine foliar spray; T₆: 75% RDF + cow urine foliar spray; T₇: 75% RDF + PGPR; T₈: 75% RDF + PGPR + Cow urine foliar spray with three replications. The land preparation was done by one deep ploughing with disc plough followed by two cross harrowing with disc harrow and planking. Application of recommended dose of fertilizers (80:30:30 kg/ha, N: P₂O₅: K₂O, respectively) were applied as per treatment. The N, P₂O₅ and K₂O were applied through urea, DAP, and muriate of potash, respectively. The half of N and full doses of P and K were applied before final harrowing and remaining half dose of nitrogen was top-dressed in two split doses as per the treatment. The PGPR liquid culture (100 ml/10 kg seeds) contains 10⁹ CFU/ml was diluted in one litres of water, and applied on seeds. Thereafter, inoculated seeds were dried in shade for 60 minutes, after drying seeds was manually sown.

Nutrified variety of fodder pearl millet was sown using 10 kg seed per hectare with maintaining spacing 30cm × 10cm row to row and plant to plant, respectively. Other package of practices was followed as per standard for cultivation of fodder pearl millet. The 10% cow urine was applied as foliar spray in early morning hours, when the dew has been evaporated at 30 and 45 DAS as per treatments.

Analysis of nutrients content in plant sample: The crop was harvested manually at 50% flowering stage. The harvested chopped plant samples from net plot area were dried in hot air oven at 70° C for at least 72 hours until a constant weight was reached. Further, these oven-dried plant samples were ground to pass through two mm sieve in a Wiley mill. The sieved samples were used for chemical analysis, viz., nitrogen (Jackson 1967), phosphorus (Olsen and Sommers 1982) and potassium (Richards 1954). Thereafter, the uptake of nutrients by plant was calculated through following equation:

$$\text{Nutrient uptake (kg/ha)} = \frac{\text{Nutrient content (\%)} \times \text{Dry matter yield (kg)}}{100}$$

Analysis of soil biological properties: At 40 DAS and harvest of crop, soil samples randomly collected from five spot at 0-15 cm depth in each net plot area and make it a composite sample. Taken 500g soil from composite sample after quartering for further soil analysis. Microbial biomass carbon, dehydrogenase activities, alkaline phosphatase activities, bacteria count, actinomycetes count and fungal count in fresh soil was estimated by using standard procedure given by Nunan et al (1998), Casida et al (1964), Tabatabai and Bermner (1969), Gordon et al (1973), Kenknight and Muncie (1939) and Martin (1950), respectively.

Statistical analysis: All the data recorded were processed in Microsoft excel 2010 and analysed with the help of analysis of variance at 5% level of significance. Simple Pearson's correlation coefficient was computed by using mean values of different nutrient contents in plant sample with the help of SPSS 23.0 Version.

RESULTS AND DISCUSSION

Dry matter yield: The dry matter yield of pearl millet was significantly influenced with different nutrient management practices and recorded significantly higher dry matter yield (11.33 t/ha) at harvest with application of 100% RDF+PGPR+CU, which was found statistically at par with 100% RDF+PGPR and both were significantly higher than other treatments (Fig. 1). Balanced and regular supply of essential plant nutrients, PGPR produce phytohormones (Enebak and Carey 2000) and cow urine supply enzyme and hormones that attributed to stimulate plant physiological

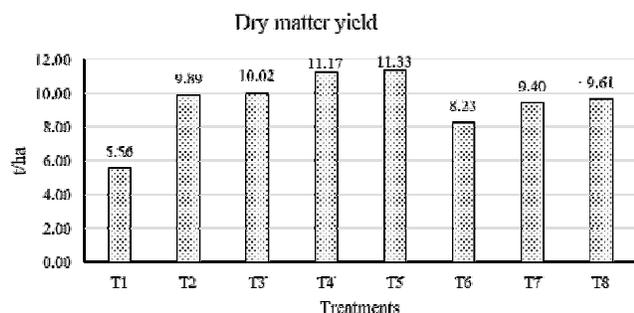


Fig. 1. Effect of nutrient management practices on dry matter yield of fodder pearl millet

processes leads to increase leaf area index that responsible for higher interception of solar radiation and produce more photosynthates and nutrients acquired, resulted in to increase dry matter assimilation in different part of plant leads to increase dry matter yield. Further, higher biomass production and dry matter content attributed to increase dry matter yield. The similar results also reported by Chattha et al (2017).

Nutrient content and uptake: The chemical analysis of fodder pearl millet showed that nutrient content and their uptake significantly influenced with different nutrient management practices and recorded maximum nitrogen (1.565%), phosphorus (0.273%) and potassium (1.663%) contents in pearl millet fodder on dry matter basis with application of 100% RDF+PGPR+CU, which was statistically at par with 100% RDF+PGPR (Table 1). Nutrients supplied with 100% RDF+PGPR+CU improved nitrogen by 3.27, 6.65, 8.78 and 21.33%; phosphorus by 1.23, 14.85, 15.49 and 41.38% and potassium by 1.42, 8.24, 8.89 and 38.96% over 100% RDF+PGPR, 100% RDF+CU, 100% RDF and absolute control, respectively. The pearl millet supplied with 100% RDF+PGPR+CU approached maximum nutrients contents in fodder on dry matter basis due to nutrients

concentration in soil solution increase with application of fertiliser, PGPR supplement N by biological fixation of atmospheric nitrogen, P by solubilizing of fixed inorganic soil phosphate to available form and K by secretion organic acid that increase availability to plant (Cakmakci et al 2007). The cow urine additionally supplies N, P and K to plant foliage leads to quickly supply of nutrients to plant. Optimum and continues availability of nutrients to plant increase uptake as well as assimilation in plant tissue, leads to increase nutrients content. Likewise, contents the uptake of nutrients reported to follow the similar trend (Table 1) and recorded maximum nitrogen (177.40 kg/ha), phosphorus (30.62 kg/ha) and potassium (188.56 kg/ha) uptake with application of 100% RDF + PGPR + CU, which was statistically at par with 100% RDF + PGPR and both were significantly higher than other treatments. Higher assimilation of nutrients in plant tissue as well as biomass production attributed to increase nutrients uptake.

Soil biological properties: The analysis of soil showed that soil biological properties significantly influenced with different nutrient management practices and recorded maximum microbial biomass carbon (163.47 and 192.44 µg/g dry soil), dehydrogenase activities (13.25 and 23.83 µg TPF/g soil/day), alkaline phosphatase activities (84.55 and 106.96 µg PNP/g soil/hr.), bacterial counts (14.46×10^6 and 22.09×10^6 CFU/g soil), actinomycetes counts (26.40×10^5 and 37.15×10^5 CFU/g soil) and fungal counts (14.07×10^4 and 18.85×10^4 CFU/g soil) at 40 DAS and harvest respectively, with application of 100% RDF+PGPR+CU, which was statistically at par with 100% RDF+PGPR followed by 75% RDF+PGPR+CU and 75% RDF+PGPR at 40 DAS, while, at harvest it was found at par with 100% RDF+PGPR and both were found significantly higher over rest of the treatments. In addition to fertiliser, PGPR increase availability of phosphorus through solubilization and

Table 1. Effect of nutrient management practices on nutrient content and uptake by fodder pearl millet

Treatments	Nutrient content (%)			Nutrient uptake (kg/ha)		
	N	P	K	N	P	K
T ₁ : Absolute control	1.290	0.193	1.197	71.82	10.76	66.59
T ₂ : 100% RDF	1.439	0.237	1.528	142.28	23.40	150.92
T ₃ : 100% RDF+CU	1.468	0.238	1.537	147.19	23.75	154.24
T ₄ : 100% RDF+PGPR	1.516	0.270	1.640	169.33	30.55	183.24
T ₅ : 100% RDF+PGPR+CU	1.565	0.273	1.663	177.40	30.62	188.56
T ₆ : 75% RDF+CU	1.356	0.220	1.403	111.61	18.11	115.53
T ₇ : 75% RDF+PGPR	1.395	0.237	1.463	131.23	22.26	137.57
T ₈ : 75% RDF+PGPR+CU	1.418	0.240	1.477	136.37	23.10	142.08
CD (P=0.05)	0.057	0.013	0.097	10.14	2.16	13.59

nitrogen by biological nitrogen fixation that increase root growth, surface area and biomass leads to produce more exudates resulted increase SOC. However, SOC considered as food for microbes leads to increase microbial counts in soil attributed to increase microbial biomass carbon. Similar result also reported by Lestari and Andrian (2017) and Cakmakci et al (2007). Soil enzymatic activities are important indicator of soil health and play vital role in organic matter decomposition and nutrient cycling of carbon, nitrogen, phosphorus and sulphur, which indicate higher metabolic activity of microbes (Dinesh et al 2013). Soil dehydrogenase enzymes indicate oxidation activity of soil microorganisms. Higher microbial population in soil attributed to increase dehydrogenase activities. Alkaline phosphatase responsible for organic phosphorus transformation in soil. PGPR attributed to increase native

phosphorus availability by solubilization leads to increase alkaline phosphatase activities. Similar result also reported by Rana et al (2012).

Correlation studies: The correlation between N, P and K uptake and dry matter yield of fodder pearl millet (Table 4) revealed that N uptake ($r = 0.992$), P uptake ($r = 0.985$) and K uptake ($r = 0.994$) showed significant highly positive correlation with dry matter yield of fodder pearl millet. However, dry matter yield increase with nutrients uptake.

Regression studies: The regression analysis exhibited (Fig. 2) a significant polynomial correlation between nutrients uptake and dry matter yield of fodder pearl millet at harvest. The R^2 value between dry matter yield and N, P and K uptake was 0.99, 0.99 and 0.99, respectively. This indicates that N, P and K at harvest accounted 99% of total variation in dry matter yield.

Table 2. Effect of nutrient management practices on soil microbial biomass carbon and enzymatic activities under fodder pearl millet cultivation

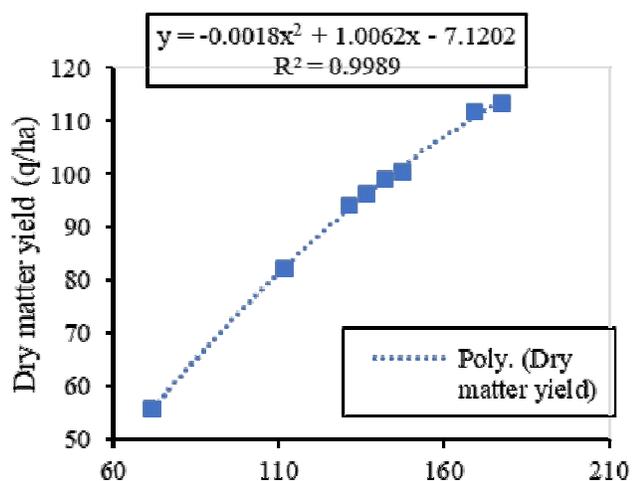
Treatments	MBC ($\mu\text{g/g}$ dry soil)		DHA ($\mu\text{g TPF/g}$ soil/day)		APA ($\mu\text{g PNP/g}$ soil/hr.)	
	40 DAS	Harvest	40 DAS	Harvest	40 DAS	Harvest
T ₁ : Absolute control	130.00	149.07	10.15	15.63	72.86	82.84
T ₂ : 100% RDF	149.10	174.11	12.02	19.36	79.37	93.29
T ₃ : 100% RDF+CU	149.10	174.13	12.03	19.43	79.85	93.37
T ₄ : 100% RDF+PGPR	163.46	192.41	13.12	23.68	84.08	106.24
T ₅ : 100% RDF+PGPR+CU	163.47	192.44	13.25	23.83	84.55	106.96
T ₆ : 75% RDF+CU	140.59	166.30	11.33	18.68	77.79	88.62
T ₇ : 75% RDF+PGPR	156.30	181.53	12.63	20.54	81.54	98.03
T ₈ : 75% RDF+PGPR+CU	156.31	181.73	12.70	20.70	82.03	98.19
CD (P=0.05)	9.81	10.43	0.93	1.77	3.60	4.95

MBC: Microbial biomass carbon, DHA: Dehydrogenase activities, APA: Alkaline phosphatase activities

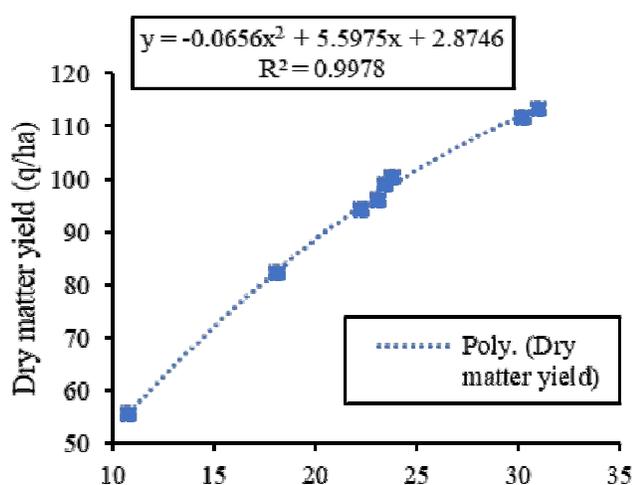
Table 3. Effect of nutrient management practices on soil microbial population under fodder pearl millet cultivation

Treatments	BC (10^6 CFU/g soil)		AC (10^5 CFU/g soil)		FC (10^4 CFU/g soil)	
	40 DAS	Harvest	40 DAS	Harvest	40 DAS	Harvest
T ₁ : Absolute control	10.07	14.36	19.80	26.88	9.71	13.07
T ₂ : 100% RDF	12.98	18.34	23.78	32.15	12.17	16.04
T ₃ : 100% RDF+CU	13.03	18.35	23.95	32.20	12.23	16.07
T ₄ : 100% RDF+PGPR	14.34	21.97	26.13	37.10	13.98	18.80
T ₅ : 100% RDF+PGPR+CU	14.46	22.09	26.40	37.15	14.07	18.85
T ₆ : 75% RDF+CU	12.07	17.25	22.47	30.78	11.32	14.90
T ₇ : 75% RDF+PGPR	13.41	19.46	24.90	34.00	13.07	17.00
T ₈ : 75% RDF+PGPR+CU	13.63	19.48	25.02	34.17	13.10	17.27
CD (P=0.05)	1.14	1.34	1.67	2.29	1.09	1.34

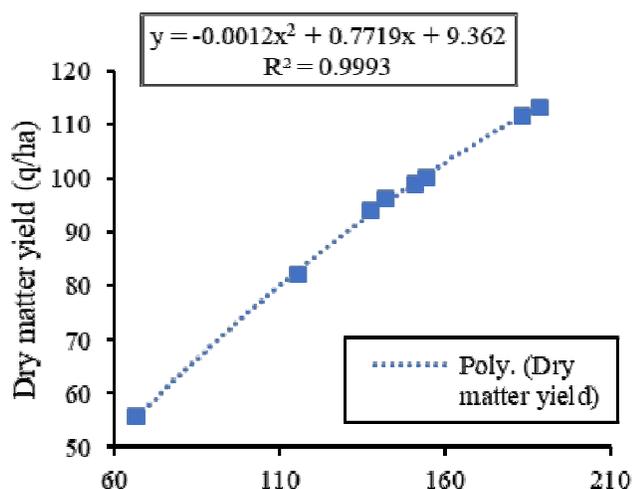
BC: Bacterial count, AC: Actinomycetes count and FC: Fungal count



2.a Nitrogen uptake (kg/ha)



2.b Phosphorous uptake (kg/ha)



2.c Potassium uptake (kg/ha)

Fig. 2. Relationship between nutrient uptake and dry matter yield of fodder pearl millet

Table 4. Correlation coefficient (r) between dry matter yield and nutrients uptake by fodder pearl millet

Pearson	Correlations			
	DMY	N	P	K
DMY	1			
N	0.992**	1		
P	0.985**	0.995**	1	
K	0.994**	0.999**	0.995**	1

Note: DMY - Dry matter yield, ** - Correlation is significant at the 0.01 level (2-tailed)

CONCLUSION

Nutrient management is an important aspect, where intensive cropping system are dominates. Judicious use of organic and inorganic sources of nutrients sustains fodder quality and soil health. Maximum nitrogen, phosphorus and potassium content and uptake, as well as soil biological properties recorded with T_5 treatment under fodder pearl millet cultivation. For future line of work, as like pearl millet, different cereal fodder crops can be explored location wise along with proper dose and sources (inorganic and organic) of nutrients for better fodder quality.

ACKNOWLEDGEMENTS

Authors sincerely acknowledge Indian Council of Agricultural Research for financial support and Director, ICAR-NDRI, Karnal for assistance in conducting this research.

REFERENCES

- Anonymous 2020. 20th livestock census-2019. All India reports. Department of animal husbandry and dairying, Ministry of fisheries, animal husbandry and dairying, Government of India. p. 16.
- Bashan Y, Holguin G and De-Bashan LE 2004. Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances 1997-2003. *Canadian Journal of Microbiology* **50**(8): 521-577.
- Cakmakci R, Donmez MF and Erdogan U 2007. The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. *Turkish Journal of Agriculture and Forestry* **31**(3): 189-199.
- Casida LEJ, Klein DA and Santaro T 1964. Soil dehydrogenase activity. *Soil Science* **98**: 371-376.
- Chattha MB, Iqbal A, Chattha MU, Hassan MU, Khan I, Ashraf I, Faisal M and Usman M 2017. PGPR inoculated-seed increases the productivity of forage sorghum under fertilized conditions. *Journal of Basic and Applied Sciences* **13**: 150-153.
- Dinesh R, Anandaraj M, Kumar A, Srinivasan V, Bini YK, Subila KP, Aravind R and Hamza S 2013. Effects of plant growth-promoting rhizobacteria and NPK fertilizers on biochemical and microbial properties of soils under ginger (*Zingiber officinale*) cultivation. *Agricultural Research* **2**(4): 346-353.
- Enebak SA and Carey WA 2000. Evidence for induced systemic protection to Fusarium rust in Loblolly pine by plant growth promoting rhizosphere. *Plant Disease* **84**: 306-308.

- Gomez KA and Gomez A 1984. Statistical Procedures for Agricultural Research, *John Willey and Sons*, Singapore, p. 680.
- Gordon RE, Haynes WC, Pang CHN and Smith NR 1973. The Genus *Bacillus*. Agriculture Handbook No. 427. Washington, D.C., United State Department of Agriculture.
- Jackson ML 1967. *Soil Chemical Analysis*. Prentice Hall of India Private Limited, New Delhi, pp. 111-203.
- Kenknight G and Muncie JH 1939. Isolation of phytopathogenic Actinomycetes. *Phytopathology* **29**: 1000-1001.
- Kumar R, Ram H, Kumar R, Meena RK, Meena BL, Manisha and Kumar D 2022. Proximate Composition and Fibre Fraction of Pearl Millet Fodder as Influenced by Different Nutrient Management Practices. *Indian Journal of Animal Research* 1-6,
- Lestari SU and Andrian A 2017. Effects of urine cow dosage on growth and production of sorghum plant (*Sorghum Bicolor* L) on Peat Land. *Earth and Environmental Science* **97**(1): 132-136.
- Martin JP 1950. Use of acid, rose Bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Science* **69**: 215-232.
- Nunan N, Morgan MA and Herlihy M 1998. Ultraviolet absorbance (280 nm) of compounds released from soil during chloroform fumigation as an estimate of the microbial biomass. *Soil biology and biochemistry* **30**(12): 1599-1603.
- Olsen SR and Sommers LE 1982. Phosphorus. *Methods of Soil Analysis: Part 2 [Agronomy: A Series of Monographs 9]*, 2nd edn, pp 403–430. Page AL (Eds). Madison WI: ASA and ASSA.
- Olsen SR, Cole CV, Watanabe FS and Dean LA 1954. *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. USDA Circular 939, U.S. Government Printing Office, Washington D.C., p. 19.
- Pal K, Dey R, Bhatt DM and Chauhan SM 1999. Enhancement of groundnut growth and yield by plant growth promoting rhizobacteria. *International Arachis Newsletter* **19**: 51-53.
- Piper CS 1942. Soil and Plant Analysis: *A laboratory manual of methods for the examination of soils and the determination of the inorganic constituents of plants*. Hans, Bombay, p. 152.
- Rana A, Saharan B, Nain L, Prasanna R and Shivay YS 2012. Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. *Soil Science and Plant Nutrition* **58**(5): 573-582.
- Richard LA 1954. *Diagnosis and improvement of saline and alkaline soils*. Agriculture Handbook No. 60, USDA, Washington DC, p 160.
- Saunders WHM 1982. Effects of cow urine and its major constituents on pasture properties. *New Zealand Journal of Agricultural Research* **25**(1): 61-68.
- Shormy SAS, Chowdhury MAH, Saha BK and Haque MA 2013. Effects of different sources of organic materials on nutrient contents and their uptake by T. aman rice. *Journal of Agroforestry and Environment* **7**(1): 37-40.
- Shukla AK, Malik RS, Tiwari PK, Prakash C, Behera SK, Yadav H and Narwal RP 2015. Status of micronutrient deficiencies in soils of Haryana. *Indian Journal of Fertilisers* **11**(5): 16-27.
- Subbiah BV and Asija GL 1956. A rapid procedure for determination of available nitrogen in soil. *Current Science* **25**: 259-260.
- Surve V, Patil P and Arvadia M 2011. Forage production potential of sorghum (*Sorghum bicolor*), maize (*Zea mays*) and cowpea (*Vigna unguiculata*) under sole and intercropping systems. *Madras Agricultural Journal* **98**(4): 372-374.
- Tabatabai MA and Bremner JM 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* **1**: 301-307.
- Walkley A and Black IA 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science* **37**(1): 29-38.
- Wu SC, Cao ZH, Li ZG, Cheung KC and Wong MH 2005. Effects of biofertilizer containing N-fixers, P and K solubilizers and AM fungi on maize growth: A greenhouse trial. *Geoderma* **125**(1-2): 155-166.