Evaluation of Available Soil Nutrients and Nitrogen Fixing Bacterial Population in Rhizosphere of Wild Grasses From agricultural land of Newasa Tahasil of Ahmednagar District (MS) India

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ABSTRACT

Soil is a wonderful gift of nature for living creatures. It supports crop growth. It is an important environmental factor, and has a close reciprocal relationship with the plants growing in it and the soil microbes that exist in it. The forest soil consists of high humus with variety of microbes. In this experiment forest area of Newasa was selected for evaluation of nutrient status and bacterial population. On the basis of density of grasses *Iseilema prostratum* L, *Setaria pumila* (Poir.) Roem. & Schult, *Aristida hystrix* L., *Dactyloctenium aegyptium* (L.) Willd, *Fimbriristylis miliacea* (L.) Vahl. were selected. 100 soil samples from different locations from rhizosphere of these monocots were collected and analyzed for soil nutrient status and nitrogen fixing bacterial population. It was recorded that, out of five plants, the rhizosphere soil of *Setaria pumila* L. showed maximum bacterial population (11700540.00). Available nitrogen (320 kg/ha) and % organic carbon (0.60 %) followed by *Setaria pumila* (Poir.) Roem. & Schult the bacterial population was (2558265.00), available nitrogen (203 Kg/ha) and % Organic Carbon (0.59%). Other soil nutrients were also measured.

Keywords: Monocots, Nutrient status, Bacterial population, Available nitrogen, % Organic carbon.

Introduction

Soil generally contains variety of nutrients required for plants. Nutrient content in agricultural lands is slowly reduced because it is absorbed by plants to meet its growth needs (Sutejo *et al*. 1991). Manure can improve the physical, chemical and biological properties of soil through its role as a source of food for microbes in the soil (Sugito *et al*. 1995) and increase the varieties of microbes and populations (Sarief, 1989).

Soil plays an important role in the mineral cycles viz. nitrogen, phosphorus, sulfur and carbon cycles. The bacteria that play roles in the nitrogen cycle are Azotobacter. Azotobacter, for example, is the bacteria that live in rhizosphere areas which are heterotrophic. These bacteria bind free nitrogen which influence the physical and chemical properties and increase soil fertility. The population of nitrifying bacteria in the soil affects the concentration ratio of nitrogen in the soil, so that the microbial population is an indicator of soil fertility levels (Allen and Allen, 1981). The use of manure can improve soil fertility for a longer period of time. Soil physico-chemical characteristics influence the composition of the soil microbial community, their activity and the level of microbial mass (Dick, 1994).

Newasa tahasil is present in Ahmednagar district (MS) India. In this Tahasil 91.1% land, is under agriculture and 1.02% land under forest. Forest soil is not contaminated; there is no use of chemical fertilizers and pesticides. Hence the soil was selected to evaluate the available nutrient status and population density of nitrogen fixing bacteria. With this view we select five wild monocots to evaluate nitrogen fixing bacterial population from their rhizosphere soil and available plant nutrients. The survey of the forest grasses was done. On the basis of density, grasses viz. *Iseilema prostratum* L, *Setaria pumila* (Poir.) Roem.& Schult, *Aristida hystrix* L., *Dactyloctenium aegyptium* (L.) Willd, and *Fimbriristylis miliacea* (L.) Vahl. were selected.

Materials and Methods

Collection of Soil Samples

The 100 soil samples were collected in June 2016 from rhizosphere of monocotyledons. five monocots designated as 1A, 2A, 3A, 4A, 5A which were identified from Botanical survey of India Gov. of India Ministry of Environment, Forest and Climate change western regional centre, Koregaon road, Pune viz. *Iseilema prostratum* L. (1A), *Setaria pumila* (Poir.) Roem. & Schult.(2A), *Aristida hystrix* L.(3A), *Dactyloctenium aegyptium* (L.) Willd. (4A), *Fimbriristylis miliacea* (L.) Vahl. (5A). 20 soil samples from each monocot at 20 different places of forest area of Newasa tahasil of Ahmednagar district Maharashtra state India were collected. These soil samples were placed in sterile bags, numbered and carried to the laboratory and stored in a refrigerator prior to processing.

Culture media

Jensen's agar Medium (Jensen 1965) was used for the evaluation of nitrogen fixing Bacterial population. It contained (g/liter) Sucrose 20 g, di-potassium hydrogen phosphate 1 g, magnesium sulfate 0.5 g, sodium chloride 0.5 g, ferrous sulfate 0.1 g, sodium molybdate 0.005 g,1 tablet of flucanozole 500mg, Agar 20 g, pH (ph.7.1 ± 0.2) of culture media was adjusted by using 1N NaOH or 1N H_2So_4. Media was sterilized by autoclaving at 120^0c temperature, 15 lb pressure for 15- 20 minutes.

Isolation

The collected soil samples were used for the culturing of nitrogen fixing microorganisms on Jensen's nitrogen free Agar medium by serial dilution agar plate technique (Aneja 2002). Ten gram of soil from each sample was aseptically weighed and transferred to 250 ml conical flask containing 90 ml of sterile double distilled water. The flasks were shaked in orbital shaker at 200 rpm for 30 minutes. Samples were allowed to settle for 1 minute. Aliquots of 1 ml of the supernatant from the samples were transferred to 9 ml of (0.85% NaCl) saline solution in test tube and serially diluted up to 10^{-7}. 2 ml of sample from each of these dilutions was transferred to sterilized 18 ml of Jensen's agar medium before solidification (45^0C temperature) and poured in sterile Petri dish after mixing. After solidification, plates were kept in an incubator at 28^0 C (± 2) for 4-5 days. After incubation grown microbial colonies were counted on colony counter.

Analysis of collected soil samples

The collected rhizosphere soil samples of selected five monocots were analyzed for pH and Electrical conductivity (Thomas 1996), % Organic Carbon (Walkley Black Method 1947), Available Nitrogen Kg/ h (Alkaline permanganate method 1956), Available Phosphates Kg /h (Ascorbic acid method 1954), Available Potassium Kg/h (Ammonium Acetate method Thomas 1982), Available Zn, Fe, Mn, Cu. DTPA-TEA method (Lindsay and Norvell 1978). The method consists of use of DTPA (Diethylene tetra amine penta acetic acid) as an attractants which has been widely accepted for the simultaneous extraction of metal micronutrient cations viz., zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) in neutral and alkaline soils.

The contents of these cations in the extract were determined on an Atomic Absorption Spectrophotometer. (A.A.S)

The enumeration of Azotobacter was done by serial dilution method. The soil samples were inoculated on nitrogen free Jensen's Agar medium, were kept for incubation. These plates were analyzed for total viable count of bacteria by serial dilution method
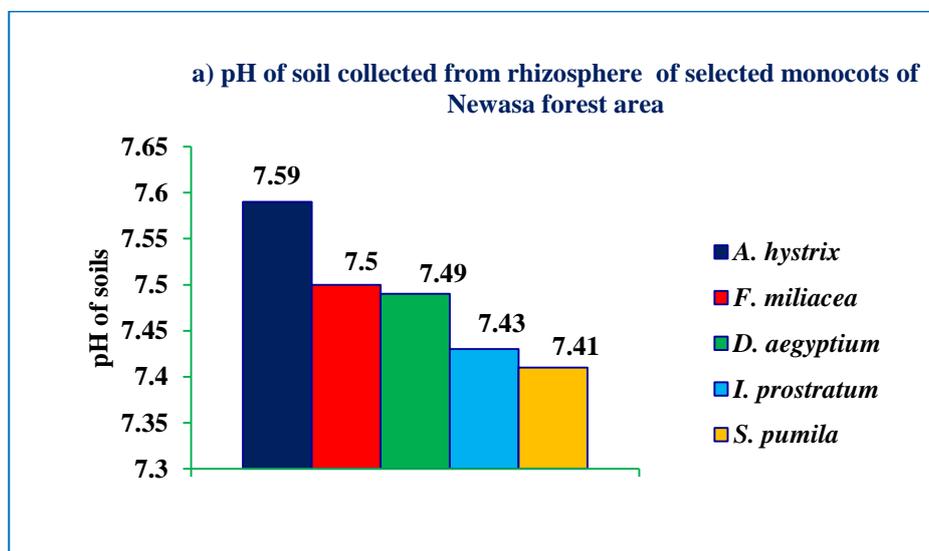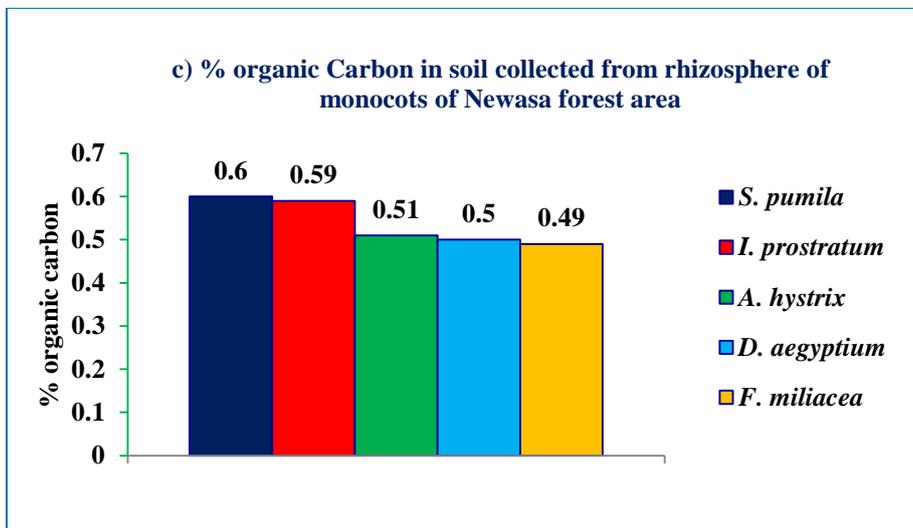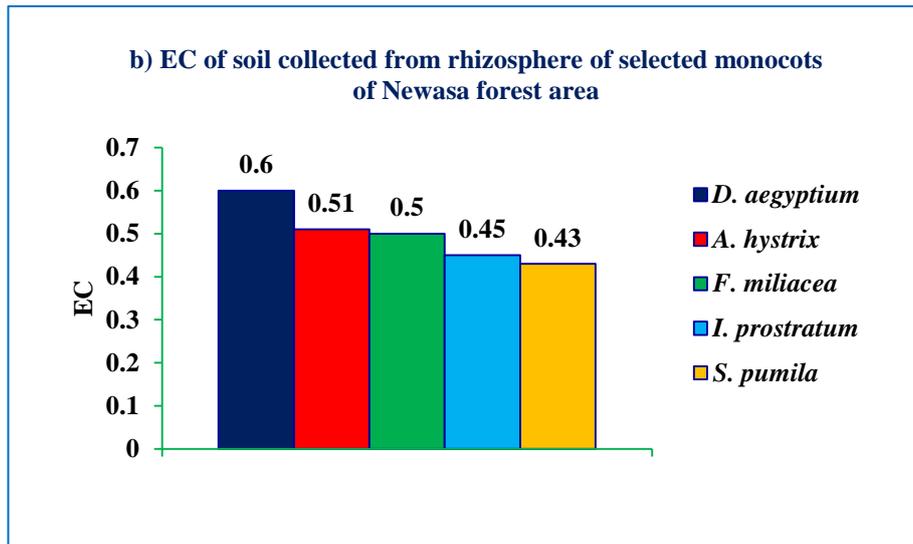
Result Table:-

Table No 6: Chemical Analysis of soil and nitrogen fixing bacterial population in Rhizosphere of four Wild grasses & one sedge plant of forest area of Newasa tahasil, Dist. Ahmednagar (MS)

Name of the monocot plants	pH	EC. Ml moh/ cm2	Organic Carbon %	Available Nitrogen kg/h	Available Phosphate Kg/h	Available Potassium	Fe in ppm	Cu in ppm	Zn in ppm	Mn in ppm	Number of colonies/g soil
Iseilema prostratum L.	7.43a ±0.07	0.45a ±0.05	0.59a ±0.09	203.75a ±41.73	8.42a ±1.23	352.46a ±23.95	2.88a ±0.31	0.53a ±0.11	0.57a ±0.12	1.97a ±0.28	2558265.00a
Setaria pumila (Poir.) Roem.& Schult.	7.41ab ±0.06	0.43ab ±0.03	0.60a ±0.13	320.05b ±23.53	0.43b ±0.03	352.74a ±30.64	2.52a ±0.22	0.81b ±0.13	0.28b ±0.05	0.80b ±0.14	11700540.00b
Aristida hystrix L.	7.59ac ±0.10	0.51a ±0.06	0.51a ±0.10	198.05a ±14.44	5.65c ±0.77	321.54a ±26.41	2.51a ±0.22	0.78b ±0.17	0.59ac ±0.04	1.13c ±0.19	1659650.00c
Dactyloctenium aegyptium (L.)Willd.	7.49ad ±0.04	0.60c ±0.05	0.50a ±0.12	182.95a ±24.39	7.95a ±0.78	317.56 ±19.71	2.40b ±0.25	0.67a ±0.08	0.66ac ±0.05	0.81b ±0.10	1454440=00c
Fimbristylis miliacea (L.)Vahl	7.50ad ±0.08	0.50ab ±0.04	0.49a ±0.09	178.10a ±21.47	7.84a ±0.49	290.10b ±24.41	2.44b ±0.21	0.66a ±0.11	0.52acd ±0.04	1.89c ±0.16	493530.00c
CD at 0.05%	0.12	0.08	0.18	44.65	1.28	42.29	0.41	0.2	0.11	0.3	
P-value at 0.05%	0.43	0.12	0.92	0.001	2.05E-11	0.36	0.67	0.51	0.001	9.14E-07	0.16

(Where Mean values are of average of twenty readings. Data was analysed using single factor ANOVA at 0.05% P-value followed by CD at 0.05% and Tukey's test. ±: standard error of means.)
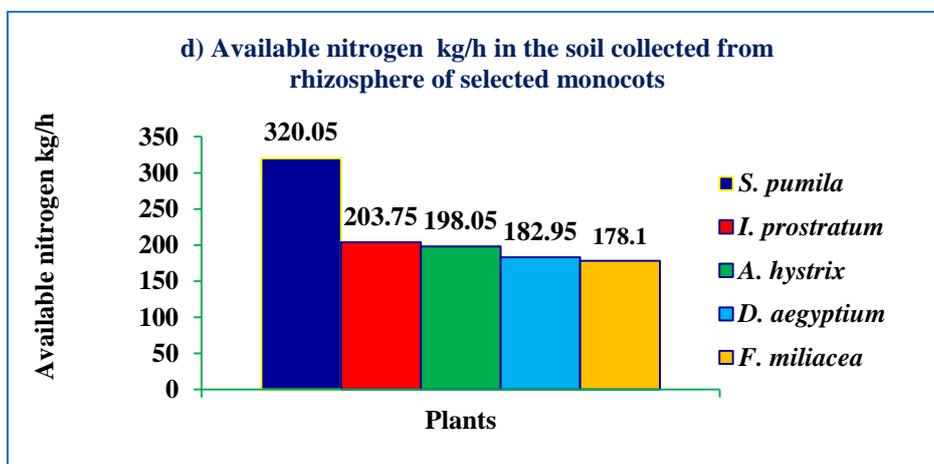
Graph NO 1 (A): Soil analysis of rhizosphere of wild monocots from forest area of Newasa tahasil, Dist. Ahmednagar
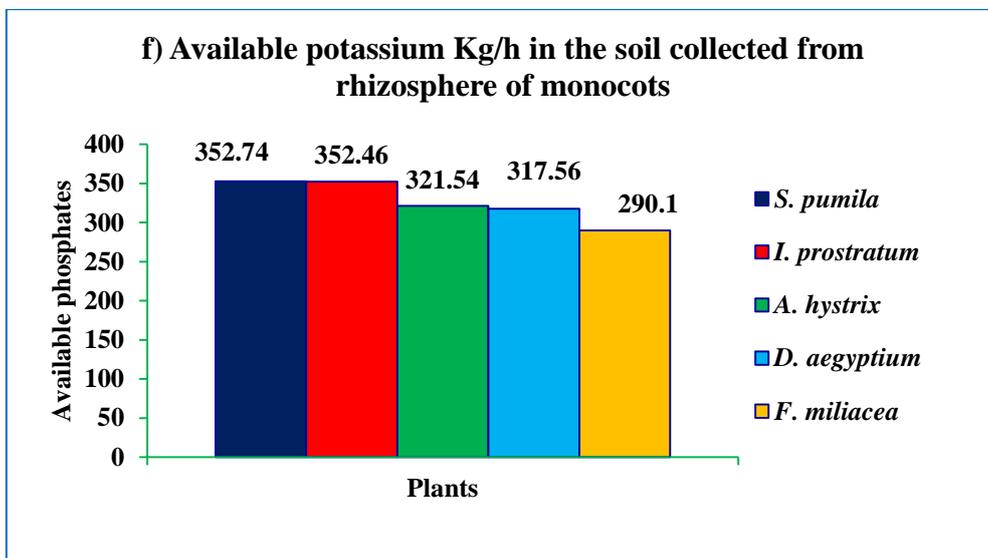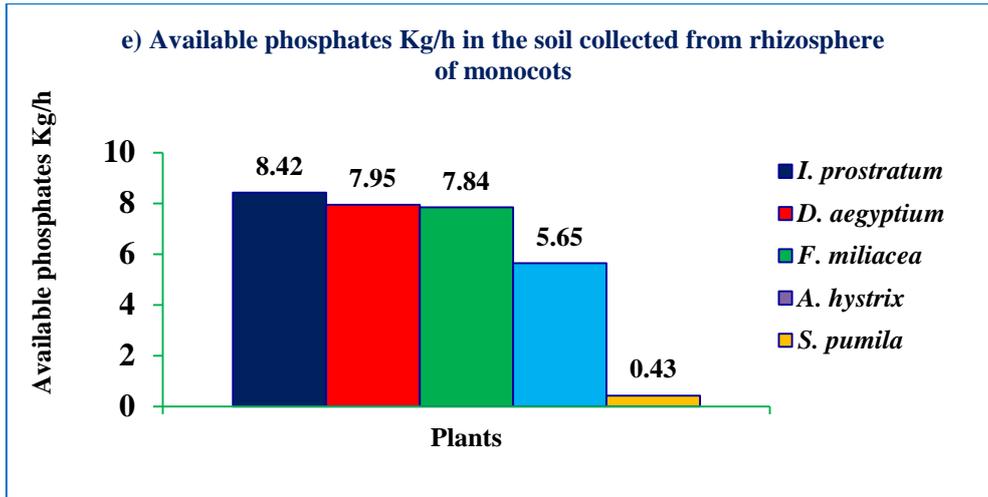
b) EC of soil collected from rhizosphere of selected monocots of Newasa forest area

c) % organic Carbon in soil collected from rhizosphere of monocots of Newasa forest area

Where*: Iseilema prostratum* L.*; Setaria pumila* (Poir.) Roem. & Schult. ; *Aristida hystrix* L.*; Dactyloctenium aegyptium* (L.) Willd. And *Fimbristylis miliacea* (L.) Vahl

Graph NO 1(B): Soil analysis of rhizosphere of wild monocots of forest area of Newasa tahasil, Dist. Ahmednagar (MS)

d) Available nitrogen kg/h in the soil collected from rhizosphere of selected monocots

e) Available phosphates Kg/h in the soil collected from rhizosphere of monocots

f) Available potassium Kg/h in the soil collected from rhizosphere of monocots

Where: *I: Iseilema; S: Setaria.; A:ristida; D: Dactyloctenium a*nd *F: Fimbristylis*

Graph NO 1(C): Soil analysis of Rhizosphere of Wild Monocots of forest area of Newasa tahasil, Dist. Ahmednagar (MS) India
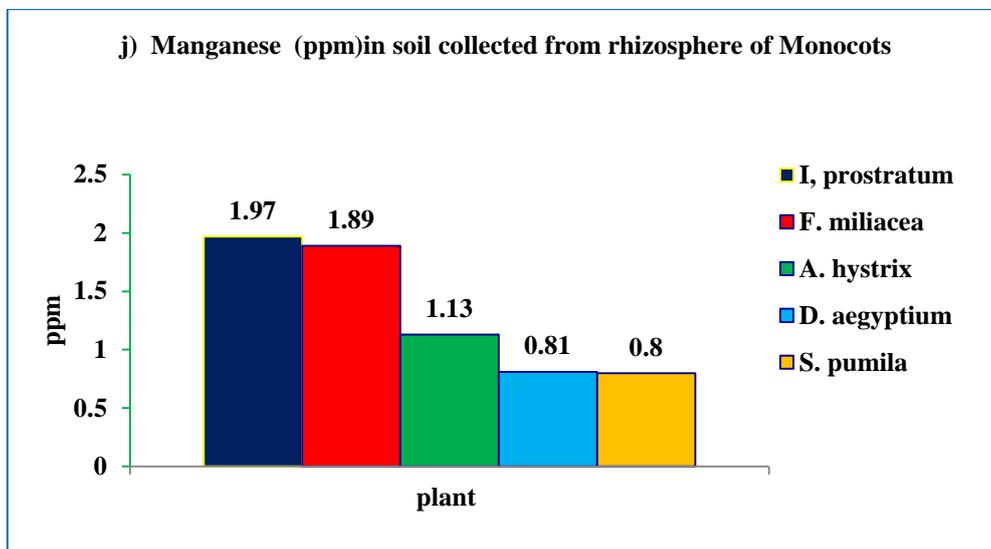
j) Manganese (ppm)in soil collected from rhizosphere of Monocots

Where*: Iseilema prostratum* L.*; Setaria pumila* (Poir.) Roem. & Schult. ; *Aristida hystrix* L.*; Dactyloctenium aegyptium* (L.) Willd. and *Fimbristylis miliacea* (L.) Vahl

Result and Discussion

The concentration of nitrogen ratio in the soil is affected by the population of nitrifying bacteria in the soil (Deokar, 2015). Mujiyatil, and Supriyadi,(2009) reported that, the sandy clay loam, sandy loam, and loam showed the highest bacterial populations *Azotobacter* population, without any treatment reached the mean of 4333.33 cfu/mg, whereas Azotobacter population, with NPK treatment reached the mean of 6666. 67 cfu/mg. From these observations, it can be concluded that the giving of NPK may increase the population of Azotobacter to the highest value as compared to control. *Azotobacter* population got higher value when compared with manure was understandable due to the granting of NPK fertilizer that can decrease soil pH to 6.31 compared to soil pH with manure treatment which was 7.08 and with no treatment (control) which was 7.08.

Rao (1994) reported that, *Azotobacter* bacteria are commonly found in neutral or acid soil. *Azotobacter* has a beneficial effect in the rate of seed development, plant growth, standing crop and vegetative growth with the increase in *Azotobacter.*

Boddey and Dobereiner (1995) reported that improvement of nutrient uptake and plant nutritional status can be achieved by using selective bacteria as observed with legumes and grasses. Soil pH of rhizosphere of grasses and a sedge plant: Twenty readings were taken and collected data was subjected to single factor ANOVA analysis. pH of soils collected from rhizosphere of grasses and a sedge plant were not significantly different at 05% p-level. pH of soil from *Setaria pumila* (Poir) Roem &Schult was lowest(7.41±0.06). pH of soil from *Aristida hystrix* L. was highest (7.59±0.10).

pH of soil from a sedge plant of Cyperaceae family *Fimbristylis miliacea* (L.) Vahl. was (7.50±0.08).Soils were not too much alkaline. Electric conductivity MI Moh/cm^2 in the rhizosphere was in the range of 0.43 to 0.60. Soil organic carbon % in soil samples was in range of 0.49 to 0.60. Available potassium kg/h in a range of 290.10 to 352.46 Kg/h.Fe (ferrous form) in ppm in a range of 2.40ppm to 2.88ppm.Cu (ppm) was in a range of 0.53 to 0.81. Electric conductivity, organic carbon %, Available potassium kg/h and Cu (ppm) were not significantly different (at 0.05% P-level) in the rhizosphere of all five monocot plants.

Available nitrogen Kg/h was significantly different in rhizospheres of all five plants. Analysis of Rhizosphere soil of *Fimbristylis* revealed lowest (178.10 kg/h) available nitrogen while in the rhizosphere soil of *Setaria pumila* it was recorded highest (320.05 kg/h). Available phosphates Kg/h was in a range of 0.43 kg/h to 8.42 kg/h. ppm ; Zn (ppm) was in a range 0.28 to 0.66ppm and Mn (ppm) was in a range from 0.80 to 1.97ppm. These were significantly different at 0.05% probability level. Number of bacterial colonies in the soils samples of rhizosphere of grasses was in a range of 1454440 to 11700540.

Conclusions

Soils from rhizospheres of five Monocot plants were not too much alkaline. Electric conductivity, organic carbon %, Available potassium kg/h and Cu (ppm) were not significantly different in the rhizosphere of all five monocot

plants. Rhizosphere soil of *Fimbristylis* revealed lowest available nitrogen while *Setaria pumila* the highest. Available phosphates and Zn (ppm) were significantly different at 0.05% probability level. Bacterial colonies in the soils samples of rhizosphere of *Setaria pumila* (Poir.) Roem.& Schult were maximum as compared to other four Monocots (Table No 1)

References

[1] Allen ON, Allen EK. (1981) The leguminosae: A source book of characteristics, uses, and nodulation, University of Wisconsin Press. Madison, USA.

[2] Deokar. S.C. (2015) Effect of Mangnous Oxide, Zinc Sulphate on plant growth promoting rhizobacteria - Azotobacter chrococcum. International Journal of Scientific Engineering and Applied Science (IJSEAS) - Volume-1, Issue-9, pp50-56

[3] Dick, R.P. (1994) Soil Enzyme Activities as Indicators of Soil Quality. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F. and Stewart, B.A., Eds., Defining Soil Quality for a Sustainable Environment, Soil Science Society of America.

[4] Rao NSS. (1982) Biofertilizers in agriculture. Oxford and IBH Publishers. New Delhi.

[5] Rao NSS. (1994) Soil microorganisms and plant growth. UI Press. Jakarta. [Indonesia].

[6] Sarief S. (1989) Fertility and fertilization of agricultural land. Pustaka Buana. Bandung. [Indonesia]

[7] Sugito Y, Nuraini Y, Nihayati E. (1995) Organic farming systems. Faculty of Agriculture. Brawijaya University. Malang. [Indonesia]

[8] Lindsay, W.L. and Norvell. (1978)Development of a DTPA soil tests for Zn, Fe, Cu, and Mn Soil Sci. Soc. Am. J., 42:421- 428

[9] Thomas G.S. (1996) Soil pH and Soil acidity p. 475-490. In methods of Soil Analysis. Parts. Chemical Methods (Ed.) DL Sparts, SSSA ASA, Madison WI.

[10] Thomas G.S. (1996) Soil Electrical conductivity p. 475-490. In methods of Soil Analysis. Parts. Chemical Methods (Ed.) DL Sparts, SSSA ASA, Madison WI.

[11] Walkley A. (1947) A critical examination of rapid method for determining organic carbon in soil: effect of variations in digestion condition and organic soil constituents soil science. 63; 251-263. In Somawanshi R, Kadlag A, Deshpande A, Tamboli B, 2012 Soil and water testing methods soil science and Agricultural chemistry Mahatma phule Krushi vidhyapeeth Rahuri Ahmednagar MS India.

[12] Subbiah B.V. and Asija G.I. (1956) A rapid procedure for the estimation of available nitrogen in soil current science 25; 259- 260. In Somawanshi R, Kadlag A, Deshpande A, Tamboli B, (2012) Soil and water testing methods soil science and Agricultural chemistry Mahatma phule Krushi vidhyapeeth Rahuri Ahmednagar MS India.

[13] Olsen S.R. Cole C.V. Watanable F.S. and Dean L.R. (1954) Estimation of available phosphate in soil by extraction with sodium bi carbonate VSDA circular 939 VSDA Washington D.C. In Somawanshi R, Kadlag A, Deshpande A, Tamboli B, (2012) Soil and water testing methods soil science and Agricultural chemistry Mahatma phule Krushi vidhyapeeth Rahuri Ahmednagar MS India.

[14] Thomas G.W. (1982) Exchangeable cations p159-165 In Page A.L. et al. (ed) Methods of soil Analysis part 2 second (ed) Agron monger and ASA and SSSA Madison W.I. In Somawanshi R, Kadlag A, Deshpande A, Tamboli B, (2012) Soil and water testing methods soil science and Agricultural chemistry Mahatma phule Krushi vidhyapeeth Rahuri Ahmednagar MS India.

[15] Lindsay W.L. and Narvell W.A. (1978) Development of a DTPA soil test for Zn, Fe, Cu and Mn Soil Science AM 5. 42: 421-428 In Somawanshi R, Kadlag A, Deshpande A, Tamboli B, (2012) Soil and water testing methods soil science and Agricultural chemistry Mahatma phule Krushi vidhyapeeth Rahuri Ahmednagar MS India.

[16] Aneja K.R. (2002) Experiments in Microbiology, Plant Pathology, Tissue Culture and Mushroom production technology New Age international p. Ltd. New Delhi. PP 632.