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Diversity of *Azotobacter* and *Azospirillum* in Rhizosphere of Different Crop Rotations in Eastern Uttar Pradesh of India

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ABSTRACT

Azotobacter and *Azospirillum* are known as non symbiotic free living nitrogen fixing biofertilizer microorganisms which actively participate in nutrients cycles. In eastern Uttar Pradesh of India, there is a great diversity in various cropping systems that may possess variation in these important biofertilizer microorganisms. The present study was conducted in order to chalk out the diversity of *Azotobacter* and *Azospirillum* sp. in different districts of eastern Uttar Pradesh in India with respect to the prevailing cropping system. Sixty two soil samples were collected from different crop rotations namely rice-wheat, vegetables, agroforestry and grassland for enumerating the diversity of *Azotobacter* and *Azospirillum*. The result showed that the *Azotobacter* population in rice-wheat, vegetables, agroforestry and grassland based crop rotations varied from 10×10^5-13×10^5, 12×10^5-16.5×10^5, 9×10^5-15.5×10^5 and 7×10^5-10.5×10^5 CFU g^{-1} soil, respectively. Population of *Azospirillum* in rice-wheat, vegetables, agroforestry and grassland based crop rotations varied from 5.5×10^5-10.5×10^5, 6.5×10^5-12×10^5, 5×10^5-13×10^5 and 4.5×10^5-10×10^5 CFU g^{-1} soil, respectively. The diversity in population density of *Azotobacter* was maximum in soil of agroforestry followed by vegetables, grassland and lowest in rice-wheat based crop rotations, while in case of *Azospirillum* diversity was maximum in agroforestry and the lowest was observed in the rice-wheat but similar diversity present in vegetables and grassland crop rotations soil of eastern Uttar Pradesh. Overall maximum diversity of both the biofertilizer micro-organisms occurred in agroforestry based crop rotation.

Key words: Biofertilizer microorganisms, microbial diversity, *Azotobacter*, *Azospirillum*, crop rotations

INTRODUCTION

Soil beneath our foot constitute rich source of microbes whose abundance are affected by both biotic and abiotic factors. The plethora of these microbes is present in rhizospheric zone and their size and activities are regulated by physical and physicochemical status (Aira *et al.*, 2007). The surplus type of microorganisms present in soil are indispensable and directly responsible for successful completion of pedological processes and providing complimentary medium for biological reactions of nutrient cycling in any area (Maurya *et al.*, 2011). The rhizobacteria promote plant growth by improving the soil health (Jolly *et al.*, 2010).

The world population will cross the 10 billion mark by 2050. This will demand more land area for food, fiber, fuel and raw materials to support the life. This demand is creating pressure on the agriculture practices involving more chemical fertilizers input to have greater yield. This further influences the soil health. In the past few decades research has been oriented towards the biofertilisers as a substitute for the chemical fertilizer (Kannaiyan, 2000; Parr *et al*., 1983; Treto *et al*., 2002) since biofertilizer have definite advantage over chemical fertilizers. Biofertilizer provides essential nutrients along with certain growth promoting substances like hormones, vitamins, amino acids, etc. These growth promoting substances increase the efficiency of added chemical fertilisers (Mostafa and Abo-Baker, 2010; Yasari *et al*., 2008).

Among the microorganisms that have been used as biofertilisers there is a group of bacteria known as Plant Growth Promoting Rhizobacteria (PGPR). These PGPR have been recognized for their potential use in agriculture and horticulture (Lucy *et al*., 2004; Munir *et al*., 2003). Research study using PGPR like *Azotobacter*, *Azospirillum* and phosphate solubilizers had showed the beneficial influence reflected by improved plant biomass and productivity (Saxena and Tilak, 1998; Yasari and Patwardhan, 2007; Shaukat *et al*., 2006; Karthikeyan and Sakthivel, 2011). Nitrogen fixation, solubilisation of phosphorus in the rhizosphere and the production of phytohormones enhance plant growth directly.

The presence of an organism in the environment does not necessarily mean that it is contributing significantly to soil processes. Mixed cropping increases rhizospheric microorganisms viz. bacteria and free-living N_2-fixers *Azotobacter* species. Also, during germination of seeds and the growth of plants, number of microorganisms in root zone increases (10-1000 times). The increased number of microorganisms remains relatively constant in this area during the whole period of plant development (Rao *et al*., 1989).

Free living diazotrophic bacteria like *Azospirillum* and *Azotobacter* play an important role as biofertilisers. In order to have better biofetilisers for different crops a knowledge of diversity of these microorganisms is necessary. A number of studies has been made on diversity of *Azotobacter* and *Azospirillum* in rhizospheric soil of different crops (Jolly *et al*., 2010; Kumari and Lakshmi, 2009; Kalaigandhi *et al*., 2010; Kanimozhi and Panneerselvam, 2011). But no study has been conducted comparatively among different cropping patterns. *Azospirillum* species are commonly found in soils and in association with roots of rice, maize, wheat and legumes. Rhizosphere colonization by *Azospirillum* species has been shown to stimulate the growth of a variety of plant species (Yasari and Patwardhan, 2007; Mehry *et al*., 2008; Arzanesh *et al*., 2009). The growth stimulation by *Azospirillum* depends on the effective colonization in the rhizosphere.

Keeping in mind the importance of *Azospirillum* and *Azotobacter* as a biofertilizer to maintain the soil health, the present work has been done to determine the diversity of diazotrophic bacteria (*Azotobacter* and *Azospirillum*) in different crop rotation of different districts of Eastern Uttar Pradesh.

MATERIALS AND METHODS

Collection of soil samples: Soil samples were collected from rhizospheric zones of different Agro-eco-cropping systems: namely agroforestry, grass land, vegetables and rice-wheat growing areas of different districts of Eastern Uttar Pradesh, India during June 2010 to March 2011. The samples were packed in isothermic bag, labeled and brought to the laboratory and stored at 4°C for further studies.

Media used for enumerating diazotrophs: Enumeration of *Azotobacter* was done on *Azotobacter* Berks' medium having ingredients namely 0.25 g KH_2PO_4, 0.125 g $MgSO_4.7H_2O$, 0.125 g NaCl, 0.005 g $FeSO_4.7H_2O$, 0.005 g $Na_2MnO_4.2H_2O$, 0.005 g $MnSO_4.4H_2O$, 0.1 g $CaCO_3$ and 10 g glucose L^{-1} at pH 7.2. For the enumeration of *Azospirillum* Nitrogen-free medium was used that contains 0.5 g malic acid, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.02 g $CaCl_2$, 0.002 g Na_2MoO_4, 0.01 g $MnSO_4.H_2O$, 1.64% EDTA, 3 mL of Bromothymol blue 0.5% (w/w in ethanol), 4.5 g KOH, 0.1 mg Biotin and 1000 mL of distilled water at pH-6.8.

Enumeration of diazotrophs: For enumerating the diazotrophic population of soil, serial dilution and plating technique as described by Schmidt and Belser (1982) was used. The result was expressed as CFU g^{-1} soil. In brief, 10 g of soil sample was dispersed in 90 mL of 0.9% sterile saline water in 250 mL conical flask and kept for half an hour to settle down the suspended soil particles. For preparation of serial dilution, 1 mL of suspension was transferred to culture tube containing 9 mL of sterile saline water, shaken on mechanical stirrer and labeled. Serial dilution up to 10^{-5} was made for *Azospirillum* and *Azotobacter,* respectively. One milliliter of soil suspension was poured and uniformly spread on sterilized solid media plate followed by incubation at 28±2°C for 10 and 5 days to grow the *Azospirillum* and *Azotobacter,* respectively. Colony formed were counted and expressed as CFU g^{-1} soil.

RESULT

Azotobacter and *Azospirillum* population diversity under different cropping sequences in some districts of eastern Uttar Pradesh is being presented in Fig. 1 and 2.

Microbial population diversity of *Azotobacter*: The data reveals that rice-wheat crop rotation showed maximum population diversity in Bhadohi as compared to other district, while minimum in Varanasi district. Vegetable crop rotation showed prominent diversity in all districts except Varanasi. In Bhadohi, non significant difference of *Azotobacter* diversity was observed in rice-wheat, vegetable and Agroforestry crop rotations. *Azotobacter* population was recorded to be minimum in grassland rotation in all districts as compared to other crop rotations. In vegetable crop rotations, maximum population occurred in Allahabad district and other districts have almost similar population of *Azotobacter* except in Varanasi district which was lowest. The vegetable based

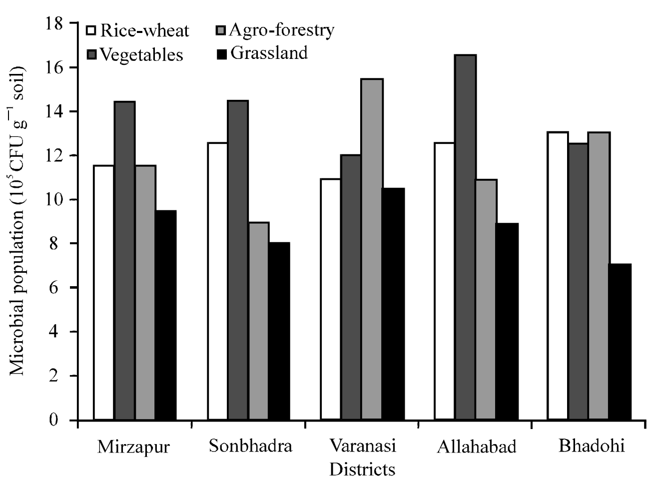

Fig. 1: Population diversity of *Azotobacter* in different crop rotations of different districts of Eastern Uttar Pradesh

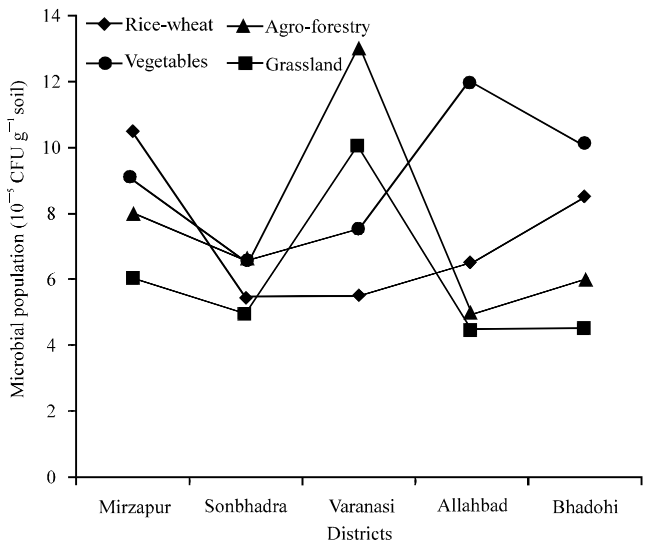

Fig. 2: Population diversity of *Azospirillum* in different crop rotations of different districts of Eastern Uttar Pradesh

crop rotation showed maximum population density of *Azotobacter* biofertilizer than any other crop rotations. Agro-forestry based crop rotation, Varanasi district have high *Azotobacter* microbial population density followed by Bhadohi and other districts has almost similar population except Sonbhadra district which was lowest. The maximum population diversity of *Azotobacter* was present in agroforestry followed by vegetables, grassland and lowest was rice-wheat based crop rotation. Vegetables based crop rotation has maximum population of *Azotobacter* followed by Agro-forestry, rice-wheat and lowest in grassland based crop rotation (Fig. 1).

Microbial population diversity of *Azospirillum*: Rice-wheat crop rotation showed lower population density of *Azospirillum* in Sonbhadra and Varanasi districts but Mirzapur district have maximum microbial population of *Azospirillum* followed by Bhadohi and Allahabad districts. *Azospirillum* population density showed fluctuation in vegetable based crop rotations in different districts. Maximum population of *Azospirillum* was present in Allahabad but lowest was noted in the Sonbhadra district while other districts had almost similar population density. The lowest population of *Azospirillum* occurred in agroforestry system of Allahabad district but Varanasi district had high *Azospirillum* population followed by Mirzapur. Other districts showed almost similar microbial population. Varanasi district showed maximum population density of *Azospirillum* in grassland but similar microbial population densities were present in all other districts. In the Varanasi district the *Azospirillum* population in grassland based crop rotation was higher than the rice-wheat and vegetables based crop rotations. With in different crop rotations, the maximum *Azospirillum* population density was recorded in Agroforestry system and minimum population density was present in grassland (Fig. 2).

DISCUSSION

The biofertilizer microorganisms are more suitable for high crop yield, protection from different pathogens, pesticides and help in maintaining soil health by decomposition of dead and decaying matters in the soils (Pedro, 2006; Verma *et al*., 2010). These biofertilizer microorganisms also help to provide suitable environment for flora and fauna. The microbial biofertilizers such as *Azotobacter* and *Azospirillum* in the rhizospheric soils were variable with crop rotations of different districts of eastern Uttar Pradesh. The *azotobacter* microbial population diversity in different crop rotations varied in following orders namely Agro-forestry, vegetable, grassland and rice-wheat based crop rotations, respectively while in case of *Azospirillum*, trend was agroforestry followed by vegetables and grasslands and rice-wheat based crop rotations. In general, the *Azotobacter* population density of biofertilizer was greater in all cropping patterns than *Azospirillum* population density. Temperature, pH, soil water content, land use changes, percentage of clay, organic matter and nutrient availability have been identified as key variables controlling the soil microbial activities. Favorable soil conditions as well as soil salinity and aerobic conditions caused microbial population density to increase (Pimentel *et al*., 1995; Goss *et al*., 2002; Stotzky, 1985). These facts are in evident to the experimental findings of Anderson and Nilsson (2001), Allison *et al*. (2008), Ye *et al*. (2009), Kang *et al*. (2003), Jia *et al*. (2006) and Enowashu *et al*. (2009).

The *Azotobacter* population density in rhizospheric soil samples of rice-wheat crop rotation ranged from 10×10^5-13×10^5 CFU g^{-1} soil due to high organic matter in the soil. A similar result was reported by Akond *et al*. (2007) and Kalaigandhi *et al*. (2010). The *Azospirillum* population of rice-wheat crop rotation varied from 5.5×10^5-10.5×10^5 CFU g^{-1} soil so that population density increased with the help of percentage of clay matter. Similar result was also reported by Karthikeyan *et al*. (2008).

Azospirillum population in the vegetables based crop rotation varied from 6.5×10^5-12×10^5 CFU g^{-1} soil due to high organic matter content in soil and suitable for environmental condition of soils. Similar results were reported by Jolly *et al*. (2010) and Kumari and Lakshmi (2009). The *Azotobacter* microbial population in vegetables based crop rotation varied from 12×10^5-16.5×10^5 CFU g^{-1} soil due to more suitable environmental condition of soil, percentage of clay and organic matter for increased population. The *Azotobacter* population density in CFU g^{-1} soil was higher in vegetables based crop than any other crop rotations such as rice-wheat, agroforestry and grassland.

The agroforestry based crop rotation showed to possess maximum population density of *Azospirillum* and *Azotobacter*. Maximum population density of *Azospirillum* in agroforestry based crop rotation varied from 5×10^5-13×10^5 CFU g^{-1} soil so that maximum population of *Azospirillum* was recorded in this crop rotation than any other rotation viz. rice-wheat, vegetables and grassland due to high humus formation in the soil which support the increase in the microbial population density. The *Azotobacter* population density in agroforestry based crop rotation ranged from 9×10^5-15.5×10^5 CFU g^{-1} soils due to more support soil for increased in the population. The similar results were reported by Karthikeyan *et al*. (2008) in medicinal plants and Rao *et al*. (1989) in various trees.

Grassland based crop rotation showed that the population density of *Azotobac*ter varied from 7×10^5-10.5×10^5 CFU g^{-1} soils. It increased with increase in the organic matter, environmental condition of soil and soil salinity. *Azospirillum* population density in grassland based crop rotation varied from 4.5×10^5-10×10^5 CFU g^{-1} soils. It possessed least population density due to less organic carbon. In general, grassland based crop rotation was recorded to have lowest population density of both (*Azotobacter* and *Azospirillum*) microorganisms. Diversity of microbial population density of both (*Azotobacter* and *Azospirillum*) microorganisms in grassland was maximum in Varanasi and minimum of *Azotobacter* was observed in the Bhadohi and *Azospirillum* in soils of Allahabad and

Bhadohi. In general, among the both biofertilizer microorganisms i.e., *Azotobacter* and *Azospirillum*, the maximum population density of *Azotobacter* was present than *Azospirillum* in any crop rotations.

The present research study helped to evaluate the microbial population density of *Azotobacter* and *Azospirillum* in rhizospheric soil and diversity between them in different cropping systems of different districts in eastern Uttar Pradesh. The result of the present study will help to determine the cropping system and input fertilizer need suitable to have a greater and sustained yield for different soils of Eastern Uttar Pradesh.

CONCLUSION

The soils of eastern Uttar Pradesh districts are variable in nature for microbial population density of *Azotobacter* and *Azospirillum* biofertilizers microorganisms. The microbial populations of both the microorganisms in different crop rotation are different due to organic matter, salinity, and environmental conditions of soil. The maximum diversity of microbial population density of both *Azotobacter* and *Azospirillum* microorganisms are present in Agroforestry based crop rotation. The maximum population density of *Azotobacter* present in Allahabad district in vegetable based crop rotation but maximum population density of *Azospirillum* present in Varanasi district in agroforestry based crop rotation. Overall in general the *Azotobacter* has maximum microbial population density than *Azospirillum* in any cropping systems of different districts of eastern Uttar Pradesh.

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