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Salinity and Sodicity Influence Mutualistic Association of Beneficial Microorganism in Arid Soils

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Abstract

Azotobacter, phosphate solublizing bacteria (PSB) and phosphate solublizing fungi (PSF) population were assessed in soil under five different crop species grown in irrigated Indian arid environment. *Azotobacter* was most abundant in arid soil (5.6×10^4 CFU g^{-1} soil) in comparison to PSB (1.9×10^4) and PSF (1.3×10^4). The population of these organisms varied among the crop species. Maximum population was recorded for *Cuminum cyminum* L. followed by *Brassica oleracia* L., *Capsicum annuum* L., *Triticum aestivum L.* and *Brassica juncea* L. Soil properties were significantly correlated with *Azotobacter* population. Of the various soil properties, pH and EC (1:2 soil: water) showed negative correlation, while organic carbon (OC), Olsen's P and NH_4OAc extractable K were positively correlated with *Azotobacter* population. Further, *Azotobacter* population decreased drastically with the increase in EC_{iw} and SAR_{iw} of applied irrigation water. In PCA biplot, *Azotobacter* population was aligned with OC, Olsen's P. *Brassica oleracia* was most efficient in hosting P solubilizers while *Cumin cyminum* and *Brassica oleracia* were most efficient in forging mutualistic association with *Azotobacter*. Regression analysis indicated that about 50, 42, 42, 52 and 16 per cent variation in *Azotobacter* count was attributed to $pH_{1:2}$, $EC_{1:2}$, OC, Olsen's P and NH_4OAc-K, respectively.

Key words: Beneficial microorganisms, *Azotobacter*, Phosphate solublizing microorganism, Mutualistic association, Arid soil, SAR, RSC

Introduction

Microorganisms play a key role in terrestrial nutrient cycling and biochemical transformation and facilitate in rhizospheric modification for improved adaptation in stressed environments (Rashid *et al.*, 2016). Among several soil processes, nitrogen (N) and phosphorous (P) cycle are governed through biological nitrogen fixation and solublization of P, respectively, are at mainstay for nutrition of these elements to plants. Among the asymbiotic nitrogen fixing organisms, *Azotobacter* plays an important role in maintaining the soil fertility through atmospheric nitrogen fixation and production of growth promoting substances, synthesis cell protein by utilizing atmospheric N_2 (Saha *et al.,* 2017). This cell protein is then mineralized in soil after the death of *Azotobacter* cells, thereby contributing towards the N availability of the crop plants. Soil also contains large number of phosphate dissolving microorganisms and their biochemical activity enhance the phosphate assimilation by higher plants (Gaur 1990; Dubey *et al.*, 1999; Mitra *et al.*, 2020). The effective transformation of these nutrient elements depend upon the microbial abundance and conducive soil environment.

Soil salinity and sodicity are the major abiotic stress imposing limitation on the selection of the crop plants for their economic cultivation in arid regions. The soil salinity and sodicity can directly or indirectly affect the activities of soil microorganisms and soil enzymes (Wichern *et al.*, 2020; Nannipieri *et al.,* 2017). Sustainability of agriculture in arid region world over including India is always under threat because of low rain fall and dependence on poor-quality water for raising *rabi* season crops (Tomar *et al.*, 2003; Abd El-Wahed *et al.*, 2018). Use of saline and /or sodic water often results in salinization in the surface soil and decline of soil health with associated crop losses (Minhas, 2012).

Mutualistic associations of the beneficial microorganisms impart coping mechanisms to the crops against stressers (Zelicourt *et al.,* 2013). These beneficial microorganisms belong to a number of different bacterial families and improve the growth of vegetables and crops under abiotic stress conditions (Egamberdieva and Kucharova, 2009). Inoculation with *Rhizobium* and *Pseudomonas* in *Zea mays* L. increased salinity tolerance by maintaining leaf water contents and decreasing electrolyte leakage (Bano and Fatima, 2009). Some microorganisms also increased nutrient acquisition by manipulating root plasticity due to secretion of the plant growth promoting hormones, such as indole acetic acid and gibberellic acid (Egamberdieva and Kucharova, 2009). In this background it was hypothesized that associatory nitrogen fixers and P solublizers in the rhizospheric nitches of major crops *viz.* cumin (*Cuminum cyminum* L.), cabbage (*Brassica oleracia* L.), chilli *(Capsicum annuum* L.), wheat *(Triticum aestivum L.*) and mustard (*Brassica juncea* L.) may have varied response to salinity and sodicity in irrigated sandy soils. The responsiveness of these beneficial microorganisms might therefore open new applications for a sustainable agriculture. However, information regarding the native population of *Azotobacter* and phosphate solublizers are meager especially for the soils of Western Rajasthan having very low organic matter, arid climate, high mean annual soil temperature and saline SAR (sodium adsorption ratio) water irrigation. Hence, the present study was conducted with an objective to determine the abundance of *Azotobacter* and phosphate solublizing bacteria and fungi in some irrigated soils and their relationship with important soil and irrigation water properties.

Material and Methods

Soil sampling and analysis

A survey was carried out on farmer's fields in Barmer district, Rajasthan India. Rhizospheric soil was collected by uprooting the plants and soils attached to the roots were collected in aseptically in polythene bag and immediately transferred in ice box. Bulk soils (BS) of 0-15 cm depth from nearby locations of plants were also collected. After collection, soil samples were brought to the laboratory and rhizospheric samples were kept at 4°C till their analysis, while bulk samples were air dried and ground to pass through 2 mm sieve for physicochemical analysis. Soil pH and electrical conductivity were measured in aqueous soil extract in de-ionized water (1:2 soil: water ratio). Soil organic carbon (SOC) was determined by the wet chromic acid digestion method (Walkley and Black, 1934). The sodium bi-carbonate extractable P (Olsen's P) and neutral normal ammonium acetate extractable K (NH_4OAC-K) in soil were estimated by methods as described by Jackson (1973) and expressed in kg ha^{-1}. Soil texture was determined following the method described by Gee and Bauder (2006).

Water quality estimation

Irrigation water samples were also collected for each irrigation bore-wells. Standard methods were applied for the determination of pH and EC of irrigation water. Ca^{2+} and Mg^{2+} were estimated by atomic absorption spectrometer. Na^+ and K^+ were measured with a flame photometer. Chloride was measured by argentometric titration (Jackson, 1973). Carbonate and bicarbonate were determined by methyl red and phenolphthalein endpoint titration. Sulphate was determined as described by Chesnin and Yien (1951).

Sodium adsorption ratio (SAR) and RSC are the mathematical relationship shown in following equations:

$$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

$$RSC\ (me\ L^{-1}) = [CO_3^{2-} + HCO_3^-] - [Ca^{2+} + Mg^{2+}]$$

where [ ] represents the concentration of cations in cmol (p+) L^{-1}

Microbial enumeration

The culturable microbial counts of *Azotobacter* and phosphorus solubilizing bacteria and fungi were estimated through enumeration of colonies which can be cultured on the growth medium and expressed in Colony forming units (CFUs) per gram soil. The collected rhizospheric soils were serially diluted and one mL of soil suspension

from appropriate dilution was used for the study. Pour plate technique for the isolation of *Azotobacter* and P solubilizing bacteria/fungi were carried out on Ashby's mannitol agar (Clark, 1965) and Pikovskaya agar medium (Pikovskaya, 1948), respectively. Triplicate agar Ashby medium (5.0 g glucose; 5.0 g mannitol; 0.1 g $CaCl_2.2H_2O$; 0.1 g $MgSO_4.7H_2O$; 5.0 mg $Na_2MoO_4.22H_2O$; 0.9 g K_2HPO_4; 0.1 g KH_2PO_4; 0.01 g $FeSO_4.7H_2O$; 5 g $CaCO_3$ and 15.0 g agar in 1.0 L distilled water; pH 7.3) were incubated at 30°C for 72 hour. Similarly, triplicate Pikovskaya agar medium (glucose 10.0 g; rock phosphate 5.0 g; ammonium sulphate 0.5 g; sodium chloride 0.2 g; manganese sulphate; ferrous sulphate in traces; agar 20 g, distilled water 1000 ml) were incubated at 30°C for 72 hours. After incubation time microbial colonies were counted and expressed in colony forming units (CFUs) per gram soil.

Statistical analysis

Statistical analysis of the data performed using SAS 9.3. Shapiro Wilk and Bartlett test used to check the normality and heterogeneity of database, respectively. Microbial counts data were log transformed before the analysis of variance. The analysis of variance (ANOVA) carried out to determine the statistical significance of treatment effects using Independent-Samples Kruskal-Wallis Test. Multiple comparison of response variables were made after adjusting the significance value using the Bonferroni correction. Further, separate principal component analysis (PCA) was performed for *Azotobacter* with soil and water properties. Pearson correlation coefficients also computed to evaluate the relationships among the rhizospheric counts and soil and water properties.

Results and Discussion

The studied soils were mainly loamy sand and alkaline in reaction (pH 8.3-9.2) with electrical conductivity mostly less than one (Table 1). Soils were low in organic carbon content (1.0-5.4 g kg^{-1}) and low to medium in Olsen's P (3.9-23.9 kg ha^{-1}) and medium to high NH_4OAc-K (112.5-373.3 kg ha^{-1}).

The EC_{iw} representing the total concentration of soluble salts in groundwater varied between 0.83 to 10.7 dS m^{-1}; however, 56% of collected irrigation water recorded soluble salts less than 2.0 dS m^{-1} (Table 2). The pH_{iw} and SAR_{iw} ranged between 7.5 to 8.8 and 0.7 to 22.2 $cmol^{0.5}\ L^{-0.5}$, respectively. The soluble carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-) in the water samples varied from 3.8 to 11.0 me L^{-1}. This CO_3^{2-} and HCO_3^- was less than Ca^{2+} and Mg^{2+}; thereby, preventing the excessive Na^+ saturation in soil in majority of cases. The RSC of groundwater varied from non-detectable to 5.0 me L^{-1} and only 6.0 per cent sample showed problem of RSC for irrigation. The percentage of samples having SAR values <10, 10-18 and >18 $cmol^{0.5}\ L^{-0.5}$ were 62, 6 and 32 per cent, respectively (Fig. 1). Hence, majority of water samples were found under good category as suggested by Gupta *et al.* (1994) but 32% of irrigation can favour sodication of soil (Basak *et al.*, 2015). The SO_4^{2-} and K^+ content ranged from 0.1 to 1.0 me L^{-1} respectively. Besides, being

Table 1. Physicochemical characteristics of soil (n=31) from different locations in Barmer district of Rajasthan

Parameters	Mean	Range
$pH_{1:2}$	8.8	8.3-9.2
$EC_{1:2}$ (dS m^{-1})	0.39	0.11-1.1
Organic carbon (g kg^{-1})	3.4	1.0-5.4
Olsen's P (kg ha^{-1})	12.6	3.9-23.9
NH_4OAc-K (kg ha^{-1})	182.6	112.5-373.3
Sand (%)	82.6	79.9-85.1
Silt (%)	7.6	5.5-12.0
Clay (%)	9.3	7.5-11.5
Soil texture	Loamy sand	
Soil classification	*Camborthids*	

Table 2. Ionic composition of irrigation water (n=31) used for cropping at sampling sites in Barmer district, Rajasthan

Parameters	Mean	Range
pH_{iw}	7.94	7.50-8.80
EC_{iw} (dS m^{-1})	2.97	0.60-10.70
$Ca^{2+} + Mg^{2+}$ (me L^{-1})	11.60	4.20-27.40
Na^+ (me L^{-1})	21.60	1.50-80.60
K^+ (me L^{-1})	0.03	0.01-0.09
$CO_3^{2-} + HCO_3^-$ (me L^{-1})	6.37	3.80-11.00
Cl^- (me L^{-1})	25.86	2.00-96.00
SO_4^{2-} (me L^{-1})	0.39	0.10-1.00
RSC (me L^{-1})	0.40	0.00-5.00
SAR ($mmol^{1/2}\ L^{-1/2}$)	11.15	0.74-22.18

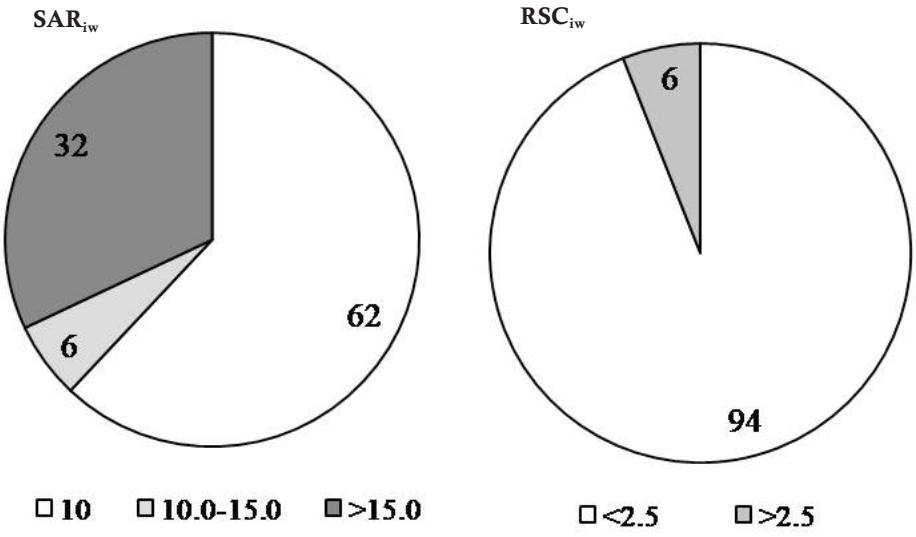

Fig. 1 SAR and RSC irrigation water (n=31) in Barmer district, Rajasthan.

essential plant nutrient sulphur is also beneficial in reducing the sodium hazards of water (Singh and Bishnoi, 1993).

All the soil samples showed the presence of *Azotobacter* but only 52.0 per cent of these showed the counting of PSB and PSF (Fig. 2). This absence of PSB and PSF may be because of low organic matter content, alkaline pH (>8.3) and medium to high Olsen's P creating unfavourable situation for establishment of native phosphate solublizing microorganisms. This is in consonance with earlier report that pH 6.0 and 4.0 is optimum for maximum activity of PSB and PSF, respectively (Bajpai and Sundara Rao, 1971).

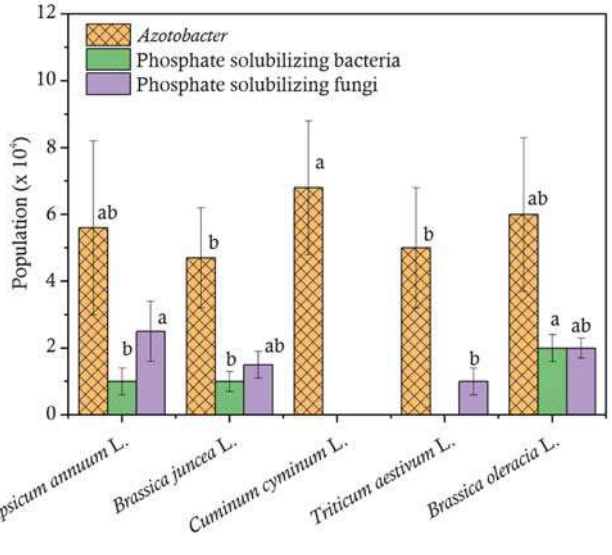

Fig. 2 Changes in microbial population in different plant rhizhisphere (bars followed by different letters (a-b) are significantly different ($p \leq 0.05$)

Besides, soil rich in organic matter will favor microbial growth and therefore favors proliferation of PSB and PSF. P availability increased in soils with pH values between 6.0 to 7.5 as P fixation limit <5.5 and between 7.5 to 8.5 by aluminum, iron, or calcium, and declined P availability for P. A negative correlation was observed between phosphate solubilized by *Bacillus cepacia* SCAUK0330 and the pH drop (Table 3) that was associated with this process. The pH drop led to an increase in phosphate solubilization. At pH 3.12, 452 µg mL^{-1} of phosphorus was solubilized, and when 154 µg mL^{-1} of P was solubilized the pH value was 4.95 (Zhao *et al.*, 2014). Gaur (1990) indicated that PSM activity was greatly reduces at pH 7 to 8 in culture medium; while, the *Azotobacter* population increased with soil pH up to 8.5 (Gandotra *et al.*, 1998).

All the crops were found to be highly infected by *Azotobacter* (Fig. 2). Maximum population (CFU g^{-1} soil) was observed in *Cumin cyminum* (6.8 × 10^4) followed by *Brassica oleracia* (6.0×10^4) and *Capsicum annuum* (5.6 × 10^4), whereas least population was found in *Brassica juncea* (4.7 × 10^4). This crop wise variation may be because of root induced rhizospheric condition, differ in many respects from those in bulk soil. This root induced modification are of crucial importance for the growth of microorganisms and the extent to which root can modify them depends on the plant species (Emmerling et al., 2001; Schoebitz et al., 2016). Different plant species showed *Azotobacter*

Table 3. Correlation matrix for *Azotobacter* population with soil and water characteristics

Soil properties	*Azotobacter*	$pH_{1:2}$	$EC_{1:2}$	Organic C	Olsen's P	NH_4OAc-K	EC_{iw}	SAR_{iw}
$pH_{1:2}$	-0.71**							
$EC_{1:2}$	-0.65**	0.52**						
Organic C	0.66**	-0.44*	-0.59**					
Olsen's P	0.73**	-0.51**	-0.44*	0.62**				
NH_4OAc-K	0.38*	-0.10ns	-0.19 ns	0.23 ns	0.50			
EC_{iw}	-0.41*	0.60**	0.39*	-0.20 ns	-0.32 ns	0.04 ns		
SAR_{iw}	-0.68**	0.79**	0.53**	-0.51**	-0.48*	-0.09 ns	0.66**	1

* and ** denote Correlation is significant at the $p \leq 0.05$ and 0.01 level (2-tailed)

population in the range of 2.0-9.0 × 10^4 CFU g^{-1} soil. Present findings corroborate with earlier reports showing variation in *Azotobacter* population with crops (Maurya *et al.,* 2012). The population density of *Azotobacter* was maximum in soil of agroforestry followed by vegetables, grassland and lowest in rice-wheat based crop rotations.

Alkaline reaction facilitates higher *Azotobacter* counts in comparison to PSB and PSF in these soils as optimum pH requirement of *Azotobacter* growth is slightly alkaline (7.5); whereas PSB and PSF prefer acidic reaction (4.0 to 6.0) (Alexender, 1977; Gaur, 1990). Besides, low nitrogen and energy source in these soils because of low organic matter may also contribute to lower proliferation of PSB and PSF. This was further evident from the very high correlation between *Azotobacter* counts and soil/water parameters (Table 4). But definite trend was not observed for PSB and PSF with soil properties due to lower number of samples showing their presence. On an average higher number of *Azotobacter* was encountered in pH range of 8.3 to 8.5 which decreased significantly with increase in pH up to 9.2. This was supported by significant negative correlation between pH and *Azotobacter* counts (Table 3). Similarly decrease in *Azotobacter* population was found with increase in soil electrical conductivity as reported in rice soil where *Azotobacter* count showed a decrement in with increment in EC (Kaushik and Sethi, 2005). *Azotobacter* population increased with the OC and Olsen's P (Fig. 3 and Table 4) and showed significant positive correlation with NH_4OAc-K. Alexander (1977) and Gandotra (1998) also reported the similar trend. Although significant increase in the number of *Azotobacter* was found with increase in

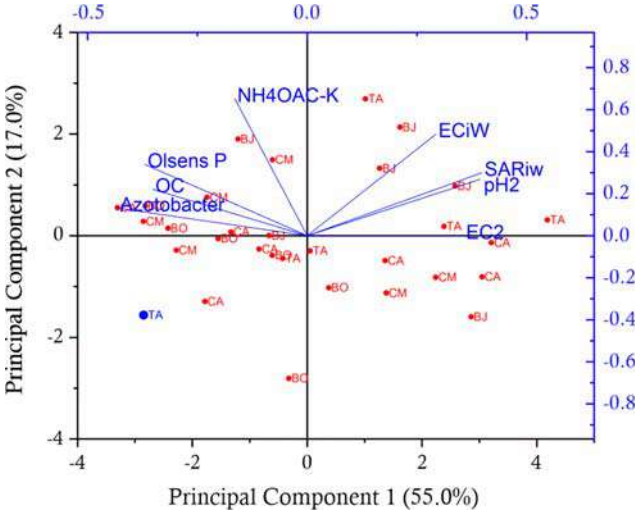

Fig. 3 Biplot for principal components (PC1 and PC2) of studied soil and water attributes in relation with *Azotobacter* population

NH_4OAc-K, but calculated correlation co-efficient was very low. This might be due to relatively higher amount of NH_4OAc-K in desert soils was not limiting for the growth of studied population. Irrigation water EC and SAR also indirectly affected the population of *Azotobacter* by its direct effect on soil reaction and EC. In consistence with trend of *Azotobacter* population decreased drastically with increase in EC and SAR of irrigation water (Table 4).

Principal component analysis (PCA) analysis performed to find out the effects of key soil and water quality parameters on rhizospheric *Azotobacter* population of different crops. Biplot analysis of soil and water attributes for *Azotobacter* shared different spatial distributions on the coordinate axes (PC1 and PC2) (Figure 3; Table 3). The first three PC axes accounting for 81% of the observed variation in data set. *Azotobacter*,

Table 4. Principal component analysis of studied soil and water attributes in relation with *Azotobacter* population

Statistics	PC1	PC2	PC3
Eigenvalue	4.40	1.36	0.75
Percentage of variance	0.55	0.17	0.09
Cumulative	0.55	0.72	0.81
Eigenvectors			
Azotobacter	**-0.43**	0.13	0.04
Olsen's P	-0.37	0.34	-0.17
OC	-0.35	0.22	**0.55**
NH_4OAC-K	-0.16	**0.65**	-0.52
EC_{iw}	0.29	0.48	0.40
$EC_{1:2}$	0.36	0.00	-0.44
$pH_{1:2}$	**0.39**	0.27	0.16
SAR_{iw}	**0.40**	0.30	0.11

Bold figures indicate highly weighted variables among the respective PC (principal component)

$pH_{1:2}$, SAR_{iw} (PC1), NH_4OAC-K (PC2) and organic carbon (PC3) were identified as key parmaters responsible for variation in the data set. The corresponding projective points suggest that the *Azotobacter* population was aligned with OC and Olsen's P in second quadrants while $EC_{1:2}$, $pH_{1:2}$, EC_{iw} and SAR_{iw} was aligned along first quadrant.

Conclusions

Infection of crop plants by *Azotobacter* was very common while presence of phosphate solublizers in general is very low in arid soils. Crop plants vary in their capability to modify rhizospheric environment to develop mutualistic association with *Azotobacter* and P solubilizers. *Cuminum cyminum* and *Brassica oleracia* were most efficient in forging mutualistic association with *Azotobacter* while, *Brassica oleracia* was most efficient in hosting P solubilizers in its rhizspheric nitches. These rhizospheric interactions were further modified by salinity and sodicity of the native soils. Such information can be effectively utilized in developing efficient rhizospheric interaction for nutrition of crop plant in salt-affected soils.

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