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Biochemical characteristics and microbial association of Isabgol (*Plantago ovata* Forks.) growing soils in Western Arid region of India

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Isabgol (*Plantago ovata* Forks.) is one of the important cash crops in arid and semi-arid regions of India. The husk of Isabgol seeds are primarily used as laxative in medicinal preparations. The cultivation of Isabgol crop is very much dependent on soils and weather conditions as this crop is highly susceptible to many biotic and abiotic stress parameters. Soil microbial population is involved in many direct and indirect interactions with crop plants. The type of microbes present in the soils also affects the plant health. Soil samples from the rhizospheric zone of the plants were collected from Barmer, Jalore and Ajmer Districts of Rajasthan State in western arid region of India. The soil EC and pH (1:2.5) recorded were in the range of 0.12 to 0.46 dS/m and 7.4 to 8.9, respectively, depicting neutral to alkaline soils, the macro nutrient viz; N, P, K were found to be in the range of 128.0 to 192.54, 19.4 to 80.4 and 149.8 to 338.8 kg/ha, respectively. The DTPA extractable micronutrients Cu, Zn, Mn and Fe were in the range of 0.29 to 3.50, 0.26 to 1.5, 0.51 to 4.51 and 1.0 to 5.38 ppm, respectively, in the soil samples of Isabgol growing regions of Rajasthan. Total viable count (TVC) gives a quantitative idea about the presence of microorganism such as bacteria and fungi in samples. The total bacterial count of soil microorganisms varied from 0.8×10^7 to 1.96×10^7 cfu/g whereas, total fungal count varied from 1.52×10^6 to 2.85×10^6 cfu/g. Beneficial microorganism population in terms of total *Azotobacter* count (0.1×10^5 to 1.0×10^5) psuedomonads counts (3.33×10^5 to 5.8×10^5 cfu/g) and phosphate solubilizing bacterial count (0.1×10^3 to 1.0×10^3 cfu/g) varied highly in different soils.

Key words: Isabgol (*Plantago ovata* Forks.), bacterial count, fungal count, N, P, K, macronutrients, micronutrients.

INTRODUCTION

Isabgol (*Plantago ovata* and *Plantago psyllium*) is grown as a cash crop in Gujarat, Punjab and Rajasthan. India is

the largest producer and exporter of this crop in the world. The seed husk is used to cure inflammation of the

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mucus membrane of gastrointestinal and genito-urinary tracts, chronic constipation, dysentery, duodenal ulcers, gonorrhoea and piles. It is also used in calico printing, setting lotions and food industry (Dhar et al., 2005). Isabgol is an irrigated rabi crop which remains in the field for about four months. The crop is grown in marginal, light, well-drained sandy-loam to loamy soils having pH between 7 and 8. It requires a cool climate and dry sunny weather as light showers cause seed shedding. Isabgol makes a moderate demand for nutrients. Usually, 25 kg of each N and P per hectare is given at plantation. The crop is given 1 or 2 hand-weedings during the entire growing period. The plants are about 50 cm high and each plant gives out between 25 to 100 tillers, depending upon the fertility of soil and weather conditions and a good crop may yield about 800-1000 kg of seeds per hectare (Aishwath and Ram, 2008). The plant bears the flowering spikes in about 60 days after sowing and matures in the next 2 months. The yellowing of the lower leaves is an indication of maturity, confirmed by pressing a spike between two fingers when the mature seeds come out. The crop is harvested close to the ground in the early morning hours to avoid losses owing to seed shedding. The harvested material is stacked for 1 or 2 days, made to be trampled by bullocks, winnowed and the separated seed crop is collected. Seeds are processed through a series of grinding mills to separate the husk. About 30% husk by weight is thus recovered. The current level of production in the country is estimated at 90,000 tonnes. Isabgol production is primarily limited to Rajasthan and Gujarat. Some areas in Haryana, Bihar and Madhya Pradesh are also reported to be under Isabgol cultivation, though production in these areas is negligible. In India, Rajasthan's share is 61,000 tonnes (67%) and Gujarat accounts for 29,000 tonnes (33%) for total national Isabgol production and Rajasthan state accounts for 95,000 hectares area under this crop with Jalore, Barmer and Jaisalmer districts being the major production centers (Maiti and Mandal, 2000).

Application of biofertilizers leads to sustainability of our cropping system that is under threat due to continuous and excessive use of chemical fertilizers and other agrochemicals. Beneficial rhizospheric microorganisms for plants that are better known as plant growth promoting rhizobacteria (PGPR) favours plant growth by fixation or solubilization of plant nutrients and the production of growth stimulants. PGPR may facilitate plant growth and development both directly and indirectly. Direct stimulation may include providing plants with fixed nitrogen, phytohormones, iron that has been sequestered by bacterial siderophores, and soluble phosphate, while indirect stimulation of plant growth includes preventing phytopathogens (biocontrol) and thus, promote plant growth and development (Bashan, 1998; Glick, 1995). Isolating of native strains adapted to the environment and their study may contribute to the formulation of inoculants

to be used in region specific crops. The present investigation was undertaken with a view to study the physical and chemical properties, its microbial diversity and isolation of rhizospheric bacteria and to work out the microbiological profile of rhizospheric soils.

MATERIALS AND METHODS

Estimation of biochemical characteristics of soil samples

Thirteen (13) soil samples (P1 to P13) from two Agro-eco sub regions viz. 2.1 and 2.3 (NBSS Publ. 51) under Western plain hot arid ecosystem desert soils (Jaisalmer and Barmer) with 100-300 mm rainfall, 1700-2000 potential evapo-transpiration, 25-30°C mean temperature, LGP <60 days and Western plain (Jalore) hot arid ecosystem desert soils with 300-450 mm rainfall, 1800-1900 potential evapo-transpiration, 24-27°C mean temperature and LGP 60-90 days, respectively were collected during the crop growth stage of pre-flowering in rabi season so that maximum microbial activity under plant root influence could be observed from the rhizospheres of Isabgol growing soils of Barmer, Jalore and Jaisalmer districts of Rajasthan (Table 1). Soil samples were analyzed for electrical conductivity (EC) and pH using aqueous soil extract (2:1), organic carbon (Walkley and Black, 1934), $\text{NH}_4^+\text{-N}$ (Keeney and Nelson, 1982); 0.5M $\text{NaHCO}_3\text{-P}$ (Olsen et al., 1954) neutral 1 N $\text{NH}_4\text{OAc-K}$ (Hanway and Heidel, 1952) and DTPA-extractable Fe, Zn, Cu and Mn (Lindsay and Norvell, 1978). The soils of Jalore, Barmer and Jaisalmer were classified taxonomically at suborders as Typic Torripsamments, Typic Calciorthis and Typic Camborthids (Soil Survey Staff, 2010). Location of sample sites under Isabgol cultivation in different districts of Rajasthan and their soil classification are presented in Table 1.

Estimation of microbial characteristics of soil samples

The rhizospheric soil samples collected from Isabgol fields were used for making serial dilutions upto 10^{-7} . Ninety milliliters of the diluents was measured into bottles used for serial dilution containing 10 g soil of each sample. The mixture was shaken using a horizontal shaker (Remi Make, India) for 30 min. Further dilutions were made and desired dilutions were pour plated in Petri dishes containing selective and non-selective growth media. The total aerobic mesophilic bacterial count and total mesophilic fungal count was determined using nutrient agar and Rose Bengal agar medium using standard microbiological methods. The isolation of rhizospheric bacteria on selective nutrient agar medium and other media were carried out for total viable bacterial count and total viable fungal count. Different beneficial microbial population selective and specific growth media such as Rose Bengal dextrose agar media for fungal population, Pikovskaya's agar medium for total phosphate solubilizing (PSB) bacteria, King's B medium for Pseudomonas and Azotobacter medium for Azotobacter were employed. Total viable microbial count of these soil samples were estimated by serial dilution technique using nutrient agar medium for bacterial count and Rose Bengal Agar medium for fungal count. All the bacterial growth media employed in this study were procured from Hi-media, India.

RESULTS AND DISCUSSION

Plant rhizosphere soils are known to be the preferred

Table 1. Location of sample sites under Isabgol cultivation in different districts of Rajasthan.

Sample no.	District	Village/location	Agro-ecological region	Soil taxonomy
P1	Jalore	Kharwa, Bhinmal-A	2.3*	Typic Camborthids
P2	Jalore	Kharwa, Bhinmal-B	2.3	Typic Camborthids
P3	Jalore	Kharwa, Bhinmal-C	2.3	Typic Calciorthids
P4	Jalore	Ghaseri, Bhinmal	2.3	Typic Camborthids
P5	Jalore	Hadetar, Sanchoor-A	2.3	Typic Torripsamments
P6	Jalore	Hadetar, Sanchoor-B	2.3	Typic Torripsamments
P7	Jalore	Hadetar, Sanchoor-C	2.3	Typic Calciorthids
P8	Barmer	Sajitada-A	2.1*	Typic Camborthids
P9	Barmer	Sajitada-B	2.1	Typic Camborthids
P10	Barmer	Nmibli	2.1	Typic Calciorthids
P11	Barmer	Bhukha	2.1	Typic Camborthids
P12	Jaisalmer	Fatehgarh, Sam	2.1	Typic Torripsamments
P13	Jaisalmer	Pokharna	2.1	Typic Torripsamments

2.1*- Western plain hot arid ecosystem desert soils (Jaisalmer and Barmer) with 100-300 mm rainfall, 1700-2000 potential evapo-transpiration, 25-30°C mean temperature, LGP <60 days; 2.3* Western plain (Jalore) hot arid ecosystem desert soils with 300-450 mm rainfall, 1800-1900 potential evapo-transpiration, 24-27°C mean temperature, LGP 60-90 days.

ecological niche for various types of soil microorganisms due to rich nutrient availability. Physio-chemical properties like pH, EC, are indicators of soil quality for understanding the nutrient status of soil and also its correlation with prevailing microbial population. The Isabgol cropping areas of Rajasthan falls under two agro-ecological regions viz; Western plain hot arid ecosystem desert soils (Jaisalmer and Barmer) with 100-300 mm rainfall, 1700-2000 potential evapo-transpiration, 25-30°C mean temperature, LGP <60 days and Western plain (Jalore) hot arid ecosystem desert soils with 300-450 mm rainfall, 1800-1900 potential evapo-transpiration, 24-27°C mean temperature, LGP 60-90 days (Table 1). Majority of soil samples under the present investigation falls under Typic Camborthids followed by Typic Torripsamments. Biochemical properties of soils under Isabgol cultivation in different districts of Rajasthan are presented in Table 2. Soil properties of the samples (P1 to P13) exhibited variations with respect to different soil sample sites. The electrical conductivity of the soils are in the range 0.12-0.46 dS/m and the hydrogen ion concentration between 7.4 - 8.9 thereby depicting neutral to slightly alkaline soils which is in confirmity with earlier findings for soils of coriander crops in Rajasthan (Mishra et al., 2013). Organic carbon content of the soils ranged from 1.6 to 4.1 g/kg across the soils (P1 to P13). In the surface soil samples from Jalore districts (P1 to P7) it was in the range 1.8 to 3.3 g/kg, 1.6 to 4.1 g/kg in the soils of Barmer district and 1.8 to 3.3 g/kg in Jaisalmer district soils. Conclusively the soil organic carbon content was low at all the sites being the inherent characteristic of the soils of the arid and semi-arid regions. The soil texture

varied between sandy loam to loamy sand. The available nitrogen content was low in all the soils with the lowest value in P13 (128.0 kg/ha) and highest in P5 (192.5 kg/ha). The P₂O₅ content in the soil samples P1, P2, P3, P5, P7 and P12 was in the lower range, medium in P4, P10 and P11 whereas slightly high in P8, P9 and P13. The K₂O content in soils P1, P2 and P3 was low (149.8-160.2 kg/ha) and medium in the rest of the soils (P4 to P13; 209.4 to 338.8). The slightly higher values for K₂O content was obtained in the soils of Barmer and Jaisalmer districts.

The concentration of DTPA extractable micronutrients was in the medium to higher range in almost all the soil samples. DTPA-Cu concentration in the soils was medium for Jalore district (P1 to P4) and higher in rest of the soils from Barmer and bordering areas of Jaisalmer district. Highest DTPA-Cu content was observed in the Typic Camborthids of Barmer district, the DTPA-Zn concentration in the soil samples varied from 0.26 to 1.5 ppm, the lowest being in Typic Torripsamments of Jaisalmer (P13) and highest in the Typic Camborthids of Barmer (P11). The soils from Jalore (P1 to P4) and Jaisalmer district (P13) were low in DTPA-Zn content, whereas rest of the soils has medium values for DTPA-Zn concentration (Hand Book of Agriculture, ICAR). Rest of the soils can be grouped under marginally deficient category in terms of DTPA-Mn and DTPA_Fe. Similar results were also reported by Chattopadhyay et al. (1996) and Shyampura and Sehgal (1996) for the arid and semi-arid soils of Rajasthan, India.

Electrical conductivity is an important factor in determining the salinity/sodicity or both in the soils. It

Table 2. Biochemical properties of soils under Isabgol cultivation in different districts of Rajasthan.

Sample no.	Soil EC (dS/m)	Soil pH (1:2.5)	Organic carbon (g/kg)	Surface texture	Soil taxonomy	Macro nutrients (kg/ha)			Micronutrients (ppm)			
						N	P ₂ O ₅	K ₂ O	Cu	Zn	Mn	Fe
P1	0.21	8.2	3.30	Sandy loam	Typic Camborthids	163.1	27.2	160.2	0.31	0.48	3.83	3.83
P2	0.33	8.3	2.80	Sandy loam	Typic Camborthids	189.1	23.1	158.7	0.29	0.42	3.21	3.80
P3	0.24	8.9	3.00	Loamy sand	Typic Calciorthids	155.7	19.4	149.8	0.30	0.45	3.27	3.74
P4	0.19	8.2	3.00	Sandy loam	Typic Camborthids	188.4	63.1	241.1	0.32	0.53	3.55	3.79
P5	0.38	8.1	1.80	Loamy sand	Typic Torripsammments	192.5	34.6	212.9	1.55	0.84	2.63	5.38
P6	0.17	8.0	2.10	Sandy loam	Typic Torripsammments	189.2	29.4	209.4	1.45	0.78	2.32	5.29
P7	0.46	8.3	2.70	Sandy loam	Typic Calciorthids	187.5	31.4	231.1	1.63	0.84	2.68	4.98
P8	0.38	7.4	2.30	Sandy loam	Typic Camborthids	163.8	80.1	222.1	1.26	0.86	2.41	5.12
P9	0.12	8.1	2.40	Loamy sand	Typic Camborthids	178.4	79.8	214.9	1.56	0.91	2.37	5.34
P10	0.17	8.3	4.10	Sandy loam	Typic Calciorthids	165.6	43.1	338.8	1.23	0.81	2.18	5.76
P11	0.26	8.1	1.60	Loamy sand	Typic Camborthids	153.4	35.6	315.2	3.50	1.50	4.51	2.21
P12	0.20	8.2	1.80	Sandy loam	Typic Torripsammments	135.2	33.2	274.2	0.44	0.31	3.65	3.47
P13	0.36	8.2	2.30	Sandy loam	Typic Torripsammments	128.0	61.1	299.8	0.51	0.26	0.51	1.00

represents the availability of salts in the soil. Increase in electrical conductivity of soil, increases the availability of soluble salts to the plants and thus effect on soil fertility status of the soil which in turn may affect plant health. The pH of soil plays an important role in the occurrence and dominance of a particular group of microorganism. Soil microorganisms, just like higher plants depends entirely on soil for their nutrition, growth and activity. The major soil factors which influence the microbial population, distribution and their activity in the soil are nutrients, moisture, temperature, aeration, pH (H-ion concentration) and salt concentration (Knight et al., 1997). All these factors play a great role in determining not only the number and type of organism but also their activities. Variations in any one or more of these factors may lead to the changes in the activity of the organisms which ultimately affect

the soil fertility level.

Microorganism requires a favorable nutritional and physical environment to grow and multiply. Isolation of microorganisms was done by using serial dilution methods followed by purification using Gram's staining and repeated streaking on nutrient agar media. These steps are essential to obtain well separated discrete colonies in different selective media. Total viable aerobic bacterial count of the samples ranged from 0.8×10^7 to 1.96×10^7 cfu/g soil while the total aerobic fungal population varied from 1.52×10^6 to 2.85×10^6 cfu/g soil. The maximum soil bacterial population was observed from soil sample of P8 locality in district Barmer. Similarly, maximum fungal population was recorded from Isabgol field soil samples of Nibla locality in Barmer district while minimum was observed from Gasedi, Bhinmal locality in Jalore district (Table 3).

Total viable count (TVC) gives a qualitative idea about the presence of microorganism such as bacteria, yeast and mold in a sample on different agar media. To isolate plant growth promoting rhizobacteria specific growth media such as Azospirillum semi-solid agar media Azotobactor agar media, King's B Media and Pikovaskaya agar media were used to enumerate the rhizobacteria on the basis of their ability to grow in the given media. The colonies grown on these specific growth media were enumerated and bacterial cultures were selected and isolated on the basis of different colony morphologies through subculture technique. Microorganism requires a favorable nutritional and physical environment to grow and multiply. Isolation of microorganisms was done by using serial dilution methods followed by purification using Gram's staining and repeated streaking on nutrient agar

Table 3. Microbial properties of Isabgol growing soils from Rajasthan.

Sample no.	District	Village/location	Total viable microbial count (CFUs/g)				
			Bacteria	Fungi	Azotobacter	Pseudomonads	Phosphate solubilizing bacteria
P1	Jalore	Kharwa, Bhinmal-A	1.1X10 ⁷	1.62X10 ⁶	0.3X10 ⁵	4.6X10 ⁵	0.7X10 ³
P2	Jalore	Kharwa, Bhinmal-B	0.9X10 ⁷	1.28X10 ⁶	0.7X10 ⁵	5.2X10 ⁵	0.4X10 ³
P3	Jalore	Kharwa, Bhinmal-C	1.2X10 ⁷	1.70X10 ⁶	0.1X10 ⁵	5.6X10 ⁵	1.0X10 ³
P4	Jalore	Ghaseri, Bhinmal	1.2X10 ⁷	1.52X10 ⁶	0.8X10 ⁵	3.8X10 ⁵	0.1X10 ³
P5	Jalore	Hadetar, Sanchoor-A	1.3X10 ⁷	2.12X10 ⁶	1.0X10 ⁵	3.3X10 ⁵	0.8X10 ³
P6	Jalore	Hadetar, Sanchoor-B	1.8X10 ⁷	1.78X10 ⁶	0.6X10 ⁵	3.8X10 ⁵	0.9X10 ³
P7	Jalore	Hadetar, Sanchoor-C	1.9X10 ⁷	1.58X10 ⁶	0.3X10 ⁵	4.3X10 ⁵	0.3X10 ³
P8	Barmer	Sajitada-A	1.96X10 ⁷	2.40X10 ⁶	0.8X10 ⁵	5.8X10 ⁵	0.7X10 ³
P9	Barmer	Sajitada-B	0.96X10 ⁷	2.40X10 ⁶	0.5X10 ⁵	3.4X10 ⁵	0.6X10 ³
P10	Barmer	Nmibli	1.9X10 ⁷	2.85X10 ⁶	0.7X10 ⁵	4.5X10 ⁵	0.9X10 ³
P11	Barmer	Bhukha	0.96X10 ⁷	1.53X10 ⁶	0.6X10 ⁵	4.3X10 ⁵	0.1X10 ³
P12	Barmer	Fatehgarh, Sam	0.90X10 ⁷	2.25X10 ⁶	0.4X10 ⁵	3.5X10 ⁵	1.0X10 ³
P13	Jaisalmer	Pokharna	1.0X10 ⁷	2.10X10 ⁶	0.9X10 ⁵	4.1X10 ⁵	0.7X10 ³

media. These steps are essential to obtain well separated discrete colonies in different selective media. Pure cultures were preserved on nutrient agar slants stored at 4°C in a refrigerator and further used for estimation of various plant growth promotion characteristics. Microbial count on nitrogen free Azotobacter medium revealed the population of Azotobacter in Isabgol field soil samples which varied from 0.1x10⁵ to 2.0x10⁵ cfu/g. The Pikovskaya medium containing tricalcium phosphate as sole source of phosphorus for bacterial growth provided the rough estimate of phosphate solubilizing bacterial (PSB) population and it ranged from 0.2x10³ to 4.8x10³ cfu/g and Pseudomonads population on Kings B medium varied from 3.3x10⁶ to 5.8x10⁶ cfu/g soil among the collected soil samples of Isabgol crop (Table 3).

It is well established that inoculation with diazotrophic bacteria like *Rhizobium*, *Azotobacter* and *Azospirillum* enhances the plant growth as a result of their ability to fix nitrogen. Growth promotion may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities (Bhashan and Bashan, 2005).

Sakthivel and Karthikeyan (2012) had studied thirty rhizospheric soil samples collected from commercially grown *Coleus forskohlii* from Perambalur and Salem districts of Tamil Nadu. The results obtained showed that among the 30 isolates of Perambalur and Salem districts the range was from 4.00-9.22x10⁶ and 4.66-10.00x10⁶ for *Azospirillum* spp., 3.00-7.66x10⁶ and 3.88-8.00x10⁶ for *Bacillus* spp, 4.66-12.00x10⁶ and 4.88-13.00x10⁶ for *Pseudomonas* spp. and 2.22-8.00x10⁶ and 3.66-9.00x10⁶ for *Azotobacter* spp., respectively, for the two districts. In the present investigation, the population of Isabgol plant beneficial rhizobacteria was found lower than reported by

Sakthivel and Karthikeyan (2012) which may be due to different agro-ecological condition prevailing in the Rajasthan as compared to Tamilnadu. In addition to plant growth promoting traits, these bacterial strains must be rhizospheric competent, able to survive and colonize in the rhizosphere soil (Grover et al., 2009). During the past couple of decades, the use of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture has increased tremendously in various parts of the world. Significant increases in growth and yield of agronomical important crops in response to inoculation with PGPR have been repeatedly reported (Biswas et al., 2000; Asghar et al., 2002; Vessey, 2003).

A general concept has been developed, where bacterial strains were identified that were able to colonize plant roots, stimulate plant growth, and/or reduce the incidence of plant disease. Importance of native strains and ecological specificity while selecting the microbial inoculants for a specific environment is also realized. Isolation of microorganisms, screening for desirable characters, and selection of efficient strains and production of inoculums are important steps for making use of this microbe-based technology.

Conclusions

Though the soils of Jalore, Barmer and Jaisalmer fall under the arid region of India and are poor to medium in terms of major and minor nutrients availability, the crop of Isabgol thrives well. The nutrient requirement of Isabgol crop can still be fulfilled by these soils and thus Isabgol cultivation is a good proposition for these areas. Plant growth promoting rhizo-bacterial population can also con-

tribute significantly towards nutrient availability. Total count of phosphate solubilizing bacteria, free living nitrogen fixing bacteria and Pseudomonads estimated for different soil samples recorded significant differences among the samples of different locations. Thus it can be concluded that the diverse agro-climatic conditions of this semi-arid Rajasthan offers a great potential of further microbiological exploration for novel microbes and especially nitrogen fixers and phosphate solubilizing bacteria for application in Isabgol and other agricultural crops.

Conflict of interests

The authors did not declare any conflict of interest.

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