International Journal of Research and Review
www.ijrrjournal.com E-ISSN: 2349-9788; P-ISSN: 2454-2237

Original Research Article

Phosphorus Solubilizing Bacterial (PSB) Population Dynamics with Seasons and Their Screening in Mulberry Rhizosphere in Different Regions of Kashmir

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ABSTRACT

In India, mulberry plant (exclusive food to silkworm *Bombyx mori* L.) is grown both in the temperate and tropical belts, but the soils are low in available nutrients which cause their deficiency limiting the mulberry growth both qualitatively and quantitatively. Application of fertilizers in soil involve high expenditure and also the fixing and leaching of nutrients in deeper layers immediately after application makes them unavailable. Population dynamics with seasons and screening of potential phosphate solubilizing isolates is recognized as an area of interest because such microbial inoculants could substantially reduce the chemical fertilizer requirement. Therefore, the present study was undertaken to study the influence of seasons on PSB population and the efficiency of best PSB strains isolated from three regions (north, central and south) of Kashmir. The results revealed differences in PSB population with respect to seasons and were found more abundant during spring season followed by autumn, summer and winter seasons, which could be due to better nutrient availability, soil moisture, temperature, etc prevailing during the favourable season. The selected isolates among the population of PSB's, however, differed in their halo zone size in qualitative method with least value ranging from 1.1mm in isolate R3L1PSB1 (southern region) to highest value of 4.9mm in isolate R1L2PSB2 (northern region) and in quantitative method, it was minimum (11.11µg/ml) in isolate R1L2PSB3 (northern region) and maximum (72.9µg/ml) in isolate R1L2PSB2 (northern region) in selected isolates from each region of Kashmir.

Keywords: - Mulberry, Population, PSB, Regions, Rhizosphere, Screening, Seasons.

1. INTRODUCTION

The basic strategy to boost the productivity of raw mulberry silk lies with the production of maximum quantity of quality leaf. This is highly linked with the growth and physiology of mulberry plants. Mulberry (*Morus sps*.) is the sole food plant cultivated mainly to rear the silkworm *Bombyx mori* (L) for cocoon production. Mulberry, being a high biomass producing plant requires perpetual and balanced availability of these nutrients. However, high levels of biomass production coupled with non application of fertilizers have resulted in prolonged exhaustion of nutrients, seasonal variability in crop performances and imbalances in nutrient availability in soils. Although leaf productivity and quality are the specific characters of a particular mulberry variety yet they are greatly influenced by nutrient availability which in turn is affected by environmental conditions and microbial population. Hiltner (1904) for the first time noticed the presence of a much higher number of microorganisms in the vicinity of

plant roots than that observed in soil away from plants. Specific soil microorganisms enhance the amount and efficiency of nutrient acquisition by the vegetation through the formation of symbiotic associations such as those of nitrogen fixing root nodules, mycorrhiza and by the activity of phosphate solubilization. Some microorganisms carry substantial potential to solubilize unavailable fraction of soil phosphorus and those are generally termed as phosphorus solubilizing microorganisms. Phosphate solubilizing bacteria (PSB) inhabiting the rhizosphere are considered as promising biofertilizers since they can supply plants with 'phosphorus' from sources otherwise poorly available by several mechanisms and hence a viable substitute to chemical phosphate fertilizers (Khan *et al.*, 2006; Chaiharn *et al.*, 2008). Screening of potential phosphate solubilizing isolates can be used as bio-inoculants to increase plant growth and yield, which is recognized as an area of interest because such microbial inoculants could substantially reduce the chemical fertilizer requirement and enhance the fertility status of soil. Therefore, it becomes quite important to use low cost biofertilizers made out of native species available in soil rhizosphere which can be isolated, screened and multiplied for the sustenance of soil fertility. Although studies have been conducted on the use of biofertilizers and their relative efficiency on mulberry world over (Baqual, 2005 a&b; Baqual and Das, 2006;) yet very scanty information is available on the seasonal trends in phosphorus solubilizing bacterial population and the influence of native species of PSB inoculants on mulberry growth and productivity. Thus, the present study entitled, "Phosphorus solubilizing bacterial population dynamics with seasons and their screening in mulberry rhizosphere in different regions of Kashmir" was undertaken to know the influence of seasons on PSB population and the efficiency of potential PSB strains so that they can be put to use in the management of soil fertility.

2. MATERIALS AND METHODS

The study entitled, "Phosphorus solubilizing bacterial population dynamics with seasons and their screening in mulberry rhizosphere in different regions of Kashmir" was conducted at Biofertilizer Research Laboratory, FoA, Wadura, SKUAST-K as per the following experimental details:-

2.1 Experimental details

1. Regions : 03- North (Baramulla and Bandipora), Central (Srinagar and Pulwama north) and South (Anantnag and Kulgam districts)
2. Number of locations : 03 from each region
3. Location names : North (TSRI-Mirgund, P_4 BSF-Manasbal and Sericulture Development Department (SDD) - Bandipora).
   : Central (SDD-Poohu, Central Silk Board-Galander and SDD- Srinagar)
   : South (SDD-Y.K. Pora, SDD-Krungsoo and SDD- Bijbehara)
4. Samples per location : 05 (Composite)
5. Year : 2014-2015
6. Seasons : 04 (Spring, Summer, Autumn, and Winter)
   Spring → 1st fortnight of April
   Summer →1st fortnight of July
   Autumn →1st fortnight of October
   Winter →1st fortnight of January
6. Design of survey : Purposive sampling.

2.2. Soil sampling

Soil samples were collected during spring, summer, autumn and winter seasons from 9 different mulberry farms of Kashmir valley from the soil adhering to the roots of mulberry in sterilized polythene bags with proper labels to prevent moisture loss and as soon as possible were refrigerated at 4ºC to avoid microbial fluctuations. From each location, during each season, five samples were collected to minimize the effect of inherent site variability. Thus a total of 180 samples were collected in all the four seasons and brought to the laboratory for isolation and enumeration (cfu's) of phosphorus solubilizing bacteria using Pikovskaya's agar medium and employing Pour Plate Serial Dilution Technique (Pikovskaya, 1948) under aseptic conditions in a laminar air flow chamber. For isolation,1gram of rhizosphere soil was taken and mixed well with 9 ml of sterile

water to get 10^{-1} dilution. After thorough mixing, one ml of sample from this test tube was pipetted out and transferred to another test tube containing 9 milliliters of sterile water and mixed thoroughly to get 10^{-2} dilution. The procedure was repeated again and again upto 10^{-7} dilution. From the respective dilution, 0.5 ml of microbial suspension was drawn aseptically with the help of 1ml sterilized pipette and spread uniformly on sterile petri dishes containing 15 ml of the respective media. Dilution of 10^{5} was used for isolation of PSB (plate-1). The inoculated plates were then inverted upside down, incubated at 28 ± 2°C in dark and were observed for 1-6 days for phosphorus solubilizing bacteria, showing solubilizing zone around their colonies, then enumerations were made with the help of digital colony counter. Finally, on the basis of visual observation, potential phosphorus solubilizers/strains were selected for further studies.

Plate- 1 isolated PSB colonies

2.3 Screening of phosphorus solubilizing bacteria isolated from mulberry gardens for phosphorus solubilization

On the basis of visual observation, three well performing strains of PSB from every location of each region were screened for the solubilization of sparingly soluble phosphorus taking the total to 27 as per the details furnished in Table -1.

Table 1: Details of 27 phosphorus solubilizing bacteria collected from different locations

No.	Isolate	Taken from	S. No.	Isolate	Taken from	S. No.	Isolate	Taken from
1	R1L1PSB1	North Mirgund)	10	R2L1PSB1	Central Poohu)	19	R3L1PSB1	South (Y.K.pora)
2	R1L1PSB2	North Mirgund)	11	R2L1PSB2	Central (Poohu)	20	R3L1PSB2	South (Y.K.pora)
3	R1L1PSB3	North Mirgund)	12	R2L1PSB3	Central (Poohu)	21	R3L1PSB3	South (Y.K.pora)
4	R1L2PSB1	North Manasbal)	13	R2L2PSB1	Central (Galander)	22	R3L2PSB1	South (Krungsoo)
5	R1L2PSB2	North Manasbal)	14	R2L21PSB2	Central (Galander)	23	R3L21PSB2	South (Krungsoo)
6	R1L2PSB3	North Manasbal)	15	R2L2PSB3	Central (Galander)	24	R3L2PSB3	South (Krungsoo)
7	R1L3PSB1	North Bandipora	16	R2L3PSB1	Central (Srinagar)	25	R3L3PSB1	South (Bijbehara)
8	R1L3PSB2	North Bandipora	17	R2L3PSB2	Central (Srinagar)	26	R3L3PSB2	South (Bijbehara)
9	R1L3PSB3	North Bandipora	18	R2L3PSB3	Central (Srinagar)	27	R3L3PSB3	South (Bijbehara)

Where "R" stands for region, "L" for location, where from three PSB strains were taken.

2.4 Qualitative method (Katznelson, 1959)

In this method, the best selected PSB strains isolated from pour plate serial dilution technique were subjected to screening by spot inoculation of all the individual colonies showing larger zones of clearance around them (Sundara Rao and Sinha, 1963) on Pikovskaya medium containing 5grams of Tricalcium phosphate as the sole source of phosphorus. The plates were then incubated at 28°C for 24 hours.

The halo zone diameter and colony diameter were measured upto 6-7 days after incubation for calculating their efficiency as per Karpagam & Nagalakshmi (2014) as:-

$$PSI = \frac{\text{Colony diameter + Halozone diameter}}{\text{Colony diameter}}$$

or

$$PSI = Z/C$$

Where, "Z" is the Colony diameter + Halo diameter
and
"C" is the Colony diameter.

2.5 Quantitative method (King, 1936)

Hundred milliliters (100 ml) of Pikovskaya's broth medium with 250 mg of TCP was prepared and sterilized, 1ml of each selected isolate was inoculated onto the broth medium. The amount of soluble phosphate was measured by colorimetric method as described by King (1936). Isolates of the phosphate solubilizing microorganisms were inoculated to 100 ml of Pikovskaya's broth in 250 ml flasks. The flasks were incubated on an incubating shaker at 28±2°C for 05 days. The amount of phosphate released in the broth in flasks was estimated after 5 days of inoculation. The broth cultures of bacteria were centrifuged at 10,000 rpm for 20 min. Then the supernatant (1 milliliter) was mixed with 10 milliliters of Chloromolybdic acid (7.5 g of ammonium molybdate in 150 milliliters of distilled water to which 162 milliliters of concentrated HCl was added and final volume was made up to 1liter with distilled water) and the volume was made to 45 ml with distilled water. Further, 0.25ml of chlorostannous acid (25grams of SnCl2.2H2O in 100 milliliters of concentrated HCL and rising to 1liter with distilled water) was added and the volume was made up to 50 milliliters with distilled water. The concentration of phosphate was determined by the absorbance of the color blue at 610 nm.

3. STATISTICAL ANALYSIS

Data recorded during different seasons and from different regions and locations was compiled and analyzed statistically to find differences among the seasons, regions and locations. The experimental data was processed using SAS statistical package licensed to SKUAST-K. All the results are expressed as means of the F values for which $p<0.05$ were considered significant.

4. EXPERIMENTAL FINDINGS

Under the study entitled, "Phosphorus solubilizing bacterial population dynamics with seasons and their screening in mulberry rhizosphere in different regions of Kashmir" the observations recorded are described under the following headings:

4.1 Phosphorus solubilizing bacteria (cfu/g soil × 10^5)

The population of phosphorus solubilizing bacteria was maximum (18.60) during spring season being significantly more than the rest of the seasons ranging from 9.66 in winter to 14.08 in autumn season. Among the regions, central region registered the maximum value (14.11) for phosphorus solubilizing bacteria and was statistically at par with the population (13.91) recorded in northern region but significantly higher than the population (12.65) recorded in southern region (Table 2).

Table 2: Seasonal variation in phosphorus solubilizing bacterial (cfu/g soil × 10^5) population of mulberry soils of Kashmir

Region / Season	North				Central				South				Overall Mean
	Mirgund	Manasbal	Bandipora	Sub Mean	Poohu	Galander	Srinagar	Sub Mean	Y.K.pora	Krungsoo	Bijbehara	Sub Mean	
Spring	19.40	18.40	18.00	18.60	19.00	19.40	18.80	19.06	18.40	18.20	17.80	18.13	18.60
Summer	13.20	12.40	11.80	12.46	12.40	13.60	12.00	12.66	11.00	10.40	10.20	10.53	11.88
Autumn	15.00	14.60	13.20	14.26	14.40	13.80	15.80	14.66	12.80	13.40	13.80	13.33	14.08
Winter	11.20	10.00	9.80	10.33	9.80	10.80	9.60	10.06	9.20	8.20	8.40	8.60	9.66
Mean	14.70	13.85	13.20	13.91	13.90	14.40	14.05	14.11	12.85	12.55	12.55	12.65	

C. D ($p \leq 0.05$)
Seasons : 0.46
Regions : 0.40

4.2. Screening of phosphorus solubilizing bacteria isolated from mulberry gardens for phosphorus solubilization

Qualitative analysis

The efficiency of PSB with regard to phosphorus solubilization varied considerably among the selected isolates. As evident from Table 3, the halo zone diameter was the least (1.1 mm) in isolate 1 of location 1 from southern region (R3L1PSB1) where as it was the highest (4.9 mm) in isolate 2 from location 2 of northern region (R1L2PSB2). Six isolates registered the halo zone diameter less than 2 millimeter, eight more than 2 millimeter where as the rest had halo zone of more than 3millimeter diameter.

Phosphorus solubilization efficiency (PSI) in the isolates from northern region ranged from 2.2 in isolate 2 from location 1 (R1L1PSB2) and also in isolate 2 of location 3 (R1L1PSB2) to 7.1 in isolate 2 from location 2 (R1L2PSB2). Among the isolates from central region the values for PSI were almost in the same range as in the northern region with the lowest (2.1) recorded in isolate 1 from location 3 (R2L3PSB1) to 5.7 in isolate 2 from location 2 (R2L2PSB2). Besides this, the isolate 3 from location 3 (R2L3PSB3) also recorded PSI more than five (5.0). The isolates taken from southern region showed that PSI was the least 2.3 in isolate 1 from location 3 (R3L3PSB1) where as isolate 2 from location 2 (R3L2PSB2) registered the maximum PSI of 5.2.

4.3 Quantitative analysis

The selected PSB isolates when studied for phosphorus solubilization in liquid culture depicted great variation. The potential for solubilization of inorganic phosphorus varied among the isolate as indicated by the increase of quantum of phosphorus in the PVK broth medium. The phosphorus solubilization varied from 11.1 µg/ml to 72.9 µg/ml. The highest value was exhibited by R1L2PSB2 (72.9 µg/ml), followed by R2L3PSB3 (69.0 µg/ml), R2L2PSB2 (68.1 µg/ml) and R1L1PSB1 (58.4 µg/ml) to the least in (11.1 µg/ml) in R1L1PSB3 (Table 4).

Table 3: Comparative efficiency of PSB Isolates collected from different regions (qualitative screening)

Region	Isolates	Halo zone (mm)	Colony diameter (mm)	PSI
Northern	R1LIPSB1	3.6	0.9	5.0
	R1L1PSB2	1.5	1.2	2.2
	R1L1PSB3	2.5	1.2	3.1
	R1L2PSB1	3.0	1.3	3.3
	R1L2PSB2	4.9	0.8	7.1
	R1L1PSB3	2.7	1.9	2.4
	R1L3PSB1	3.1	1.5	3.0
	R1L3PSB2	2.2	1.7	2.2
	R1L3PSB3	2.8	1.4	3.0
Central	R2LIPSB1	1.8	1.3	2.3
	R2L1PSB2	3.6	1.7	3.1
	R2L1PSB3	4.0	1.5	3.6
	R2L2PSB1	3.4	1.4	3.4
	R2L2PSB2	4.7	1.0	5.7
	R2L1PSB3	4.2	1.5	3.8
	R2L3PSB1	2.1	1.8	2.1
	R2L3PSB2	2.8	1.0	3.8
	R2L3PSB3	4.5	1.1	5.1
Southern	R3LIPSB1	1.1	0.5	3.2
	R3L1PSB2	2.9	1.8	2.6
	R3L1PSB3	1.9	1.2	2.5
	R3L2PSB1	2.8	1.6	2.7
	R3L2PSB2	3.8	0.9	5.2
	R3L2PSB3	3.2	1.4	3.2
	R3L3PSB1	1.5	1.1	2.3
	R3L3PSB2	2.5	1.0	3.5
	R3L3PSB3	1.6	0.8	3.0

Table 4: Quantitative screening of PSB (phosphorus solubilizing bacteria)

Region	Isolates	Solubilization (µg/ml)
Northern	R1LIPSB1	58.4
	R1L1PSB2	38.5
	R1L1PSB3	47.1
	R1L2PSB1	37.9
	R1L2PSB2	72.9
	R1L1PSB3	11.1
	R1L3PSB1	34.5
	R1L3PSB2	24.4
	R1L3PSB3	35.3
Central	R2LIPSB1	23.5
	R2L1PSB2	31.6
	R2L1PSB3	27.2
	R2L2PSB1	44.2
	R2L2PSB2	68.1
	R2L1PSB3	39.3
	R2L3PSB1	21.4
	R2L3PSB2	43.1
	R2L3PSB3	69.0
Southern	R3LIPSB1	39.7
	R3L1PSB2	34.5
	R3L1PSB3	11.5
	R3L2PSB1	33.9
	R3L2PSB2	55.9
	R3L2PSB3	13.9
	R3L3PSB1	20.9
	R3L3PSB2	38.2
	R3L3PSB3	28.5

4. DISCUSSION

The results obtained in the study, "Phosphorus solubilizing bacterial population dynamics with seasons and their screening in mulberry rhizosphere in different regions of Kashmir"are discussed below:-

Phosphorus solubilizing bacteria were highest during spring season, being significant over other seasons and the lowest in winter. Among the regions, highest values were recorded in northern region and the lowest values in southern region. Highest population of PSB during spring could be due to the presence of nitrogen in larger quantities which they utilize as nitrite, nitrate or in amino form and in turn greatly influence phosphorus solubilization activity (Habte and Osorio, 2012). The decreased number of phosphorus solubilizing bacteria during summer and winter may be due to less carbon and unfavorable temperature during these two seasons as compared to the other two seasons. Bajpai and Rao (1971) stated that efficient phosphate solubilizers always prefer soils having good carbon content for their survival. However, the population of phosphorus solubilizing bacteria being more in northern and central region than the southern region could be explained due to the highest population of AM fungal population (Kumar, 2002) and also due to alkaline pH prevailing in the regions, as worked out during the investigations on other parameters during the course of study and was also reported by Ravikumar *et al*. (2012), in Mangrove ecosystem.

4.1 Screening of phosphorus solubilizing bacteria

Qualitative assessment showed great variation in the efficiency of PSB with regard to phosphorus solubilization as evident from the diameter of clearing zone, which ranged from 1.1 mm to 4.9 mm. Similar observations with regard to the halozone diameter were reported by Keneni *et al.* (2010) for PSB isolated from Faba bean rhizosphere and Karpagam *et al* (2014) for PSB isolates from Tomato plant rhizosphere. The ability of solubilization depends upon the production of organic acids and presence of Phosphatase enzyme in microbes (Park, 2010). As per Widawati (2011), the ability to solubilize the inorganic phosphate also depends upon the properties of soils which give knowledge of diversified groups of microbes.

Quantitative results obtained on the phosphorus solubilization of the selected isolates revealed that majority of the isolates (81.48%) exhibited medium efficiency; whereas, 18.52 per cent (%) exhibited high efficiency in mulberry soils, being neutral to slightly alkaline in nature. Krishnaveny (2010) while studying the phosphorus solubilization in bacteria from rhizosphere and non rhizosphere soils in different varieties of Foxtail Millet (*Setaria Italica*) got similar results and reported that the efficiency levels varied with the variety and soil type. Islam *et al* (2007) while working on phosphate solubilizing bacteria have also reported that the strains isolated from alkaline soil have rapid growth and the potential to solubilize phosphates at high salt and pH concentration.

5. CONCLUSION

The findings of this study led to the following conclusion:-

- Mulberry soils depict great variations among the seasons in the population of PSB's which differ in relative density as well as in their activities.
- Phosphorus solubilizing bacteria, in general, thrived well during spring and autumn as compared to summer and winter owing to favorable climatic conditions especially moisture, temperature, etc.
- Spring represented the most favourable season for the multiplication of PSB's thereby enhancing the health and activities in soil.
- Population of PSB's being more during spring season and in the northern region among the regions could be due to the presence of nitrogen in larger quantities in the season.

- Efficient PSB's isolated from the gardens of mulberry, could be supplied to the soil during the growing period of foliage (spring) when the mulberry soils are having the availability of nitrogen in larger amounts and in this way help the plant at such a stage when it has a high demand for mineral nutrients.
- Various phosphorus solubilizing bacteria also differed in their halo zone diameter and phosphorus solubilizing efficiency (PSE) qualitatively and also in phosphorus solubilization quantitatively.
- Majority of the isolates exhibited medium efficiency of phosphorous solubilization where as 18.5% exhibited high efficiency.
- The highly efficient ones in both qualitative and quantitative methods (from northern and central region) could be exploited for the healthy growth of mulberry in the valley.

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How to cite this article: Khursheed S. Phosphorus solubilizing bacterial (PSB) population dynamics with seasons and their screening in mulberry rhizosphere in different regions of Kashmir. International Journal of Research and Review. 2018; 5(7):153-160.

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