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Role of PGPR in Rice Fields of Bihar

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Abstract: ***Currently, the rising human race drives massive load on agricultural field for enhancing crop yield and sustainable agro-ecosystem at global level that chiefly turn to extreme utilization of synthetic fertilizers, but indiscriminate use of fertilizers has triggered soil infertility and affect crop productivity. PGPR are the soil-dwelling bacterium that facilitates plant growth and reduce the risk of chemical fertilizers. Weeds are undesirable, non-valuable plant that compete with main crops, reduce their growth and yield and affect soil fertility, yet their rhizospheric zone accommodate countless PGPR (Arthrobacter, Pseudomonas, Burkholderia, Serratia, Bacillus); a source of PGP traits. One of the PGP trait is Phosphate solubilization for which Eclipta alba (Bhringraja), the most common weed in the Paddy field of Aurangabad, Bihar is more relevant. The rhizospheric soil of Eclipta alba provide horizon to PGPR that stimulates growth and yield of rice cultivar and diminish dependency on chemical fertilizers through Phosphate solubilization. An assemblage of beneficial PGPR associated with Eclipta alba rhizosphere secrete organic acids that solubilize insoluble phosphate compound into soluble inorganic phosphorus to increase P-availability in the soil known as PSBs (Phosphate Solubilizing Bacteria). The novel PSBs like Bacillus, Pseudomonas and Serratia showed PS activity due to secretion of PGP substances by them and hence, act as natural biofertilizers. In this study, certain isolates were identified as Bacillus (3), Pseudomonas (5) & Serratia (7). Despite, one Bacillus strain M13; 3 Pseudomonas strains F6, K11 & N14; 2 strains of Serratia P16 and R18 were remarkably potent for PSBs in the rice field of Aurangabad, Bihar which is giving a new dimension in modern agro-ecosystem and biofertilizer. These PSBs could be used as an efficient alternative approach to chemical fertilizers to enhance soil fertility and promote growth and yield of rice cultivar.***

Keywords: PGPR, Weeds, PSBs

1. Introduction

In present scenario, dynamic nature of the soil and agro-ecosystem mainly rely upon excessive and steady applications of chemical fertilizers to check the growth of weeds, pests, improve soil nutrients and stimulate plant growth and yield. Injudicious use of synthetic fertilizers creates the problem in soil health and affects crop productivity. Weeds are un required and uninvited plat that grows wildly with desired crop plant to compete main crops for nutrient, space, moisture, light and reduce growth and yield of main crop. Apart from being unwanted plant in the crop field the roots of weeds provide horizon of growth to the soil micro organism; Rhizoacteria a source of PGP factors. PGPR (Plant Growth Promoting Rhizobacteria) are the soil dweling bacteria in the rhizospheric region close to plant root and are involved in the beneficial effects on plant growth. *Eclipta alba* commonly known as "Bhringraja" is very common weed found in abundance in the rice fields of Aurangabad district, Bihar. Weed rhizospheric soil of Ecliptaalba plant is associated with many potential rhizobacteria that promotes the growth of rice cultivar and stimulate crop productivity. Some of the important PGPR of rice field reported (*Pseudomonas*; **Looper et al**., 2007, *Burkholderia & Serratia*; **De Vleesschauwer** and **Hofte**, 2003, *Arthobacter*; **Sarathmbal et al**., 2014, and Azospirillum; **Huang et al**., 2004) are *Burkholderia*, *Pseudomonas*, *Serratia*, *Arthobacter*, Azospirillum remain present in the rhizospheric soil of *Eclipta alba* found in rice field. PGPR are beneficial bio-inoculants which are considered as novel and potential implement for providing substantial benefits to improve plant growth and yield of rice cultivar in nutrient deficient agro-ecosystem by application of biological inputs and minimize the risk of excessive chemical fertilizers. PGPR traits such as Nitrogen fixation, Phosphate solubilization, Siderophore production, Phytohormones Synthesis. Impact of PGP traits of Ecliptaalba Rhizobacteria reduces the demand for chemical fertilizers and promotes the growth of rice cultivar. One of te PGP traits is Phosphate Solubilization for which Eclipta alba is most efficient source. A large group of beneficial PGPR is associated with rhizosphere of Eclipta alba that are capable to solubilize insoluble phosphate compound in to solubilizing Bacteria (PSBs). The novel PSB like Serratia, Bacillus, Pseudomonas have exhibited Phosphate Solubilizing Activity by secreting organic acid such as Gluconic acid (Goldstein, 1995) & Ketogluconic acids (Deubel et al.2000) in the soil that convert insoluble phosphate to available 'P' form (Orthophosphate ions) without disturbing the biochemical composition of soil. The soluble ions form can easily be assimilated by rice cultivar. PSBs act as biofertilizer and used as reasonable alternative to chemical fertilizers due to PGP substances secreted by them (table 1.0).

In this context, the objectives of my present investigation are: To isolate and characterize PSB isolates from weed rhizospheric soil of Eclipta alba plant and assess their PGP traits in order to enhance fertilization efficiency.

Table 1: Phosphate Solubilizing Bacteria with PGP substances

Sl. No.	PhosphateSolubilizing bacteria	PGPsubstances	References
1	*Burkholderia*	Accdeaminase, IAA, Siderophore	Jianget. al (2008)
2	*Bacillus*	IAA, Siderophore	Banerjeeet. al (2010)
3	*Klebsiella*	Ammonia, IAA, Siderophore	AhmadandKhan (2011)
4	*Enterobacter*	IAA, Siderophore, HCN	Deepaet. al (2010)
5	*Pseudomonas*	ACCdeaminase, IAA, Siderophore, antifungal	Ganeshan (2008)
6	*Serratia*	IAA, Siderophore, HCN	Dastageret. al (2011)

2. Materials and Methods

1) Collection of Rhizospheric Soil Sample:
Seven soil samples were collected from rhizopheric region of *Eclipta alba* plant grown in rice field of different plots of Aurangabad district, Bihar, India. Each rhizosphere soil were collected in aseptic polythene bags and immediately stored in laboratory condition at 4^0c for further studies.

2) Isolation and Purification of Bacterial isolates from Weed Rhizospheric Soil:

a) Isolation and purification of isolates were done by serial dilution and spread plating method (**PVK** agar media was used)

b) Counting of Bacterial colony:
After 72-96 hours incubation, a large number of Bacterial colonies with clear halo zone were observed on the culture plates. Counting was done with the help of a colony counter (**Labtronics Colony Counter; LT - 37**, PanchKula, Haryana India). Number of colonies present in the media plates was used to determine the number of cells present in the dilutions.
Purification of single PSB isolates by Streak plating method:

c) The most prominent bacterial isolates were purified by Streaking on **Pikovaskaya Agar media** and incubated in inverted form for 5-7 days at 28+_2°c. The pure bacterial strains were appeared as transparent halo zone indicating phosphate solubilizing ability. The pure PS Bisolate were maintained (as glycerol stocks at-80^0c).

Identification of Bacterial Isolates:

After 5 days of incubation, purified phosphate solubilizing isolates were identified by morphological and biochemical characterization through phenotypictests like Gram-Staining, catalase test, citrate utilization test, MRVP test according to Bergey's Manual of determinative Bacteriology.

A. Morphological Characterization:
Morphological identification of isolates based on the colony morphology and microscopical cellular morphology.

(a) Colony Morphology Characteristics:
The colony morphology of PSB isolates were identified on the basis of shape, size, colour, margin, elevation, opacity (surface), and pigmentation. Colony morphology were examined under light microscope.

(b) Cellular Microscopical Morphology Characteristics:
Microscopical morphology of isolates was done by Gram - staining method (**Gerhardt et. al.1**994.

B. Bio - chemical Characterization:
Selected isolates of phosphate solubilizing bacteria were biochemically characterized by different tests such as Catalase test, citrate utilization test, voguspraskeur (VP) test and Methyl red (MR) test which were performed according to Bergey's manual systematic Bacteriology (**Whitman etal**., 2015)

a) Catalase test:
The catalase test was carried out by taking overnight grown bacterial colony from NAM plates at 28 degree c on a clear glass slide.1-2 drops of 30% Hydrogen peroxide (H2O2) was added to old bacterial colony on a clear glass slide and mixed well by using a sterile tooth-pick to observe the evolution of oxygen gas.

b) Citrate Utilization test:
This test was performed on agar media (**SIMMONS AGAR MEDIA**). Picked a loopfull of bacterial culture and streaked inside the slant of SCA media back and forth and was incubated at 37 c for 24-48 hours to observe colour change along the slant indicating citrate utilization.

c) VP (Vogeus Prausker) test:
Take overnight grown isolates on a clean glass slide and inoculate it with MRVP broth. Incubate it at 35-37c for 24-48 hours.6 drops of VP reagent I (Alpha-naphthaol) and 2 drops of VP reagent II (40% KOH) was added to observe the colour change in broth medium.

d) MR (Methyl Red) test:
18-24 hour old pure bacterial culture was taken in a clean test tube having 1ml aliquot of MRVP broth (pH6.9).2-3 drops of 0.02% MR indicator was added to aliquot and colour change was observed.

C. Plant Growth Promoting Activity Shown by Bacterial Isolates:
Phosphate Solubilization Activity:
This activity was shown by bacterial isolates. The ability of bacterial isolates to solubilize inorganic phosphate was tested by streaking a loop full of fresh over night grown bacterial isolates on the centre of agar plates inoculated with **Pikovaskaya Agar media** and dyed with **Bromophenol blue** indicator and incubated it for 5-10days at 37°c to observe transparent halozone (Solubilization zone) around the bacterial colony. The diameter of halozone surrounding the developed bacterial colony was measured by PSI (Phosphate Solubilization Index).

PSI= A\B X100

where, A=Total diameter of Bacterial colony and halozone diameter. B= Diameter of Bacterial colony.
This test was done intriplicate.

3. Result & Discussions

In the Present consideration, about 6rhizobacterialisolates were isolated from weed rhizospheric soil sample of *Eclipta alba* plant grown in rice field with maximum Phosphate solubilizing activity. A total of 18 bacterial isolates from 7 soil sample were cultured in PVK agar media and then morphological and Biochemical tests were tested. Growth of isolates was observed as transparent halozone around colony on PVK plates exhibited PS activity of isolates. Out of 18 isolates, 15 isolates; A1, B2, C3, D4, F6, G7, H8, I9, J10, K11, M13, N14, O15, P16, R18 showed ability for P-solubilization on PVK media with different efficacy. Based on Bergey's Manual of Systematic Bacteriology, isolates were tentatively identified as Pseudomonas (5), Serratia (7) and Bacillus (3) sps. Out of 5, 3 of Pseudomonas sps; F6, K11, N14, 2 of Serratia; P16, R18 and 1 of Bacillus; M13 were screened as good PSBs and can be applied as effective biofertilizers to improve and maintain soil fertility.

Isolation & screening of PSBs:

PSBs isolation and selection were done in PVK agar medium by Serial dilutions and Spread plating method. The 18 strains of PSBs were selected according to morphological variation in colony formation and halozone formation by isolates.

18 bacterial isolates were successfully isolated grown in rice field from different areas of Aurangabad District in Bihar, India (Table2.0). They were isolated from 3 different plots i. e Plot 1 (Gaini, Daudnagar, Field 1 & Field 2), plot 2 (Gaini, Obra, Metallic road, Field 1 & Field 2) & Plot 3 (Gaini, Panchayat Bhawan, Near PakkaIndraF1, F2 & F3)

Soil sample 1 & Sample 2 were isolated from Plot 1 total 8 bacterial isolates were identified out of 8, 5 strains were isolated from field 1 & designated as P1F1EA1, P1F1EA2, P1F1EA3, P1F1EA4, P1F1EA5 and 3 stains were isolated from field 2 of Plot 1 Gaini, Daudnagar, designated as P1F2EA6, P1F2EA7 & P1F2EA8. The total number. of isolates from plot 2 Gaini metallic road were 3; One isolates from soil no.9, Sample 3 Field 1 denoted as P2F1EA9 and the rest two strains were isolated from sample 4, field 2 expressed as P2F2EA10 & P2F2EA11. Rest 7 bacterial isolates were successfully isolated from 3 different fields of Plot 3 (Gaini, Near PakkaIndra). Two isolates, P3F1EA12 & P3F1EA13 were isolated from sample 5 of field 1, Plot 3; 4 Strains P3F1EA14, P3F1EA15, P3F1EA16 and P3F1EA17 were represented as sample 6 of field 2, Plot 3 and single isolates P3F1EA18 is considered as sample 7 of field 3, Plot 3. (**Table 2.0)**

Table 2: Sample Survey for isolation of different Isolates

Sl. No.	Number of Sample	Location of Sample collection	Number of Isolates	Code of Isolates
1	1	Gaini, Daudnagar; Aurangabad, Bihar Plot 1, Field 1	5	P1F1EA1, P1F1EA2, P1F1EA3, P1F1EA4, P1F1EA5,
2	2	Gaini, Daudnagar; Aurangabad, Bihar Plot 1, Field 2	3	P1F2EA6, P1F2EA7, P1F2EA8,
3	3	Gaini, Near Metallic road, Field 1, Plot 2	1	P2F1EA9
4	4	Gaini, Near Metallic road, Field 2, Plot 2	2	P2F2EA10, P2F2EA11
5	5	Gaini, Near PakkaIndra, Plot 3, Field 2	2	P3F1EA12, P3F1EA13,
6	6	Gaini, Near PakkaIndra, Plot 3, Field 2	4	P3F2EA14, P3F2EA15, P3F2EA16, P3F2EA17
7	7	Field 3, Plot 3, Gaini, Near PakkaIndra	1	P3F3EA18
	Total Sample - 7		Total Isolates-18	

Morphological Characterization of Rhizobacterial Isolates:

For the Prior morphological identification of novel PGPR form used rhizospheric soil sample of *Eclipta alba*, 18 bacterial colonies were isolated and characterized for their colonial morphological properties like shape, size, opacity, margin, Elevation and pigmentation and also characterized for cellular morphology like Gram's staining [Table 3.0]

Table 3: Morphological Characterization of bacterial Isolates

S. No.	Sample No.	No. of Isolates	Isolates Code: Code Name	Colony Morphology					
				Shape	**Size**	**Opacity**	**Margin**	**Elevation**	**Colour**
1	**1**	**5**	P1F1EA1: **A1**	Circular	Small	Opaque	Entire	Convex	Red
2			P1F1EA2: **B2**	Rod	Small	Opaque	Entire	Slightly	White
3			P1F1EA3: **C3**	Rod	Small	Opaque	Entire	Raised	Red
4			P1F1EA4: **D4**	Rod	Small	Translucent	Entire	Slightly	Light Green
5			P1F1EA5: **E5**	Circular	Large	Opaque	Entire	Raised	Yellow
								Slightly Raised Flattened	
6	**2**	**3**	P1F2EA6: **F6**	Circular	Large	Translucent	Irregular	Slightly	Yellow
7			P1F2EA7: **G7**	Rod	Small	Opaque	Entire	Raised	Red
8			P1F2EA8: **H8**	Rod	Small	Opaque	Irregular	Raised	White
								Raised	
9	**3**	**1**	P2F1EA9: **I9**	Circular	Small	Opaque	Entire	Slightly Raised	Red
10	**4**	**2**	P2F2EA10**: J10**	Rod	Small	Opaque	Entire	Flattened	Red
11			P2F2EA11**: K11**	Rod	Small	Opaque	Entire	Flattened	Light Green

12			P3F1EA12**: L12**	Circular	Small	Opaque	Entire	Flattened	Nil
13	**5**	**2**	P3F1EA13**: M13**	Rod	Small	Opaque	Irregular	Raised	Off White
14			P3F1EA14**: N14**	Rod	Large	Opaque	Entire	Raised	Yellow
15	**6**	**4**	P3F1EA15**: O15**	Circular	Small	Opaque	Irregular	Flattened Raised	Yellow
16			P3F1EA16**: P16**	Circular	Small	Opaque	Entire	Slightly	Red
17			P3F1EA17**: Q17**	Irregular	Small	Opaque	Entire	Raised	Nil
18	**7**	**1**	P3F1EA18**: R18**	Rod	Small	Opaque	Entire	Convex	Red

Biochemical Characterization of Rhizobacterial Isolates:
Out of 18, 15 PSB isolates were further analysed and characterized via cellular Morphological (Gram's Stain Technique) test and different biochemical test such as catalase test, Citrate utilization test, MRVP test which were performed on the basis of Bergey's Manual Systematic (Whitman et al., 2015) (Table 4.0)

a) Catalase Activity Test:
18-24 hour old PSB strains were taken from NAM plates on clear glass slide.1-2 drops of 30% Hydrogen peroxide was added on the slide and mixed well to observe evolution of O2 gas in the bubble form on the slide. All the strains showed +ve catalase activity which were observed by formation of effervescence on the glass slide after addition of H2O2.

b) Citrate Utilization Test:
Picked a loopful of pure PSB isolates from the centre of well isolated bacterial colony, Streaked inside the slant of SCA media back & forth to observe a colour change along the slant. Total 15 isolates show citrate utilization as a carbon source & growth of isolates along the slant were observed as change in colour from green to intense blue along the slant.

c) MR test:-
18-24 hr old pure bacterial isolates had taken aliquot to observe colour change (Red Colony) immediately. All 15 isolates exhibited no any colour changeWhich showed negative MR test.

d) VP test:-
Overnight grown bacterial culture were taken & inoculatedit with MRVP broth.6 drops of VP regent I (alpha - naphthol) & 2 drops of VP reagent II (40 % KOH) was added to observe color change (Crimson to Ruby Pink Color) in the broth medium. Out of 15, 10 isolates of strain A_1, B_2, C_3, G_7, H_8, I_9, J_{10}, M_{13}, P_{16}, R_{18}showed +ve VP test & 5 isolates coded as D_4, F_6, K_{11}, N_{14}, O_{15} showed no color change which indicates -ve VP test.

Table 4: Biochemical Characterization of diff. PSB isotes

Sl. No	Isolates Name	Code of Isolates	Cellular Morphology	Biochemical Traits			
			Gram's Staining	Catalase test	Citrate Utilization test	VP test	MR test
1	*Serratiasps.*	A_1	-Ve	+	+	+	-
2	*Bacillus sps*	B_2	+Ve	+	+	+	-
3	*Serratiasps.*	C_3	-Ve	+	+	+	-
4	*Pseudomonas sps.*	D_4	-Ve	+	+	-	-
5	*Pseudomonassps.*	F_6	-Ve	+	+	-	-
6	*Serratiasps.*	G_7	-Ve	+	+	+	-
7	*Bacillus sps.*	H_8	+ Ve	+	+	+	-
8	*Serratiasps.*	I_9	-Ve	+	+	+	-
9	*Serratiasps.*	J_{10}	-Ve	+	+	+	-
10	*Pseudomonas sps.*	K_{11}	-Ve	+	+	-	-
11	*Bacillus sps.*	M_{13}	+ Ve	+	+	+	-
12	*Pseudomonas sps.*	N_{14}	-Ve	+	+	-	-
13	*Pseudomonas sps.*	O_{15}	-Ve	+	+	-	-
14	*Serratiasps.*	P_{16}	-Ve	+	+	+	-
15	*Serratiasps.*	R_{18}	-Ve	+	+	+	-

+Ve: Gram Positive +: Positive Activity
-Ve: Gram Neagative-: No Activity

Bacterial Isolated Screened for PGP traits:
The 18 bacterial strains were screened for phosphate solubilization activity (PSI activity) on **Pikovsakya Agar media** in triplicates. Out of which, 7 isolates of *Serratia* coded as A1, C3, G7, I9, J10, P16 & R18 exhibited intense transparent halo Zone (Phosphate Solubilization Zone or (++) ranging from diameter 9 mm to 15 mm. Three isolates of *Bacillus* B2, H8 & M13 represent blurrhalozone (+) of range 1mm to 5 mm. About 5 isolates of *Pseudomonas*D4, F6, K11, N14 & O15 offer Peak Phosphate Solubilization zone (+++) i.e. greater than 15 mm (table 5.0). Isolates were further characterized for evaluation of various PGP traits. The maximum PSI activity of *Pseudomonas* were exhibited by 3 isolates coded as F6 (16.2), K11 (18.5) & N14 (19.5); 2 isolate code P16 (14.5) & R18 (15.0) show maximum PS activity & a single isolate M13 (4.9) of *Bacillus* exhibit high PS activity.

Table 5: Screening of Bacterial Isolates for Phosphates Solubilization

Bacterial Isolates	Code For Isolates	Name of Isolates	Phosphate Solubilization Halo Zone formation	PSI (Diameter of Halozone) In mm
P1F1EA1	A1	*Serratia*	++	9.1
P1F1EA2	B2	*Bacillus*	+	1.5
P1F1EA3	C3	*Serratia*	++	12.5
P1F1EA4	D4	*Pseudomonas*	+++	15.5
P1F1EA5	E5	*Pseudomonas*	-	-
P1F1EA6	F6	*Pseudomonas*	+++	16.2
P1F1EA7	G7	*Serratia*	++	13
P1F1EA8	H8	*Bacillus*	+	3.0
P2F1EA9	I9	*Serratia*	++	10.2
P2F1EA10	J10	*Serratia*	++	11.5
P3F1EA11	K11	*Pseudomonas*	+++	18.5
P3F1EA12	L12	*Pseudomonas*	-	-
P3F1EA13	M13	*Bacillus*	+	4.9
P3F2EA14	N14	*Pseudomonas*	+++	19.5
P3F2EA15	O15	*Pseudomonas*	+++	16.0
P3F2EA16	P16	*Serratia*	++	14.5
P3F2EA17	Q17	*Pseudomonas*	-	-
P3F2EA18	R18	*Serratia*	++	15.0

Phosphate Solubilizing Activity:
+++: Peak Clear halozone
++: intense Halozone
+: BlurrHalozone
- : No Halo zone

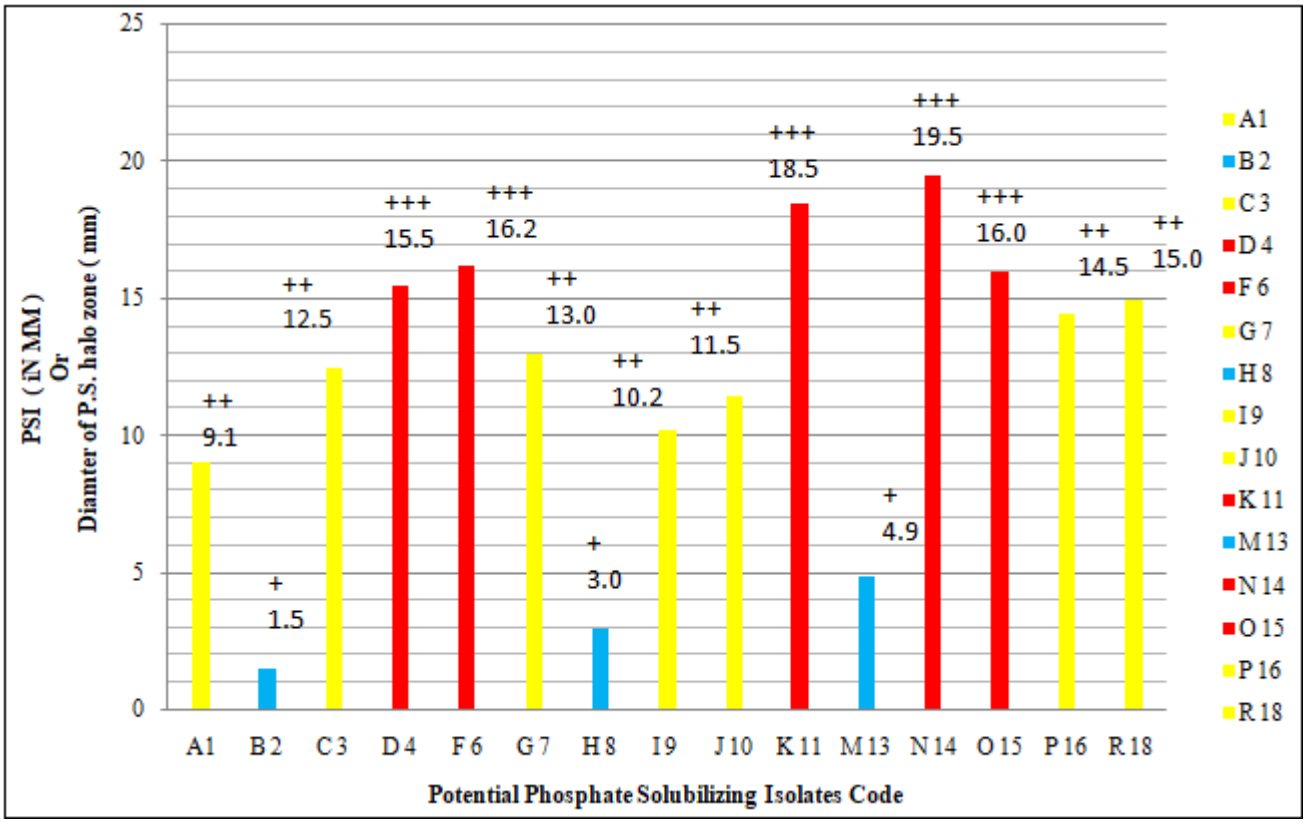

Figure 5.1: Comparison of PS Halozone formation by Potential Isolates

+++-: Peak Halozone; PSI: 15 mm to 20 mm
++-: Intense halozone; PSI: 9 mm to 15 mm
+-: Blurrhalozone; PSI: 1 mm to 5 mm

The results showed that 83.3 % of the isolates presented Phosphate solubilizing activity based on the clear halozone formation around colonies. out of which 33 % of the isolates have maximum Phosphate Solubilization activity (+++) as they form peak clear halozone of diameter 15 mm to 20 mm around the bacterial colony; 46 % of the isolates show moderate Phosphate solubilzation activity (++) with acute transparent halozone of range 9 mm to 15 mm; 20 % of isolates have low phosphate solubilization activity (+) as they form hazy halozne of range 1 mm - 5 mm diameter. (Fig: 5.1, 5.2)

Based on morphological and biochemical traits of PSB, 3 genera were tentatively identified: *Pseudomonas*, *Serratia*, *Bacillus*. Due to Bergey's manual of systematic Bacteriology, only 5 isolates with code D_4, F_6, K_{11}, N_{14}, 0_{15} with high PSI value were identified as *Pseudomonas; 7* Isolates with code A_1, C_3, G_7, I_9, J_{10}, P_{16} & R_{18} were identified as *Serratia*and 3 isolates with code B_2, H_8 and M_{13} were *Bacillus*

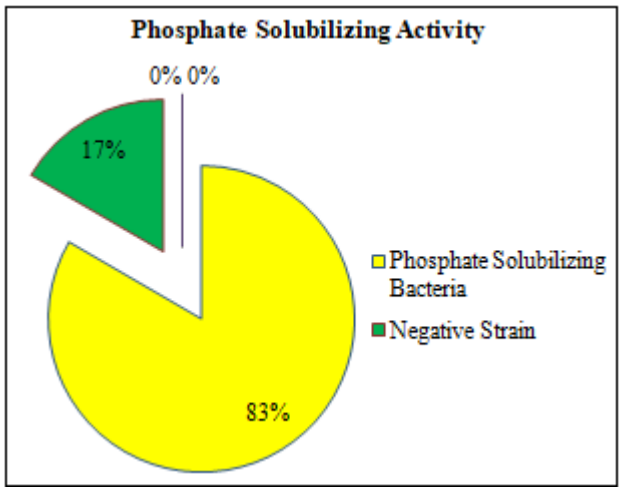

Figure 5.1: Result showing Positive PSB Strain & Negative Strain

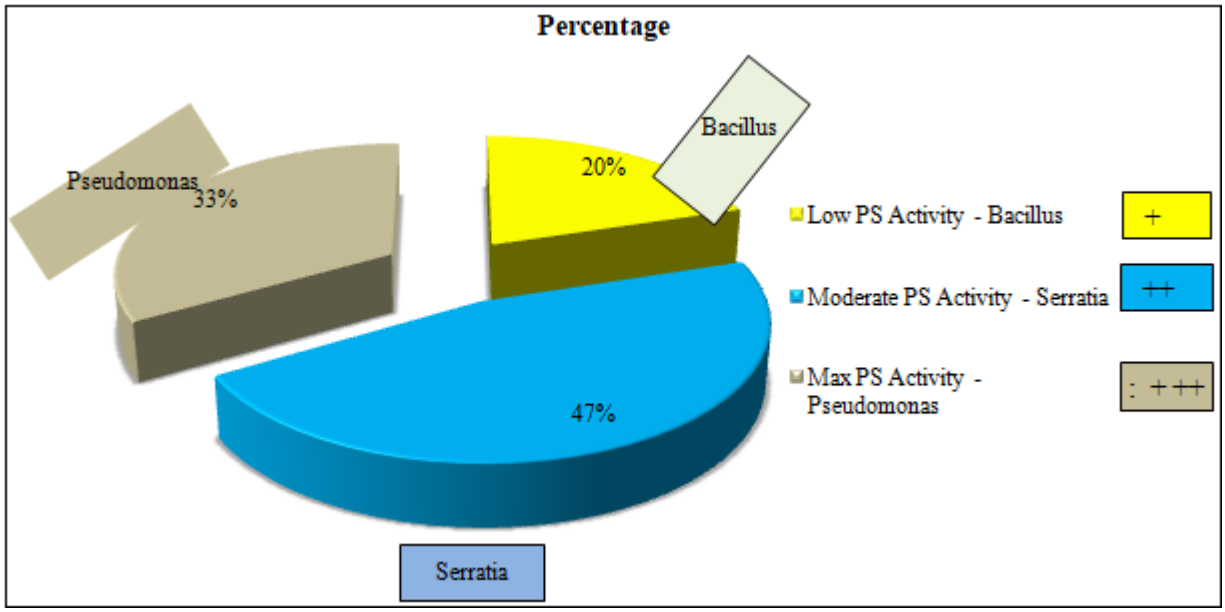

Figure 5.2: Result Showing percentage of PS activity by Bacterial Strain

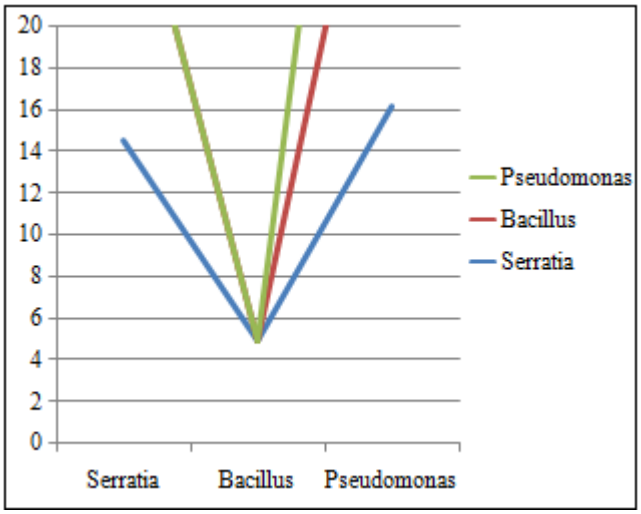

Table 5.3 Maximum PS potential of Isolates

The maximum PS activity of *Pseudomonas* isolate was exerted by 3 strain F_6, (16.2mm), K_{11} (18.5mm) N_{14} (19.5 mm). The maximum PS activity of *Serratia* was exterted by 2 strain P_{16} (14.5 mm) and R18 (15.0 mm) while the maximum PS activity of *Bacillus* were shown by single isolate M_{13} (4.9mm) (Table 5.3). The Phosphate Solubilizing activity Characterises the rhizobacterial strain to release orange acids such as gluconic acid and Ketogluconic acid that convert insoluble phosphate into soluble forms (**Pikovskaya et. al., 1948**) that could be used as alternative to chemical fertilizer that helps to increase soil fertility and growth of rice plants.

4. Conclusion

The modern agro - ecosystem used synthetic fertilizer degrade the fertility of soil making it unfit for stimulating crop plant. Development of novel PGPR from rhizospheric soil of *Eclipta alba* weed plant can be used as sustainable, low impact tool and eco-friendly way. The rhizospheric part of *E. alba* plant havingbenificalPSB such as *Pseudomonas*, *Serratia*, *Bacillus* that stimulate plant growth and development of plants directly through Phosphate Solubilation, It can be concluded from the above results and discussion that out ot 18 PGPR isolates from *E. alba*rhizosphereF_6, K_{11}, N_{14} of *Pseudomonas*; P16 and R18 strain of *Serratia* and M13 strain of *Bacillus* isolates are most potent strains in our research finding that endeavour mainly Phosphate Solubilization as they were produce transparent halozone around the colony and also produce different organic acid like gluconic acid and Ketogluconic acid which solubilize insoluble phosphate into soluble form and may be used in rice field as a biofertilization or an efficient alternative approach to chemical fertilizer.

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