Eco. Env. & Cons. 20 (3) : 2014; pp. (959-966)
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ISSN 0971–765X

Isolation, screening and characterization of *Azotobacter* from rhizospheric soils for different Plant Growth Promotion (PGP) & antagonistic activities and compatibility with agrochemicals: an *in vitro* study

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(Received : 21 January, 2014; accepted 22 February, 2014)

ABSTRACT

Plant Growth Promoting Rhizobacteria (PGPR) are known to influence plant growth by various direct or indirect mechanisms. In search of efficient PGPR strains with multiple activities, a total of twenty four (24) *Azotobacter* isolates of plant growth promoting rhizobacteria from rhizospheric soils of Rajendranagar were isolated and identified based on their morphological, physiological and biochemical characteristics using standard methods. These test isolates were screened *in vitro* for PGPR properties like phosphate solubilization, siderophore, IAA, HCN productions, antagonistic activity against *Rhizoctonia solani, Sclerotium rolfs*ii and compatibility with commonly used pesticide molecules. The results revealed that 91.66% *Azotobacter* isolates showed positive for ammonia production, 58.3% for phosphate solubilization, 83.33% for siderophores, 54.16% for HCN and 29.16% for IAA productions. Out of 24 *Azotobacter* isolates 3 isolates CBuAB1, CBpAB2, SBpAB1 showed inhibition potential against both *Rhizoctonia solani* and *Sclerotium rolfsii.* The maximum per cent inhibition against *Rhizoctonia solani* was showed by CBuAB1, CBpAB2, CRpAB1 and ABpAB1 with 36.05%. The maximum per cent inhibition against *Sclerotium rolfsii* was showed by SBpAB2 (38.25%). The isolate that showed maximum inhibition potential against *Rhizoctonia solani* was also inhibitory to *Sclerotium rolfsii* to a lesser extent based on per cent inhibition and vice versa. Hence it can be inferred that the *Azotobacter* isolates CBuAB2, CRuAB2, CRpAB1, CRpAB2, SBuAB2, SBpAB2, SRuAB1, ARuAB1 and ARpAB1 could be considered for their bio control activity. Among the pesticides tested Azoxystrobin (fungicide), Imidacloprid (insecticide) were found to inhibit *Azotobacter* at recommended / half recommended dosage. However other fungicides, insecticides and all herbicides were compatible with all the isolates tested. Out of the 24 isolates tested for their compatibility with the four each of the fungicides, insecticides, herbicides based on their PGPR attributes and antagonistic activity, the isolate of *Azotobacter* isolate CBuAB1 showed potential as PGPR.

Key words : *Azotobacter, Biochemical characterization, PGPR tests, Antagonistic activity and compatibility with pesticide molecules.*

Introduction

Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. The rhizosphere concept was first intro-

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duced by Hiltner to describe the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities (Hiltner, 1904).

The original concept has now been extended to include the soil surrounding a root in which physical, chemical and biological properties have been changed by root growth and activity (McCully, 2005). A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates (Lynch, 1990) creating a very selective environment where diversity is low (García, 2001; Marilley, Aragno, 1999;). Since bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization (Antoun, Kloepper, 2001; Barriuso, 2008).

The rhizospheric soil contains diverse types of PGPR communities, which exhibit beneficial effects on crop productivity. Several research investigations are conducted on the understanding of the diversity, dynamics and importance of soil PGPR communities and their beneficial and cooperative roles in agricultural productivity. Some common examples of PGPR genera exhibiting plant growth promoting activity are: *Pseudomonas, Azospirillum, Azotobacter, Bacillus, Burkholdaria, Enterobacter, Rhizobium, Erwinia, Mycobacterium, Mesorhizobium, Flavobacterium,* etc.

The growth promoting effect of PGPR includes N_2 fixation, solubilization of insoluble phosphorus (Khan, *et al.* 2009), sequestering of iron by production of siderophores (Soltani, *et al.* 2012), production of phytohormones such as auxins, cytokinins, gibberellins and lowering of ethylene concentration. On the contrary, indirect mechanisms of plant growth promotion by PGPR includes antibiotic production, depletion of iron from the rhizosphere, synthesis of antifungal metabolites, production of fungal cell wall lysing enzymes, competition for sites on roots and induced systemic resistance.

The use of PGPR offers an attractive way to replace chemical fertilizer, pesticides, and supplements; most of the isolates result in a significant increase in plant height, root length, and dry matter production of shoot and root of plants. PGPR help in the disease control in plants. Some PGPR especially if they are inoculated on the seed before planting, are able to establish themselves on the crop roots. PGPR as a component in integrated management systems in which reduced rates of agrochemicals and cultural control practices are used as biocontrol agents. Such an integrated system could be used for transplanted vegetables to produce more vigorous transplants that would be tolerant to nematodes and other diseases for at least a few weeks after transplanting to the field (Kloepper, 2004).

Material and Methods

Isolation of Rhizobacteria

The rhizospheric (10) / non rhizosheric (2) soil samples were collected from Rajendranagar of Rangareddy district, Andhra Pradesh, India. All the *Azotobacter* isolates were isolated on Azotobacter agar medium and they were maintained in Azotobacter agar slants.

Identification of Bacterial Isolates

Morphological Characterization of rhizobacteria

All the 24 *Azotobacter* isolates were verified for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950).

Biochemical and Physiological Characterization of rhizobacteria

Selected isolates of *Azotobacter* (24) were biochemically characterized by IMViC tests, carbohydrate fermentation, oxidase test, Catalase test, H_2S production, Denitrification test, starch hydrolysis and gelatin liquefaction test as per the standard methods (Cappuccino and Sherman, 1992).

Phosphate Solubilization

Sterilized Pikovskaya's agar was poured as a thin layer on to the sterilized petri plates and incubated for 24h, after solidification. After incubation the pikovskaya's plates were spot inoculated with 24 isolates of *Azotobacter spp.* incubated at 28±2^{0}C for 4-5 days. Formations of a clear zone around the colonies were considered as positive result for phosphate solubilisation according to Pikovskaya, R.E. (1948). It was calculated by following formula

PSE (Phosphate Solubilization Efficiency) = Z/C x 100

Z- Clearance zone including bacterial growth

C- Colony diameter

Ammonia production

The isolates were tested for ammonia production by inoculating the isolates in to 10 mL of pre-sterilized peptone water in the test tubes. The tubes were incubated for 48-72h at 36±2°C. Nessler's reagent (0.5 ml) was added in each tube. Change in colour of the medium from brown to yellow colour was taken as positive test for ammonia production.

Indole Acetic Acid Production

Indole acetic acid production was tested according to Gorden and Weber (1951). The active culture of each test isolate was raised in 5 mL respective broth tubes and incubated at determined temperature and time. After incubation these cultures were centrifuged at recommended rpm and time. Two drops of O- phosphoric acid was added to 2 mL of supernatant to develop the colour. Development of pink colour considered as positive for IAA production.

Siderophore Production

Siderophore production was estimated qualitatively. 0.5% of cell free culture supernatant was added to 0.5 mL of 0.2% aqueous Ferric chloride solution. Appearance of orange or reddish brown colour indicated the presence of siderophore (Yeole and Dube, 2000).

Hydrogen Cyanide Production

The HCN production was tested by the method of Castric and Castric (1983). First Azotobacter agar *(Azotobacter)* media plates were prepared and incubated for 24h. After that, 1 mL of culture of each test isolate was inoculated on media plates. A disc of whatman filter paper No.1 of the diameter equal to the petri plate size, impregnated with alkaline picric acid solution (0.5% picric acid (w/v) in 1% sodium carbonate) was placed in the upper lid of the inoculated petri plates under asceptic condition. The control plate did not receive the inoculum. The plates were incubated-upside up at 28±2°C for 48-72h. Change in color from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production.

Antagonistic activity

Azotobacter isolates were screened for antagonistic activity against common disease causing phytopathogens like *Sclerotium rolfsii* and *Rhizoctonia solani* following dual culture technique (Skidmore and Dickinson, 1976). The percent inhibition of the test pathogen will be calculated using the following formula.

$$\% I = \frac{CD - TD}{CD} \times 100$$

Where,

I = % Inhibition of test pathogen.

CD = Diameter of test pathogen colony in control (mm)

TD = Diameter of test pathogen in treatment (mm)

Compatibility of efficient *Azotobacter spp.* with agrochemicals

The pure isolates having PGPR properties were estimated for compatibility with agrochemicals. Based on the dual culture studies effective rhizobacterial antagonist were identified and tested for its compatibility with commonly used fungicides, insecticides and herbicides using inhibition zone technique (Nene and Thapliyal 1993). Zone of inhibition of biocontrol agent were measured.

1) Fungicides: Tebuconazole, Azoxystribin, Carbendazim and Captan.
2) Insecticides: Spiromecifin, Thiacloprid, Imidacloprid and Flubendiamide.
3) Herbicides: Metribuzin, Propaquizafop, Pretilachlor and Pendimethalin.

Results and Discussion

Twenty four *Azotobacter* isolates were isolated from the total of twelve soil samples from different sites of Rajendranagar. All the *Azotobacter* isolates were designated as shown in (Table 1). A total of 24 *Azotobacter* isolates were identified and characterized based on gram reaction, biochemical, morphological and cultural characteristics. All isolates showed significant PGPR activity.

All the 24 isolates took 3 days to show small-medium, milky white, round, raised/flat colonies and formed non-spreading type of colonies with light brown pigmentation. These isolates were Gram negative, rod shaped with no sporulation.

All the 24 isolates of *Azotobacter* showed negative results for Voges Prausker's test, gelatin liquefaction. For indole production, starch hydrolysis, H_2S test, methyl red, citrate utilization, oxidase test, catalase test, denitrification and ammonia production

Table 1. Description of the *Azotobacter* isolates.

Sl. No.	Sample location	Type of soils	No. of isolates	Isolate codes
1	College farm	Black soil un polluted	2	CBuAB1,CBuAB2
2	College farm	Black soil polluted	2	CBpAB1,CBpAB2
3	College farm	Red soil un polluted	2	CRuAB1,CRuAB2
4	College farm	Red soil polluted	2	CRpAB1,CRpAB2
5	Agricultural Research Institute (ARI)	Black soil un polluted	2	ABuAB1,ABuAB2
6	Agricultural Research Institute (ARI)	Black soil polluted	2	ABpAB1,ABpAB2
7	Agricultural Research Institute (ARI)	Red soil un polluted	2	ARuAB1,ARuAB2
8	Agricultural Research Institute (ARI)	Red soil polluted	2	ARpAB1,ARpAB2
9	Student farm	Black soil un polluted	2	SBuAB1,SBuAB2
10	Student farm	Black soil polluted	2	SBpAB1,SBpAB2
11	Student farm	Red soil un polluted	2	SRuAB1,SRuAB2
12	Student farm	Red soil polluted	2	SRpAB1,SRpAB2

test, 7, 1, 4, 5, 5, 15, 19, 19 and 22 isolates respectively showed positive results. For mannitol, sucrose, lactose, dextrose utilization, 9, 11, 12 and 12 isolates respectively showed positive results. Biochemical and physiological characterization of *Azotobacter* isolates are presented in (Table 2).

Plant Growth Promoting Attributes of *Azotobacter* Isolates

Plant Growth Promoting Attributes of 24 *Azotobacter* isolates are shown in Table 3. Out of 24 *Azotobacter* isolates, 14 were able to form clear zone of TCP solubilisation in different isolates ranged from 10-24 mm. Among the 14 isolates, SBpAB1 isolated recorded the highest zone of 21 mm diameter and CBpAB1, CBpAB2 isolates showed minimum solubilisation zone of 10 mm. Ammonia production was shown in all isolates, except SRpAB1, SRpAB2 of which SBuAB1, SBpAB2, SRuAB1, ABuAB1 produce strongly. Seven of 24 isolates were positive for IAA production, of which CBuAB1, CBpAB2, produced more IAA followed by CBuAB1, CBpAB1.

Out of 24 *Azotobacter* isolates 20 were able to produce siderophores weakly (+) (Table 3). Out of 24 *Azotobacter* isolates, 13 produced HCN (Table 3.). Further, out of 13 isolates, SRuAB1 isolate exhibited moderate (++) HCN production. Whereas the remaining 12 isolates viz., CBuAB1, CBpAB2, CRuAB2, CRpAB1, SBuAB1, SBuAB2, SBpAB1, SRpAB1, ABuAB1, ABpAB1, ABpAB2, and ARpAB1, were scored as weak (+) for HCN producer.

Out of 24 *Azotobacter* isolates 7 isolates showed inhibition potential against *Rhizoctonia solani*, viz. CBuAB1 (36.05%), CBpAB2 (36.05%), CRuAB2 (33.85%), CRpAB1 (36.05%), SBpAB1 (34.95%), SRuAB1 (35.50%) and ABpAB1 (36.05%). The maximum per cent inhibition against *Rhizoctonia solani* was showed by CBuAB1 (36.05%), CBpAB2 (36.05%), CRpAB1 (36.05%) and ABpAB1 (36.05%) with inhibition zone 2 mm, 4 mm, 2mm and 2 mm.

Nine out of 24 isolates were inhibitory to *Sclerotium rolfsii*, viz. CBuAB1 (37.70%), CBuAB2 (34.40%), CBpAB2 (37.15%), CRpAB2 (36.05%), SBuAB2 (34.95%), SBpAB1 (36.05%), SBpAB2 (38.25%), ARuAB1 (34.95%) and ARpAB1 (36.05%). The maximum per cent inhibition against *Sclerotium rolfsii*, was showed by SBpAB2 (38.25%) with inhibition zone 4 mm (Table 3).

Out of 24 *Azotobacter* isolates 3 isolates CBuAB1, CBpAB2, SBpAB1 showed inhibition potential against both *Rhizoctonia solani* and *Sclerotium rolfsii*. The isolate that showed maximum inhibition potential against *Rhizoctonia solani* was also inhibitory to *Sclerotium rolfsii* to a lesser extent based on per cent inhibition and vice versa. Hence it can be inferred that the *Azotobacter* isolates CBuAB2, CRuAB2, CRpAB1, CRpAB2, SBuAB2, SBpAB2, SRuAB1, ARuAB1 and ARpAB1 could be considered for their bio control activity.

Out of 7 *Azotobacter* isolates 4 isolates viz., CBpAB1, SBpAB1, ABpAB1 and ARpAB1 affected by Azoxystrobin with inhibition zone of 2 mm, 2 mm, 1 mm and 1 mm at recommended dosage respectively. The remaining 3 fungicides did not affect the growth of *Azotobacter* isolates (Table 4). Among the fungicides tested Azoxystrobin was found to be inhibit *Azotobacter* at recommended / half recommended dosage. However other fungicides were compatible with all the isolates tested

Table 2. Biochemical and physiological characterization of *Azotobacter* isolates

Sl. No	Isolates	Ind	MR	Vp	Ci	Cata	Oxi	Starch	Gelatine	H_2s	Denitrification	Lactose	Sucrose	Dextrose	Mannitol	Ammonia
1	$CBuAB_1$	+	+	−	−	−	−−	−	−	+	−	+	+	+	−	+
2	$CBuAB_2$	+	−	−	−	−	+	−	−	−	+	+	+	−	+	+
3	$CBpAB_1$	+	−	−	+	+	−	−	−	−	+	−	+	−	−	+
4	$CBpAB_2$	+	−	−	+	+	−	−	−	+	+	−	−	−	+	+
5	$CRuAB_1$	+	−	−	−	+	−	+	−	−	+	−	−	−	+	+
6	$CRuAB_2$	−	+	−	−	+	−	−	−	−	+	+	+	−	−	+
7	$CRpAB_1$	+	−	−	−	+	+	−	−	−	+	−	−	+	−	+
8	$CRpAB_2$	+	−	−	−	−	−	−	−	−	+	+	−	−	+	+
9	$SBuAB_1$	+	−	−	−	−	+	−	−	−	+	+	−	+	−	+
10	$SBuAB_2$	+	−	−	−	+	+	−	−	−	+	−	+	+	−	+
11	$SBpAB_1$	+	−	−	−	+	+	−	−	−	+	−	+	+	−	+
12	$SBpAB_2$	−	−	−	−	+	+	−	−	−	+	−	−	−	−	+
13	$SRuAB_1$	+	−	−	−	+	+	−	−	+	−	+	−	−	−	+
14	$SRuAB_2$	−	+	−	−	+	−	−	−	−	+	−	−	−	−	+
15	$SRpAB_1$	+	−	−	+	+	+	−	−	−	−	+	+	−	+	−
16	$SRpAB_2$	−	−	−	+	+	+	−	−	−	+	+	−	+	+	−
17	$ABuAB_1$	−	−	−	−	+	+	−	−	−	+	−	−	−	+	+
18	$ABuAB_2$	−	−	−	−	+	+	−	−	−	+	−	+	+	−	+
19	$ABpAB_1$	+	−	−	−	+	+	−	−	−	+	−	+	+	−	+
20	$ABpAB_2$	−	−	−	−	+	+	−	−	−	+	+	+	+	+	+
21	$ARuAB_1$	−	+	−	+	−	−	−	−	+	+	−	−	+	−	+
22	$ARuAB_2$	−	−	−	−	+	−	−	−	−	+	+	+	+	+	+
23	$ARpAB_1$	−	+	−	−	+	+	−	−	−	−	+	−	+	−	+
24	$ARpAB_2$	−	−	−	−	+	+	−	−	−	+	+	−	−	−	+

\+ Positive for the test − Negative for the test

Four out of 7 *Azotobacter* isolates viz., CBpAB2, SBpAB1, SRuAB1 and ABpAB1 affected by Imidacloprid with inhibition zone of 1 mm, 1 mm, 3 mm and 1 mm at recommended dosage respectively. The remaining 3 insecticides were compatible with *Azotobacter* isolates (Table 4). Among the insecticides tested Imidacloprid was found to be inhibit *Azotobacter at* recommended / half recommended dosage. However other insecticides were compatible with all the isolates tested.

None of the herbicides had any negative effect on the *Azotobacter* isolates and therefore all are compatible (Table 4).

Multiple PGP activities among PGPR have been reported by some other workers while such findings on indigenous isolates of India are less commonly explored. Farah *et al.* (2005) isolated a total of 21 bacterial isolates (*Azotobacter spp.* 10 and *Pseudomonas fluorescens* 11) from different rhizospheric soils in the vicinity of Aligarh city and characterized as per standard methods. These isolates were further tested for the production of indole acetic acid (IAA) and the results indicated that a low amount of IAA production was recorded by *Azotobacter* strains without tryptophan addition and production of IAA in fluorescent *Pseudomonas* isolates increased with an increase in tryptophan concentration

Forty four bacterial isolates were isolated by Mahalakshmi and Reetha (2009) from the rhizosphere of tomato grown

in Cuddalore and Nagapattinam districts of Tamil Nadu, India. These bacterial isolates were grouped into *Azospirillum* (18 isolates) *Azotobacter* (9) *Pseudomonas* (12) and *Bacillus* (5) based on their morphological and biochemical characteristics. Among the forty four isolates, three isolates of *Azospirillum*, two from *Azotobacter*, one from *Bacillus* and four from *Pseudomonas* were selected to determine IAA production, siderophore production quantitatively. The maximum IAA production of 3.6 g ml-1, siderophore production of 0.86 g ml-1 were recorded by TMPS-9 and TMPS-7 respectively

Isolated a total of 150 bacterial isolates belonging to *Bacillus, Pseudomonas, Azotobacter and Rhizobium* from different rhizospheric soils of chick pea in the vicinity of Allahabad. These test isolates were biochemically characterized and screened for their plant growth promoting traits like production of indoleacetic acid (IAA), ammonia (NH_3), hydrogen cyanide (HCN), siderophore and catalase. All the isolates of *Bacillus, Pseudomonas* and *Azotobacter* produced IAA, whereas only 85.7% of *Rhizobium* was able to produce IAA (Joseph *et al.* 2007).

Isolated 72 bacterial isolates belonging to *Azotobacter*, fluorescent *Pseudomonas, Mesorhizobium, and Bacillus* and screened for their plant growth promoting traits. According to Farah *et al.* (2008) more than 80% of the isolates of *Azotobacter*, fluorescent *Pseudomonas* and *Mesorhizobium ciceri* produced IAA, whereas only 20% of *Bacillus* isolates produced IAA. Solubilization of phosphate was commonly detected in the isolates of *Bacillus* (80%) followed by *Azotobacter* (74.47%), *Pseudomonas* (55.56%) and *Mesorhizobium* (16.67%). HCN production was more

Table 3. Plant growth promoting attributes and Antagonistic potential of *Azotobacter* isolates:

Sl. No.	Isolates	Phosphate solubilisation			Ammonia Production	IAA Production	HCN Production	Siderophore Production	Percent Inhibition (%) of *Rhizoctonia solani*	Percent Inhibition (%) of *Sleroitium rolfsii*
		Zone diameter (mm)		Solubilisation efficiency (%)						
		Inhibition zone	Culture diameter							
1	$CBuAB_1$	11	8	137.5	+	++	+	+	36.05	37.70
2	$CBuAB_2$	_	_	_	+	+++	_	+	32.20	34.40
3	$CBpAB_1$	10	7	142.8	+	++	_	_	30.50	26.60
4	$CBpAB_2$	10	6	166.6	++	+++	+	+	36.05	37.15
5	$CRuAB_1$	_	_	_	+	+	_	_	26.05	11.65
6	$CRuAB_2$	_	_	_	+	_	+	_	33.85	28.25
7	$CRpAB_1$	_	_	_	+	_	+	+	36.05	23.85
8	$CRpAB_2$	_	_	_	++	_	_	+	26.65	36.05
9	$SBuAB_1$	13	12	108.3	+++	_	+	+	31.65	11.10
10	$SBuAB_2$	16	15	106.6	++	_	+	+	27.15	34.95
11	$SBpAB_1$	21	11	190.9	++	_	+	+	34.95	36.05
12	$SBpAB_2$	_	_	_	+++	_	_	+	19.95	38.25
13	$SRuAB_1$	12	8	150	+	+	++	+	35.50	32.20
14	$SRuAB_2$	14	7	200	+++	_	_	+	25.50	12.75
15	$SRpAB_1$	_	_	_	_	_	+	+	24.40	26.05
16	$SRpAB_2$	_	_	_	_	_	_	+	24.40	29.95
17	$ABuAB_1$	14	12	116.6	+++	_	+	+	27.15	14.40
18	$ABuAB_2$	14	13	107.6	+	_	_	+	29.95	12.75
19	$ABpAB_1$	13	11	118.1	+	+	+	+	36.05	19.40
20	$ABpAB_2$	13	11	118.1	++	_	+	+	20.50	24.40
21	$ARuAB_1$	_	_	_	+	_	_	+	21.10	34.95
22	$ARuAB_2$	_	_	_	++	_	_	_	21.65	21.65
23	$ARpAB_1$	17	10	170	++	_	+	+	26.05	36.05
24	$ARp\ AB_2$	14	11	127.2	+	_	_	+	19.40	22.75
	Control								00	00
	Standard error of mean (SEM)								0.95	0.56
	CD @ 0.05 probability								2.70	1.61

HCN- Hydrogen cyanide IAA- Indole Acetic Acid
+ Weak production ++ Moderate production
+++ Strong production " No production

Table 4. Compatibility of fungicides, Insecticides, herbicides with *Azotobacter* isolates *in vitro*

Sl. No.	Percent of inhibition Azotobacter isolates	Fungicides used								Insecticides used								Herbicides used							
		Tebuconazole		Azoxystribin		Carbendazim		Captan		Spiromesifin		Thiacloprid		Imidacloprid		Flubendiamide		Metribuzin		Propaquizafop		Pretilachlor		Pendimethalin	
		R	HR	R	HR	R	HR	R	HR	R	HR	R	HR	R	HR	R	HR	R	HR	R	HR	R	HR	R	HR
1.	$CBuAB_1$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	$CBpAB_2$	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
3.	$SBpAB_1$	0	0	2	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
4.	$SRuAB_1$	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0
5.	$ABuAB_1$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.	$ABpAB_1$	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
7.	$ARpAB_1$	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

R- Recommended, HR-Half Recommended
0- No inhibition, 1 mm inhibition, 2 mm inhibition, 3 mm inhibition

common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%).

Fatima *et al.* (2009) isolated seven PGPR strains from the rhizoplane and rhizosphere of wheat from four different sites of Pakistan. These strains were analyzed for production of indole acetic acid (IAA), phosphorous solublization capability and inhibition of *Rhizoctonia solani*. Strains WPR-51, WPR-42 and WM-30 were selected to test antagonistic activity on two wheat varieties infected with *R. solani*. These three strains belonged to *Azotobacter* and *Azospirillum*. Out of these three strains, WPR-51 and mixture of all three strains showed maximum inhibition of *R. solani* growth.

Sunitha *et al.* (2007) studied the survival of *Mesorhizobium ciceri* (SP4) and *Azotobacter chroococcum* (CBD-15 and M4) on chickpea (*Cicer arietinum*) seeds treated with fungicides Bavistin [carbendazim] [methyl N-(1H-benzimidazol-2yl) carbamate] and Thiram (tetramethyl-thiuram disulfide), whereas the survival of phosphate solubilizing bacteria (PSB), *Pseudomonas striata* (27) and *Bacillus polymyxa* [*Paenibacillus polymyxa*] (H5), was examined on two cultivars (Arkel and BV) of pea (*Pisum sativum*) seeds treated with Thiram. The viability of *A. chroococcum* (W5) was also examined on wheat (*Triticum aestivum*) seeds treated with Bavistin, Captan (*cis*-N-trichloromethyl thio-4 cyclohexane-1, 2-dicarboximide) and Thiram under laboratory conditions using standard dilution and the plate count technique.

Sarkar *et al.* (2005) tested the bio efficacy of Pendimethalin and Fluchloralin in mustard and concluded that the populations of fluorescent *Pseudomonas* and *Azotobacter* were improved with the application of these herbicides.

In the present study isolates CBuAB1, CBpAB2, SRuAB1 and ABpAB1 (*Azotobacter spp.*) were found to be efficient PGPR with multiple beneficial activities, which solubilized insoluble phosphorus, IAA, ammonia, siderophore and HCN productions. Out of the 24 isolates tested for their antagonistic activity against *Rhizoctonia solani, Sclerotium rolfsii* and efficient PGPR isolates were assessed the compatibility with the four each of the fungicides, insecticides, herbicides, the isolates of *Azotobacter* CBuAB1, CBpAB2, ARpAB1 showed potential as PGPR.

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