



# Exploration of Rhizobacteria as Bioagents against *Phytophthora* Blight and Yield Attributes of Pigeonpea (*Cajanus cajan* L.): *In vitro* and *In vivo* Study

Saroj Bala<sup>1\*</sup>, Rajni Devi<sup>2</sup> and Veena Khanna<sup>1</sup>

<sup>1</sup>Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141001, India.

<sup>2</sup>Department of Microbiology, COBS and H, Punjab Agricultural University, Ludhiana-141001, India.

## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

## Article Information

DOI: 10.9734/IJPSS/2021/v33i1830572

### Editor(s):

(1) Dr. Hakan Sevik, Kastamonu University, Turkey.

### Reviewers:

(1) John Kimondo Kariuki, Jomo Kenyatta University of Agriculture and Technology, Kenya.

(2) IBATSAM KHOKHAR, Forman Christian College, Pakistan.

(3) Mohamed Ali Abdelsatar, Agricultural Research Center, Egypt.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/70197>

Received 30 April 2021

Accepted 01 July 2021

Published 14 August 2021

Original Research Article

## ABSTRACT

The latest soil management scenario is occupied by destructive chemical fertilizers, which is a serious risk to both human health as well as to the environment. Advantageous microbes present in soil are used as biofertilizers for a promising role in sustainable agriculture. Pigeon pea (*Cajanus cajan* L.) is a primitive protein rich leguminous pulse in India. Thirty-five isolates from rhizospheric soil samples were collected from twelve different locations of Punjab (India). Morphological and biochemical characterization for selection of potential plant growth promoting traits with antifungal properties was undertaken. Most of the inoculated seeds with rhizoisolates evolved a significant increase in growth parameters of pigeon pea as compared to uninoculated seeds, both in vitro and in vivo conditions. Plant growth promoting rhizobacterias (PGPRs) are environmentally safe as they lead to increased production and resistance against diseases of crops.

**Keywords:** Antagonistic and antifungal; PGPRs; pigeonpea; rhizosphere; sustainable agriculture.

\*Corresponding author: E-mail: sarojbala483@gmail.com;

## 1. INTRODUCTION

Chemical fertilizers and pesticides are used to increase the productivity of farm land. However, over the past few decades, they have been overtly used in farming. This causes environmental pollution, leading to negative impact on environment and adverse effect on human health. Enormous use of such chemical leads to short term increase in soil fertility but marring long term health of the soil by increasing acidification of soil and depleting of minerals. Fertilizers contain nitrates and phosphorus that causes eutrophication and reduces oxygen for aquatic animals. Many of the fertilizers are banned by national government. For example, Indian government has prohibited the use of aldrin, nitrofen, DDT and sodium cyanide etc. [1], [2]. Biofertilizers are ecofriendly in increasing farm land productivity. Therefore, biofertilizers have started gaining traction among scientific and farming community as a sustainable substitute of fertilizers.

Versatile and valuable pigeon pea (*Cajanus cajan* [L.] Millsp) crop has diversified uses as food, fodder and fuel. It has been recognized as a valuable source of protein (17.9 gm to 24.39 gm/100 gm) particularly in developing countries where the majority of the population depends on plant diets in sustaining their dietary requirements [3]. *Phytophthora* blight is a severe threat to pigeon pea production and under favorable conditions, *Phytophthora* blight may lead to 100% yield loss [4]. Due to harmful effects of agrochemical fertilizers, the adoption of plant growth promoting rhizobacteria (PGPR), that are naturally occurring soil bacteria having capabilities to act as biocontrol agents for soil borne pathogens, is more advisable. Many integrated management practices used PGPR leading to reduction of agrochemicals use and promotion of organic farming [5]. The antagonistic potential of PGPR is due to antifungal plant growth promoting traits that help in disease resistance and increase yield production. These are siderophore production traits efficiency to chelate iron from soil borne pathogens in soil, synthesis of volatile antifungal metabolites such as ammonia, aldehydes and ketones [6].

The objective of the investigation was to isolate rhizobacteria from pigeon pea rhizosphere with potential biocontrol agents under greenhouse conditions and to promote sustainable agricultural development, organic farming and

reduce harmful effects of chemical fertilizers on the environment.

## 2. MATERIALS AND METHODS

### 2.1 Isolation of Rhizobacteria

Isolation of rhizobacteria was done from twelve different pigeon pea growing fields of Punjab. All 35 rhizobacteria were isolated from rhizosphere soil on their respective media; *Pseudomonas* spp. on King's B medium [7], *Bacillus* spp. on Nutrient agar and *Rhizobium* on yeast extract mannitol agar [8]. The incubated temperature was  $28 \pm 2^\circ\text{C}$  and stored on agar slants at low temperature on their respective medium.

### 2.2 Characterization of Pigeonpea Rhizobacteria

Selected rhizobacterial isolates were morphologically and biochemically characterized to show—the strain characteristics after conducting various biochemical tests viz., Gram's reaction, urease test; catalase production; lactose fermentation; nitrate reduction; indole production; starch hydrolysis; methyl red and citrate utilization test as per the standard methods given by [9], [10].

### 2.3 Determination of Plant growth Promoting Traits and Screening for Biological Control Agents

Several plant growths promoting traits isolated from pigeon pea rhizosphere were studied viz., IAA production as per the method given by [11]; Plate assay for zinc solubilization on Tris-minimal medium [12]; siderophores production using blue agar plates containing the dye chrome azurol S (CAS) by [13]; Ammonia production [10] and HCN production [14].

### 2.4 Evaluation for Bioantagonistic Potential under Greenhouse Conditions

A pot assay study was conducted under greenhouse conditions during *kharif* season 2017 in order to check the efficiency of selected antagonists i.e three rhizobacterial traits for elimination of Fusarium wilt disease. Seeds of pigeon pea (*Cajanus cajan* L., variety PAU 881) were obtained from the Department of Plant Breeding and Genetics Punjab Agricultural University, Ludhiana, India. The seeds were

immersed in selected 3 rhizobacteria antagonistic strains, treated separately and in combination with recommended *Rhizobium* of pigeon pea (LAR-06) by PAU before sowing. Pots were filled with sterilized soil and surface was sterilized with 0.1% mercuric chloride and autoclaved water. Treatments were isolated rhizobacteria (S-2, S-4 and S-18), combinations of these rhizobacteria with PAU recommended rhizobacteria (LAR-06), Negative control (pathogen treated) and Control sample (without inoculation). The pots were irrigated properly and temperature was controlled throughout the trails. After 60 days, different growth parameters were measured and sample was investigated for disease after 45 days. For conducting statistical analysis, CPCS1 software was used. Seedling vigor index (SVI) [15] was calculated by formulae:

$$\text{SVI} = \text{Healthy survivals} \times (\text{mean shoot length} + \text{mean root length})$$

## 2.5 Experimental Details

During *Kharif* season of 2017, evaluation was carried out in the fields available for experimentation and maintained by Pulses Section of Department of Plant Breeding and Genetics, PAU, Ludhiana. The experiment was conducted in Randomized Block Design (RBD) in three replications. As per treatments, PAU 881 variety of Pigeon pea seeds were inoculated with recommended *Rhizobium* LAR-06 strain and isolated PGPRs. Different treatments in equal quantity were mixed with seeds and left at room temperature for 30 minutes and later on, dried under shade.

As per the recommended practice, the trial was executed as follows:

Test crop	Pigeon pea ( <i>Cajanus cajan</i> [L.] Millsp.)
Experimental design	Randomized Block Design (RBD)
Treatments	6
Replications	3
Total number of plots	6 x 3=18
Variety	PAU 88
Soil	Light textured soil (sandy clay loam)
Plot size	3 x 2 m <sup>2</sup>
Row to row spacing	30 cm
Date of sowing	June 7, 2017
Irrigation	As and when required
Date of harvesting	Nov 2, 2017

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation, Screening and Characterization of Rhizobacteria

From twelve different locations of Punjab (India) rhizobacterial strains were isolated from pigeon pea rhizospheric soil (Table 1). Isolated rhizobacteria on different respective media were evaluated for morphological and biochemical characteristics. Study for all the pigeon pea rhizobacteria's morphological characters such as gram's reaction, colony color, size, Endospore formation, starch hydrolysis, border, shape, elevation, catalase production, nitrate reduction, ammonia production, urease test, lactose fermentation and citrate utilization were conducted. Out of these isolates, after screening on different temperature 35°C, 40°C and 45°C, three rhizobacteria (S-2, S-4 and S-18) were selected for both greenhouse and field experiment. As per the biochemical attributes, all the three isolates were positive for nitrate reduction, citrate utilization, ammonia and indole production. S-18 was positive for lactose fermentation. Whereas S-2 and S-18 were positive for urease test. (Table 2). On further investigation, rhizobacterial isolates were provisionally placed into three genera: *Bacillus*, *Pseudomonas* and *Rhizobium*. The outcomes were coinciding with previous investigations documented by [16].

**Table 1. Sources of rhizospheric soil samples**

Sample	Location of collection
1.	Fields of PAU
2.	Patiala
3.	Moga
4.	Nathana
5.	Chandigarh
6.	Abohar
7.	Amritsar
8.	Rampura
9.	Jalandhar
10.	Bathinda
11.	Pathankot
12.	Samrala

### 3.2 Estimation of Plant Growth Promoting Traits for Rhizobacterial Isolates IAA Production

Indole-3-acetic acid (IAA) is an elementary hormone secreted by PGPRs facilitating plant growth. It has plenty of significance such as

activating cell elongation and cell division which consequently results in plant expansion and development [17]. On rhizobacteria screening for IAA production at 40°C, they showed different deviation ranging from 7.98-9.56 µg/ml in absence and 11.31-12.56 µg/ml in presence of tryptophan after 3 days of incubation in all set ups. It was found that increase in IAA production from 8.62-10.89 µg/ml in absence and 18.63-20.56 µg/ml when tryptophan was present after an incubation period of 5 days at 40°C. Influential IAA producers were S-18 (20.56 µg/ml), S-2, S-4 and S-30 (19.5 and 18.6 µg/ml respectively) (Table 3).

Similar study was conducted by [18] opined that the synergistic effect of *P. fluorescens* AK1 and *P. aeruginosa* AK2 led to an increase in IAA production. These isolates produced IAA during L-tryptophan presence resulting in better results as compared to uninoculated seeds.

### 3.2.1 Siderophore production by Rhizobacterial isolates

Siderophores are high-affinity iron chelators exuded by different genera of microbes such as bacteria and fungi. Siderophore helps in growth, colonization, oxidative stress and sexual and asexual development in microbes. It evokes the plant's defense through an antagonistic mechanism by preventing pathogens to attack [19]. It also stimulates the salicylic and jasmonic acid which helps in plant development.

During experiments, it was observed that a change of color from blue to golden yellow after 24 hours of incubation on inoculation of rhizobacteria on CAS medium siderophores production attained their peak after 72 hours. Out of 35 isolates, 3 rhizobacteria selected for experimentation formed well defined orange halo on CAS plates indicating siderophore production. Maximum siderophore halo zone on CAS plate were produced by S-2 (2.6 cm) followed by S-18 (2.3 cm) and S-4 (1.8 cm) respectively (Table 3). Further, testing rhizobacterial isolates for quantitative siderophores production after 3 incubation days, all the three rhizobacteria, treated with Arnow's reagent, produced distinctive color. This revealed production of catechol-type siderophore in the range of 56.2-81.2 µg/ml.

These results are complementary with the finding of [20] that siderophore was produced by *Pseudomonas* sp. on CAS medium, which ranged from 1.6-1.7 cm. The assay was further determined for quantitative studies from chickpea rhizosphere [21]. Likewise, for siderophore production [22] reported screening of *P. pseudoalcaligenes* by Chrome Azurol S assay (CAS). *P. pseudoalcaligenes* also produced maximum yield of Hydroxamate type siderophore. It was also tested as seed inoculants which exhibited positive results in promoting plant growth.

**Table 2. Morphological, Physiological and biochemical characters of rhizobacteria from Pigeon pea rhizosphere**

Characteristic of test organism	S-2	S-4	S-18
Gram's reaction	-	-	+
Shape	Rods	Rods	Rods
Pigment	+	na	na
Pigment color	Fluorescent green	White	White
Elevation	Flat	Raised	Raised
Consistency	Smooth	Smooth	Smooth
Margin	Entire	Entire	Undulated
Endospore formation	-	-	-
Starch hydrolysis	+	+	+
Catalase production	+	+	+
Methyl red test	na	na	na
Nitrate reduction	+	+	+
Ammonia Production	+	+	+
Urease test	+	+	-
Indole production	+	+	+
Lactose fermentation	-	-	+
Citrate utilization	+	+	+

Abbreviations: + = Positive; - = Negative; na = not assessed; S-2 = *Pseudomonas*; S-4 = *Rhizobium*; S18 = *Bacillus*

**Table 3. In vivo production of plant growth promoting rhizobacteria and biological antifungal traits**

PGP traits		Rhizobacterial isolates		
		S-2	S-4	S-18
Growth O.D (600 nm)	35°C	2.43	2.22	2.22
	40°C	2.83	2.04	2.56
	45°C	2.09	1.79	1.96
IAA at 40°C 5 <sup>th</sup> day (µg/ml)		19.25	18.63	20.56
Siderophore production (dia in cm)	24hr	1.1	1.0	1.9
	48hr	1.6	1.3	1.6
	72hr	2.6	1.8	2.3
Catechol siderophore (µg/ml)		81.2	56.2	68.1
Zn-solubilization (mm)		41.8	40.6	43.6
Hydrogen cyanide (HCN) (%)		100	100	100
Ammonia (NH <sub>3</sub> ) (µg/ml)		82.4	79.3	81.5
P-solubilization (mm)		18.5	19.2	21.5
Growth inhibition (%) ( <i>Phytophthora drechsleri</i> )		39.5	29.3	39.5

### 3.2.2 Zinc Solubilization by Rhizobacterial Isolates

Rhizobacteria bacteria has Zinc solubilizing characteristic which control the plant growth. Zinc triggered the promotion of chlorophyll and protein content in leaves. Shortage of zinc in plants leads to the leaf discoloration and deficiency in enzymes leading to improper development of seedling. Selected rhizobacteria for the greenhouse pot experiment were able to solubilize zinc. Maximum solubilization was shown by rhizoisolate S-18 (43.6 mm) followed by S-2 (41.8mm) and S-4 (40.6 mm) (Table 3).

These findings were in line with isolates MDSR7 and MDSR14 of rhizospheric soybean and wheat produced high amount of soluble zinc [23].

### 3.2.3 Production of Hydrogen cyanide (HCN) and Ammonia (NH<sub>3</sub>)

A wide variety of compounds with antifungal and biocontrol properties are produced by rhizobacteria. HCN producing rhizobacteria is considered to be a potential biofertilizers with biocontrol properties which enhances crop production. Production of HCN was measured by intensity of color variation from yellow to light brown, moderate brown or red-brown. The analysis suggested that the three rhizobacterial isolates S-2, S-4 and S-18 were depicted by color variation implying that they were the robust producers of HCN (Table 3).

Similar studies [24] reported that HCN have antimicrobial properties that helps in biological root disease control with the help of rhizobacteria. These findings are reinforced in

other studies [25], demonstrating that 21 strains of *Pseudomonas* from pigeonpea rhizosphere, three isolates were strong producers of HCN and eleven were moderate producers.

Ammonia produced by rhizobacteria is released in soil promoting plant growth, improves seed and fruit production and help in conversion of light energy into chemical energy. The three isolates produced ammonia in lab. as well as pot experiment. The production of ammonia was estimated by the strength and stability of color development. Ammonia produced by rhizoisolates S-2 (82.4 µg/ml), S-4 (79.3 µg/ml) and S-18 (81.5 µg/ml) illustrating they were vigorous producers. (Table 3).

From findings, more than 90% rhizobacterial isolates from rice field produced ammonia which accelerated plant growth. [26].

### 3.3 Assessment of Treatments on Plant Growth Parameters and *Phytophthora* blight Control under Greenhouse and field Conditions.

#### 3.3.1 Assessment of Rhizobacteria on seedling emergence

The effects of seed treatment with PGPR<sub>S</sub> and co-inoculation of *Rhizobium* with PGPR<sub>S</sub> on pigeonpea variety of PAU 881 were encouraging. S-2 (81.83%), S-4 (80.5%) and S-18 (83.03%) revealed a synergistic interaction with the inoculants. In treatments with PGPR<sub>S</sub> *Rhizobium*, an enhancement in the germination percentage was observed if compared to treatments with PGPR<sub>S</sub> alone. Almost all the rhizobacterial

isolates enhanced seedling emergence in comparison to recommended *Rhizobium* (Table 4). Moreover, inoculation with *Rhizobium* and PGPR together enhanced the symbiotic parameters as compared to uninoculated control. In conclusion, the combination of bioantagonist with *Rhizobium* synergistically enhanced the seedling emergence in S-2 (87.07%), S-4 (84.07%) S-18 (87.37%).

Many phytohormones like IAA, gibberellins secreted by rhizobacteria lead to the increase in seed germination percentage [27].

### 3.3.2 Effect of Rhizobacteria against *Phytophthora* blight infected plant in greenhouse conditions

For greenhouse pot experimentation, three bioantagonists S-2, S-4 and S-18 further were identified for *in vitro* tests. The tests provided a strong confirmation regarding the efficiency of these isolates in suppressing *Phytophthora* blight in pigeonpea. Symptoms of *Phytophthora* blight resembles damping off disease that cause seedling to die suddenly and leaves become necrotic. The lesions appear on healthy plant from brown to black on stem. The most significant reduction in percentage of *Phytophthora* blight was shown by dual inoculation of *Rhizobium* with S-18 (38.93%), S-4 (39.1%) and S-2 (39.37%) as compared to recommended *Rhizobium* alone (Table 3).

*Phytophthora* blight reduction was reported in co-inoculation with PGPR and *Rhizobium* due to antagonistic effect of PGPR [28]. This could be possible due to production of antifungal metabolites such as phenolic compounds, cyanides, ammonia and siderophores [29].

After treatment with bioantagonist alone, S-18 showed decrease in disease incidence (44.77%) followed by S-2 (48.47%) and S-4 (49.83%). In case of negative control 77.67% of incidences of *Phytophthora* blight were recorded. However, seed treatment with *Rhizobium* reduced the disease severity to 56%. Plant growth promoting efficiencies of the bacterial isolates were suggested by Seedling Vigor Index (SVI) values ( $P < 0.05$ ) [15]. Highest SVI were showed by dual inoculation of *Rhizobium* with S-18 (8949.26), followed by S-2 (8901.12) and S-4 (8426.28%) (Table 4). Thus, the potential of antifungal strains of *Rhizobacteria* may be concluded from *in vitro* experimentation against the test fungi. Thus, S-2 and S-18 may prove to be the potent bio-control agents.

Similar studies were found in accordance with [30]. Seed inoculated together with *Trichoderma* with SVI 1314 and *Rhizobium* with 69 induced high seedling vigor when compared to control.

### 3.3.3 Effect on growth of pigeonpea in field condition

*Rhizobium* inoculation gave higher number of pods per plant (112.1) in contrast to control group (109.8). *Rhizobium* co-inoculated with S-2, S-4, S-18 and S-30 further increased the number of pods per plant (115, 116.7, 115 and 113.4) respectively. *Rhizobium*, co-inoculated with S-2, S-4, and S-30, led to further increase in the number of seeds per pod as compared to *Rhizobium* alone although the increase was not markedly higher. *Rhizobium* when co-inoculated with S-18, S-2, and S-30 increased the number of seeds per pod (3.02), (2.94), (2.74) respectively when compared to *Rhizobium* (2.66) alone, although the increase was not steep (Table 5).

**Table 4. In vivo assessment of bioantagonist on plant growth to examine the control of infectivity due to *Phytophthora drechsleri* Tucker var. *cajani* in Pigeon pea**

Treatments	Seedling emergence (%)	Shoot length (cm)	Root length (cm)	Incidence of blight (%)	Seedling index
Negative control (pathogen)	60.63	67.80	8.37	77.67	4618.24
Control	71.80	76.77	11.00	65.87	6301.65
<i>Rhizobium</i>	78.90	81.43	14.27	56.00	7550.73
S-2	81.83	84.50	15.70	48.47	8199.70
S-4	80.50	82.30	14.50	49.83	7792.40
S-18	83.03	83.93	15.37	44.77	8245.21
<i>Rhizobium</i> + S-2	87.07	86.17	16.07	39.37	8901.12
<i>Rhizobium</i> + S-4	84.07	84.70	15.53	39.10	8426.28
<i>Rhizobium</i> + S-18	87.37	85.33	17.10	38.93	8949.26
CD (p=0.05)	3.85	1.96	1.69	4.07	481.36

**Table 5. In vitro effect of dual-inoculation on yield attributing traits and yield in pigeon pea**

Treatments	No of pods/plants	No of seeds/pod	Grain Yield (kg/ha)
Control	109.8	2.37	1037
<i>Rhizobium</i>	112.1	2.66	1062
<i>Rhizobium</i> + S-2	115.0	2.94	1086
<i>Rhizobium</i> + S-4	116.7	2.80	984
<i>Rhizobium</i> +S-18	115.0	3.02	1091
<i>Rhizobium</i> + S-30	113.4	2.74	1000
CD @ 5%	NS	NS	NS

These findings were in line with [31] reported that plant height and number of pods showed increasing trend in single and multiple inoculated plants in pigeonpea.

The grain yield was noted at the stage of harvesting. Inoculation of *Rhizobium* and PGPR made significant improvement in the grain yield while the effect was more pronounced when they were applied in combination. The highest grain yield was produced by S-2 and S-18 along with *Rhizobium* (1091 and 1086 kg/ha) over inoculated *Rhizobium* alone. (Table 5)

Enhancement in grain yield as a result of PGPRs might be due to their IAA production thus, increasing availability of auxins resulted in better nodulation, growth and development ultimately leading to increased grain yield. Enhancement in yield of grains as a result of *Rhizobium* inoculation has been speculated in various legume crops [32].

#### 4. CONCLUSION

Application of rhizobacteria include protection against pathogens as well as improving, compared to control, plant growth and grain yield (1091kg/ha). These antagonistic combinations are ecofriendly way of protecting plants against diseases as incidence of blight (%) are lower when compared to control. Rhizobacterial isolates S-2 and S-18 encouraged growth promoting traits in plants and should be recommended for similar agro-ecologies. However, this study should be repeated both over locations and seasons in order to give complete overall implication for practical application.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Srimurali S, Govindaraj S, Kumar SK, Rajendran RB. Distribution of organochlorine pesticides in atmospheric air of Tamilnadu, southern India. *International Journal of Environmental Science and Technology*. 2015;12(6):1957-64.
2. Subramaniam K, Solomon J. Organochlorine pesticides BHC and DDE in human blood in and around Madurai, India. *Indian Journal of Clinical Biochemistry*. 2006;21(2):169.
3. Ali M, Kumar S. Advances in pigeonpea research. *IIPR Kanpur*. 2005;3.
4. Sharma M, Ghosh R. Isolation, Identification, and Pathogenicity of *Phytophthora* Blight of Pigeonpea. *Plant Health Progress*.2018;19(3):233-6.
5. Mishra P, Singh PP, Singh SK, Verma H. Sustainable agriculture and benefits of organic farming to special emphasis on PGPR. In *Role of Plant Growth Promoting Microorganisms in Sustainable Agriculture and Nanotechnology*. Woodhead Publishing. 2019;75-87.
6. El-Katatany M, Hetta A, Sliaban G, El-Komy H. Improvement of cell wall degrading enzymes production by alginate encapsulated *Trichoderma* sp. *Food. Technology and Biotechnology*. 2003; 41:219-225.
7. King EO, Ward MK, Randey DE. Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine*. 1954;44:301-307.
8. Vincent JM. A manual for the practical study of the root nodule bacteria. Blackwell Scientific publications Oxford and Edinburgh. 1970;1-3.
9. Kleczkowska J, Nutman PS, Skinner FA, Vincent JM. The identification and

- classification of Rhizobium. Identification Methods for microbiologists. 1968;51–65.
10. Cappuccino JC, Sherman N. In: Microbiology: A Laboratory Manual, New York. 1992;125–179.
  11. Gordan AS, Weber RP. Calorimetric estimation of indole acetic acid. Plant physiology. 1951; 26:912–195.
  12. Fasim F, Ahmed N, Parsons R, Gadd GM. Solubilization of zinc salts by bacterium isolated from the air environment of tannery. FEMS microbiology letters. 2002;213:1-6.
  13. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophore. Analytical Biochemistry. 1987;160:47-56.
  14. Bakker AW, Schippers B. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biology Biochemistry. 1987;19:249-56.
  15. Mishra SN, Chaurasia AK, Bara BM, Kumar A. Assessment of different priming methods for seed quality parameters in pigeon pea (*Cajanus cajan* L.) seeds. Journal of Pharmacognosy and Phytochemistry. 2017;6(3):522-526.
  16. Sayyed RZ, Jamadar DD, Patel PR. Production of Exo-polysaccharide by *Rhizobium* sp. Indian Journal of Microbiology. 2011;51(3):294–300.
  17. Turan M, Topcuoglu B, Kitir N, Alkaya U, Ercelik F, Nikerel E, Gunes A. Plant Growth Promoting Rhizobacteria's (PGPRS) Enzyme Dynamics in Soil Remediation. Soil Contamination: Current Consequences and Further Solutions. 2016;209.
  18. Karnwal A. Production of indol acetic acid by *Fluorescent Pseudomonas* in the prescence of L-tryptophan and rice root exudates. Journal of Plant Pathology. 2009;91(1):61-3.
  19. Olanrewaju OS, Glick BR, Babalola OO. Mechanisms of action of plant growth promoting bacteria. World Journal of Microbiology and Biotechnology. 2017; 33(11):197.
  20. Akhtar MS, Siddiqui ZA. Use of plant growth promoting rhizobacteria for the biocontrol of root-rot disease complex of chickpea. Australasian Plant Pathology. 2009;38:44-50.
  21. Kotasthane AS, Agrawal T, Zaidi NW, Singh US. Identification of siderophore producing and cynogenic fluorescent *Pseudomonas* and a simple confrontation assay to identify potential bio-control agent for collar rot of chickpea. 3 Biotech. 2017;7(2):1-8.
  22. Gamit, DA, Tank SK. Effect of siderophore producing microorganism on plant growth of *Cajanus cajan* (Pigeon pea). International Journal of Research in Pure and Applied Microbiology. 2014;4(1):20-27.
  23. Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP. Inoculation of zinc solubilizing *Bacillus aryabhatai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. Applied Soil Ecology. 2014;73:87-96.
  24. Alizadeh O, Azarpanah A, Ariana L. Induction and modulation of resistance in crop plants against disease by bioagent fungi (arbuscular mycorrhiza) and hormonal elicitors and plant growth promoting bacteria. International Journal of Farming and Allied Sciences. 2013;982-998.
  25. Siddiqui ZA, Shakeel U. Biocontrol of wilt disease complex of pigeon pea (*Cajanus cajan* L.) Millsp.) by isolates of *Pseudomonas* spp. African Journal of Plant Science. 2009;3(1):1-12.
  26. Samuel S, Muthukkaruppan SM. Characterization of plant growth promoting rhizobacteria and fungi associated with rice, mangrove and effluent contaminated soil. Current Botany. 2(3):22-25.
  27. Khan N, Bano A, Ali S, Babar MA. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. Plant Growth Regulation. 2020;1-15.
  28. Vargas LK, Volpiano CG, Lisboa BB, Giongo A, Beneduzi A, Passaglia LMP. Potential of rhizobia as plant growth-promoting rhizobacteria. In Microbes for legume improvement. 2017; 153-174 Springer, Cham.
  29. Kumari S, Khanna V. Biocidal Mechanisms in Biological Control of Fusarium Wilt in Chickpea (*Cicer arietinum* L.) by Antagonistic Rhizobacteria: A Current Perspective in Soil Borne Fungal Pest Management. International Journal of Current Microbiology and Applied Sciences. 2019; 8(10):1494-1510.
  30. Kumar A, Puniya RC, Bhuker A, Sharma R, Devi R. Seed quality as influenced by



- seed invigouration treatments in pigeonpea [*Cajanus Cajan* (L.) Millsp.]. Legume Research: An International Journal. 2017;40(5).
31. Tewari S, Sharma S. Rhizobial exopolysaccharides as supplement for enhancing nodulation and growth attributes of *Cajanus cajan* under multi-stress conditions: A study from lab to field. Soil and Tillage Research. 2020;198: 104545.
32. Singh AK, Singh RS, Singh SP, Kumawat N, Kumar R. Productivity, profitability and soil health of pigeonpea as influenced by phosphorus levels and bioinoculants under eastern Uttar Pradesh. International Journal of Current Microbiology and Applied Science. 2017;6(6):1723-32.

© 2021 Bala et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*  
*The peer review history for this paper can be accessed here:*  
<https://www.sdiarticle4.com/review-history/70197>