INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 20, No. 1, p. 12-20, 2022

RESEARCH PAPER OPEN ACCESS

Effect of Plant Growth Promoting Rhizobacteria of the Growth of *Cicer arietinum*

Arooj Qaiser[1], Arsalan Fazal[1], Maryam Khan[1], Saba Shamim[1*]

Institute of Molecular Biology and Biotechnology, The University of Lahore, Defence Road Campus, Lahore, Pakistan

Key words: Plant growth promoting rhizobacteria (PGPR), *Cicer arietinum* L., Black chickpea, Biochemical tests, *Bacillus velezensis*.

http://dx.doi.org/10.12692/ijb/20.1.12-20 Article published on January 04, 2022

Abstract

This research was conducted to isolate the rhizospheric bacteria of chickpea plant and to check their effect on its growth. Out of ten bacterial strains isolated, six were checked. They included two strains of *Pseudomonas* sp., three strains of *Bacillus* sp. and one strain of *Brevibacterium* sp. Out of all strains, one *Bacillus* strain showed good results. The 16s rRNA sequencing showed it *Bacillus velezensis* MN611255. Early germination, enhanced number of leaves, shoots, roots, and increase in their weight were notable features of *B. velezensis* as a PGPR. Furthermore, its effect on the flavonoids, total flavonoids, phenols, carbohydrates and chlorophyll content of chickpea plant was more pronounced as compared to the control. PGPR did not show siderophore production but were positive to indole acetic acid and phosphate solubilization. It can be concluded from the observations that indigenous isolated *B. velezensis* showed promising results as a PGPR. Field trials can help in further elaborating its role as a biofertilizer.

* **Corresponding Author:** Saba Shamim ✉ sabashamimgenetics@gmail.com

Introduction

Approximately 7,000 different plants species have been used as food for people. Fabaceae of pea/ bean family of plants (Rahman and Parvin, 2014) holds a special importance being third most popular flowering plant's family. Consumption-wise notable plants include *Medicago sativa* (alfalfa), *Glycyrrhiza glabra* (liquorice), *Pisum sativum* (pea), *Glycine max* (soybean), *Ceratonia siliqua* (carob), *Arachis hypogaea* (peanut), *Phaseolus* (beans) and *Cicer arietinum* (chickpeas). These plants are also the source of paper production, fuel-woods, oils, chemicals and medicines (Lewis *et al*., 2005). Among all, chickpea (botanical name: *Cicer arietinum L.*) is an important member of pulse crop grown which is consumed globally (Morel *et al*., 2012). In South Asia, it is the largest produced legume while globally it is the third largest produced food legume. Pakistan is one of the major producers of chickpea.

In developing countries, it is an important source of protein for millions of people predominantly in South Asia, where the people are mostly vegetarians either by choice or due to financial causes (Gaur *et al*., 2010). Improvement in crop production is a necessity that can pave a way for supplying the increasing population with their food need (Lwin *et al*., 2012). One effective way is the application of chemical fertilizers. It is a known fact that soil-borne microorganisms have direct influence on the growth of plants thus maintaining the ecosystem.

The idea of plant growth-promoting rhizobacteria (PGPR) is at present well-established for plant growth-promotion (Yadav *et al*., 2017). It may prove helpful for using PGPR in order to facilitate plant growth in any environmental conditions. The purpose of this study was to screen and isolate PGPR exhibiting bacterial strain from the soil and to check its effect on the growth of black chickpea. During the study, different features of plant including flavonoids, phenolics, carbohydrates and chlorophyll as well as bacterial strain (siderophore production, indole acetic acid production and phosphate solubilization) were ascertained.

Materials and methods

Isolation of PGPR and its biochemical characterization

For the isolation of PGPR, rhizospheric soil of chickpea plant was collected in a sterile container. The bacterial strains were isolated using standard microbiology techniques. The cultural, morphological and biochemical characterization of the bacterial strains were carried out. Out of 10, six bacterial strains were selected for further experimentation.

Plantation experiments

Soil and pots preparation

The soil was purchased from a local nursery. It was sieved properly to remove the pebbles. It was placed in a clean dry beaker, covered properly and autoclaved under standard conditions of temperature and pressure. It was air-dried by placing in a hot air oven. Dry soil (100 grams) was transferred to pots. Pots were labeled properly.

Selection of seeds

Healthy seeds of black chana (*C. arietinum* L.) were purchased.

Sterilization of seeds

The seeds were dipped in 1 % detergent for 30-45 minutes and rinsed with distilled water. In a clean beaker, following reagents were added: 98 ml distilled water, 0.005 g giberellic acid, 0.005 g amphotericin B, and 10 ml hydrogen peroxide). The sterilized seeds were placed in it for 24 hours. Next day, seeds were ready for placing in pots (Kannahi and Kowsalya, 2013).

Preparation of inoculum

A loopful inoculum of the bacterial strain was given in 5 ml Luria Bertani (LB) broth followed by 24 hours incubation at 37 °C.

Plantation experiment

The experiment was done in two ways; without bacterial culture which was "Control" and with bacterial culture known as "Experimental". For experimental set, seeds were placed for 30 minutes in

a beaker containing bacterial inoculum. After 30 minutes, 5 seeds were placed per pot. The pots were watered with distilled water. They were placed in dark until they germinate. They were observed for germination on daily basis. The plants were harvested on day 15.

Harvesting

The plants were harvested on 15th day. They were carefully taken out of pot, soil was gently removed.

Observations of parameters

Following features of the plants were noted: number of seeds germinated, number of leaves, shoots, root and their lengths.

Biochemical tests

In order to have deep insight into plant-microbe interaction, the biochemical tests of the germinated plants were performed. For this, the plants were cleaned properly to remove any soil particle. They were oven dried. Their parts (leaves, roots, shoots) were separated carefully. They were grinded finely to form powder. They were collected in vials for preparation of their extracts.

Preparation of extracts

Three different solvents (ethanol, hexane and water) were taken. One gram of sample was placed in 100 ml of solvent. It was left for five days at room temperature. All samples were mixed in three solvents separately. The mixture was completely evaporated in rotary evaporator. The sample was taken in a clean dry vial and stored in refrigeration till further experimentation.

Biochemical tests for plants

Following tests were performed.

Flavonoids

Before performing it, 20 % NaOH solution was prepared. In 200 ul sample (extract prepared above), 1-2 drops of NaOH were added. The yellow colored appeared which was converted to colorless solution by the addition of 70 % dilute HCl. The colorless solution confirmed flavonoids (Hossain *et al.*, 2013).

Total flavonoids

For it, three solutions were prepared; 5 % NaOH, 5 % $NaNO_3$ and 10 % $AlCl_2$. In 125 µl water, 25 µl extract was added followed by 0.8 µl $NaNO_3$.The vial was placed away from light. After 6 minutes, 10 % $AlCl_2$ (0.150 µl) was added and re-incubated in the dark for the same time. Finally water and 5 % NaOH (1:2) were added. The absorbance was recorded at 510 nm. Quercetin was used as standard for the caliberation curve. It was calculated as mentioned by Hossain *et al.* (2013).

Phenols

Here $FeCl_3$ was used for the detection of phenols which appeared as blue color in the vial after mixing of 200 µl of $FeCl_3$ with 200 µl of extract (Prabhavathi *et al.*, 2016).

Carbohydrates

In a vial, 100 µl concentrated H_2SO_4 was mixed with 100 µl extract and 1-2 drops of Molisch reagent. The reaction was given time till red or violet color appeared which confirmed carbohydrates (Prabhavathi *et al.*, 2016).

Chlorophyll

In 100 µl extract, 900 µl acetone was added. It was centrifuged for 5 minutes at 5000-10000 rpm. As it became colorless, the supernatant was transferred to a new tube. The absorbance was recorded at 645nm and 663nm. Estimation of the chlorophyll content was done (Arnon, 1949; Rajalakshmi and Banu, 2014).

Biochemical tests for bacteria

The following tests were performed for PGPR (Kannahi and Kowsalya, 2013).

Siderophore production

It was performed on TLC plate. For it, 1 ml bacterial culture was taken and centrifuged at maximum speed for 15 minutes. The supernatant was shifted to a new tube. With the help of capillary tube, spotting on TLC

plate was done. The solvent system was prepared as methanol: chloroform (1:9). The indication of reddish brown colored spotted confirmed the presence of siderophore production (Raaijmakers and Weller, 2001).

IAA production

The milky white appearance in the Luria Bertani (LB) broth inoculated with a bacterial colony for 18 hours was observed and reported as positive (Bric *et al.*, 1991).

Phosphate solubilization

A lawn of bacterial colony was made on the phosphate growth medium (LB agar containing 1gram phosphate (NaH_2PO_4)). For control, LB agar did not contain phosphate. The incubation at 37 °C for 72 hours was done. The plates were observed for appearance of zones after every 24 hours. The zone was made due to solubilization of mineral phosphate. It was estimated as done by Neutiyal *et al.* (1999).

Statistical analysis

All experiments were run in triplicate following completely randomized design. Three readings were taken; their mean and standard error of the means were calculated. The significance of the data ($p \leq 0.05$) was checked using SPSS v.17.0.

Results

Isolation of bacterial strains

Out of ten bacterial isolates, six were selected for further experiments. Two of them were Gram negative whereas four were Gram positive bacterial species. According to the results of their biochemical tests (Table 1), four were found to *Bacillus* species whereas remaining two were *Pseudomonas* species.

Germination experiments

All six strains were tested for the germination of black chickpea. Out of all strains, B2 strain showed good results (Table 2). In case of B2 strain, germination started at day 2. On day 4, sprouting was observed.

Tiny roots were visible on day 5. On day 8, baby leaves and shoots were clearly visible. In the following days (10, 12) shoot length and root numbers increased respectively. The number of leaves increased on day 14. The plant was and harvested on day 15. On the other hand, in control set, germination was delayed. Moreover, less plant growth was observed at the day of harvesting (Fig. 1).

Table 1. Detail characterization of bacterial strains isolated from the rhizosphere of black chickpea plant.

Sr. No.	Gram staining	Morphological appearance	Biochemical characterization	Tentative identification
1.	Negative	Rods	Catalase (+), oxidase (+), indole (-), MR/VP (-), citrate (+), urease (-), coagulase (-), pigment (+), motility (+)	*Pseudomonas* sp.
2.	Negative	Rods	Catalase (+), oxidase (+), indole (-), MR/VP (-/-), citrate (+), urease (-), coagulase (-), pigment (+), motility (+)	*Pseudomonas* sp.
3.	Positive	Rods	Catalase (+), oxidase (+), indole (-), MR/VP (+/-), citrate (+), urease (-), coagulase (-), pigment (-), motility (+)	*Bacillus* sp.
4.	Positive	Rods	Catalase (+), oxidase (+), indole (-), MR/VP (+/-), citrate (+), urease (-), coagulase (-), pigment (-), motility (+)	*Bacillus* sp.
5.	Positive	Rods	Catalase (+), oxidase (+), indole (-), MR/VP (+/-), citrate (+), urease (-), coagulase (-), pigment (-), motility (+)	*Bacillus* sp.
6.	Positive	Rods	Catalase (+), oxidase (-), indole (-), MR/VP (-/-), citrate (+), urease (+), coagulase (-), pigment (+), motility (-)	*Brevibacterium* sp.

Selection of the bacterial strain

On the basis of germination experiments, B2 strain was selected for further study. The 16s rRNA sequencing showed it *Bacillus velezensis*. Its accession number as obtained by NCBI GenBank was MN611255.

B. velezensis as PGPR

B2 strain improved the black chickpea plant in size, number as well as weight (Tables 3-4). The

inoculation improved the growth of number of leaves from 36 to 60 (control versus experimental). It has accelerated the growth of number of roots, adventitious root and root length. The shoot length was also more in the inoculated plant as compared to the un-inoculated one (Table 3). The growth of plant in terms of weight of roots, leaves and shoot were found more in the experimental as compared to the control *i.e.* from 0.0883, 0.0195, 0.2171 to 0.0691, 0.0067, 0.1356 respectively (Table 4).

Table 2. Observations of different parameters of chickpea plant with B2 strain.

Days	Control	Experimental	Observations
0	-	-	No growth
1	-	-	No growth
2	-	+	No growth
3	+	+++	Germination
4	++	+++	Sprouting
5	++	+++	Tiny roots
8	++	+++	Baby leaves, shoots
10	+++	++++	Shoot length increased
12	+++	++++	Roots numbers increased
14	+++	+++++	Leaves increased
15	+++	+++++	Harvesting

Biochemical tests for plants

Test for flavonoids

Flavonoids were detected in leaves and shoots of the ethanolic, aqueous and hexane extracts but absent in roots of all these extracts (Table 5).

Table 3. Measurement of all the roots, stems and leaves of black chickpea with and without bacteria are shown below.

Control	
Parameters	Observations
No. of leaves	36
No. of baby leaves	7
No. of roots	30
No. of adventitious roots	21
Root length	3.66 cm
Shoot length	6.1 cm
Experimental	
Parameters	Observations
No. of leaves	60
No. of baby leaves	18
No. of roots	35
No. of adventitious roots	45
Root length	5.86 cm
Shoot length	7.6 cm

Test for total flavonoids

B. velezensis inoculum increased the overall production of total flavonoids in black chickpea plant. Among ethanolic, aqueous and hexane extracts, more flavonoids were extracted in ethanolic extract of leaves (0.533), hexane extract of roots (0.299) and shoots (0.801) (Table 6).

Table 4. Final weights of different plant parts.

Plant parts	Weight (in grams)	
	Experimental	Control
Root	0.0883	0.0691
Leaves	0.0195	0.0067
Shoot	0.2172	0.1356

Test for phenols

Phenols were detected in all extracts of shoots, roots and leaves of a PGPR-treated plant (Table 7).

Test for carbohydrates

Carbohydrates were detected in all extracts of shoots, roots and leaves of a PGPR-treated plant (Table 7).

Test of chlorophyll

More chlorophyll content (15.82 µg/ml) was observed in PGPR-treated plant as compared to control (13.66 µg/ml) (Table 8).

Biochemical tests for bacteria

Siderophore production test

No siderophore production was observed by *B. velezensis*.

Table 5. Flavonoids in different extracts.

Ethanolic extract		
Plant parts	Control	Experimental
Roots	No	No
Leaves	Yes	Yes
Shoots	Yes	Yes
Aqueous extract		
Plant parts	Control	Experimental
Roots	No	No
Leaves	Yes	Yes
Shoots	Yes	Yes
n-Hexane extract		
Plant parts	Control	Experimental
Roots	No	No
Leaves	Yes	Yes
Shoots	Yes	Yes

IAA production

The presence of milky growth confirmed the IAA production of *B. velezensis*.

Table 6. Estimation of total flavonoids content in different parts of chickpea plant.

Ethanolic extract		
Plant parts	Control	Experimental
Roots	0.186	0.249
Leaves	0.209	0.533
Shoots	0.169	0.268
Aqueous extract		
Plant parts	Control	Experimental
Roots	0.190	0.295
Leaves	0.119	0.291
Shoots	0.315	0.201
n-Hexane extract		
Plant parts	Control	Experimental
Roots	0.272	0.299
Leaves	0.181	0.341
Shoots	0.309	0.801

Phosphate solubilization test

The selected PGPR showed zone (Fig. 2) on phosphate growth medium which confirmed it as positive for this test.

Discussion

Most of the global human population lives on a diet based on staple crops. Increased production of edible crops is the only mean to keep up the pace with the increasing population in order to meet their food needs. *Cicer arientinum* L. or black chickpea is one of the main staple crop.

Table 7. Results of phenols and carbohydrates in black chickpea.

Phenols		
Plant parts	Control	Experimental
Root	-	+
Leaves	-	+
Shoot	-	+
Carbohydrates		
Plant parts	Control	Experimental
Root	-	+
Leaves	-	+
Shoot	-	+

In recent years scientists are looking ways to produce best quality of crops in short time period. One of the best ways is by using microorganisms as biofertilizer. This research was conducted to check the effect of plant growth promoting rhizobacteria (PGPR) on the growth of black chickpea. Here PGPR was identified as *Bacillus velezensis* which was in accordance with Kanahi and Kowsalya, (2013) who reported *Bacillus subtilis* as PGPR. Agbodjato *et al.* (2015) also reported *Bacillus* and *Pseudomonas* sp. as PGPR in case of promoting growth in maize.

Table 8. Estimation of chlorophyll in leaves with and without bacteria in ethanol solvent.

Groups	chlorophyll a (µg/ml)	chlorophyll b (µg/ml)	Total chlorophyll (µg/ml)
Control (ethanol)	18.9	-5.23	13.66
Experimental (ethanol)	19.5	-3.68	15.82

According to Kaun *et al.* (2016), *B. subtilis* PGPR improved the growth of maize plant. Similarly, *Bacillus* sp. had also enhanced the overall plants parameters of chick pea and pigeon pea (Gopalakrishnan *et al.*, 2016). Previous studies were in agreement with our findings (Prathibha and Siddalingeshwara, 2013).

The results of the present study for total chlorophyll, carbohydrates, flavonoids and total phenol contents were in accordance with (Kannahi and Kowsalya, 2013). Silva-Beltrán *et al.* (2015) reported that leaf samples of ethanol extract contained the highest percentage of flavonoids, phenols and chlorophyll.

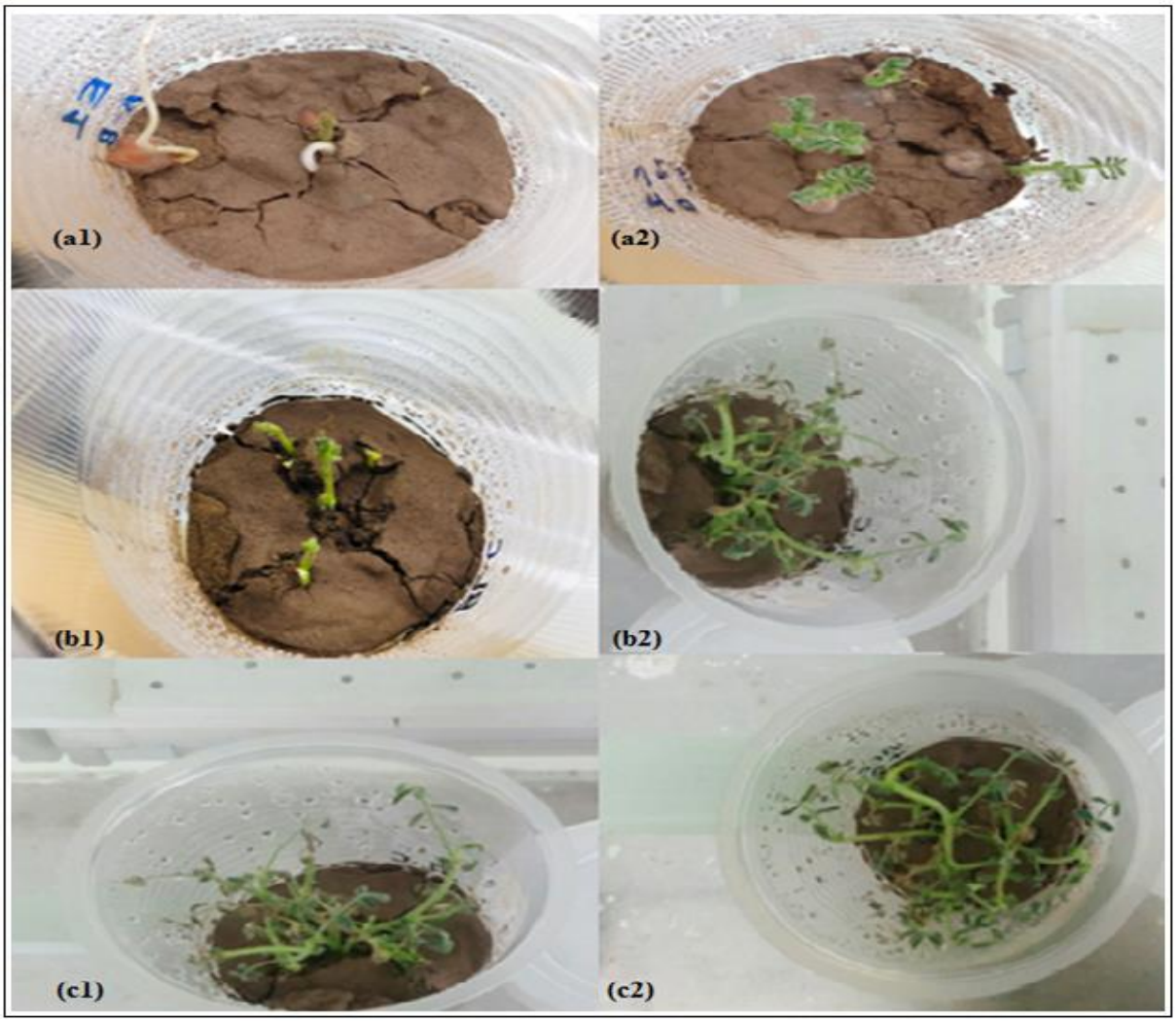

Fig. 1. Growth of black chickpea plants in the absence and presence of bacterial inoculums. (a1) without bacteria day 5, (a2) with bacteria day 5, (b1) without bacteria day 10, (b2) with bacteria day 10, (c1) without bacteria day 15 (c2) with bacteria day 15.

Prabhavathi *et al.* (2016) found the production of phenol and carbohydrate content in ethanol extract. Hossain *et al.* (2013) observed that the phenol content was high in hexane extract which was in accordance with the present study but total flavonoid content was low. Here *B. velezensis* did not show siderophore which disagree with previous findings (Hu and Xu, 2011; Pindi *et al.*, 2013; Rayavarapu and Padmavathi, 2017). *Bacillus* sp. are good producers of IAA (Joseph *et al.*, 2007; Lwin *et al.*, 2012) which in accordance with our findings.

The result of phosphate solubilization by *Bacillus* sp. was in accordance with previous findings (Kannahi and Kowsalya, 2013; Pindi *et al.*, 2013; Rayavarapu and Padmavathi, 2017).

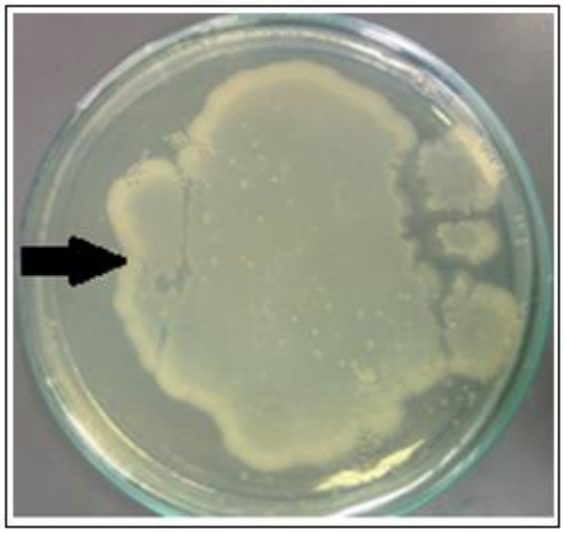

Fig. 2. Zone of PGPR (black arrowhead) on phosphate growth medium confirming positive test for phosphate solubilization.

Conclusion

It was observed in this study, that *B. velezensis* lead to increase in number of leaves, shoots and roots. Enhanced flavonoids, phenols, carbohydrates and chlorophyll contents were expressed by PGPR. *B. velezensis* did not show siderophore production but IAA and solubilized phosphate content. Field trials and further studies can help in establishing the role of *B. velezensis* as a biofertilizer.

References

Agbodjato NA, Noumavo PA, Baba-Moussa F, Salami HA, Sina H, Sèzan A, Bankolé H, Adjanohoun A, Baba-Moussa L. 2015. Characterization of potential plant growth promoting rhizobacteria Isolated from maize (*Zea mays L.*) in Central and Northern Benin (West Africa). Applied and Environmental Soil Science **2015,** 1-9. http://dx.doi.org/10.1155/2015/901656

Aron D. 1949. Copper enzymes isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. Plant Physiology **24,** 1-15. http://dx.doi.org/10.1104/pp.24.1.1

Bric JM, Bostock RM, Silverstone SE. 1991. Rapid *in situ* assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Applied and Environmental Microbiology **57,** 535-538.

Gaur PM, Tripathi S, Gowda CLL, Rao GVR, Sharma HC, Pande S, Sharma M. 2010. Chickpea seed production manual. Patancheru 502 324, Andra Pradesh, India. International Crops Research Institute for the Semi-Arid Tropics p 28.

Gopalakrishnan S, Vadlamudi S, Samineni S, Kumar CVS. 2016. Plant growth-promotion and biofortification of chickpea and pigeonpea through inoculation of biocontrol potential bacteria, isolated from organic soils. SpringerPlus **5(1),** 1882-1893. http://dx.doi.org/10.1186/s40064-016-3590-6

Hossain MA, Al- Raqmi KA, Al-Mijizy ZH, Weli AM, Al- Riyami Q. 2013. Study of total flavonoids content and phytochemical screening of various leaves crude extracts of locally green *Thymus vulgaris*. Asian Pacific Journal of Tropical Biomedicine **3(9),** 705-710. http://dx.doi.org/10.1016/S2221-1691(13)60142-2

Hu QP, Xu JG. 2011. A simple double-layered chrome azurol S agar (SDCASA) plate assay to optimize the production of siderophores by a potential biocontrol agent *Bacillus*. African Journal of Microbiology Research **5(25),** 4321-4327. http://dx.doi.org/10.5897/AJMR11.238

Joseph B, Patra RR, Lawrence R. 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). International Journal of Plant Production **1(2),** 141-152. http://dx.doi.org/10.22069/IJPP.2012.532

Kaun KB, Othman R, AbdulRahim K, Shamsuddin ZH. 2016. Plant growth promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilization of maize under greenhouse condition. PLoS One **11(3),** 1-19. http://dx.doi.org/10.1371/journal.pone.0152478

Kannahi M, Kowsalya M. 2013. Efficiency of plant growth promoting rhizobacteria for the enhancement *Vigna mungo* growth. Journal of Chemical and Pharmaceutical Research **5(5)**, 46-52.

Lewis G, Schrire B, MacKinder B, Lock M. 2005. Legumes of the world. Royal Botanical Gardens, Kew Publishing, UK.

Lwin KM, Myint MM, Tar T, Aung WZM. 2012. Isolation of plant hormone (indole-3-acetic acid- IAA) producing rhizobacteria and study on their effect on maize seedling. Engineering Journal **16(5),** 137-144. http://dx.doi.org/10.4186/ej.2012.16.5.137

Morel MA, Brana V, Aung WZM. 2012. Legume

crops, importance and use of bacterial inoculation to increase production, crop plant. Intech. p 217-241. http://dx.doi.org/10.5772/37413

Nautiyal CS. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters **170,** 265-270. http://dx.doi.org/10.1111/j.1574-6968.1999.tb13383.x

Pindi PK, Sultana S, Vootla PK. 2014. Plant growth regulation of Bt-cotton through *Bacillus* species. 3Biotech **4,** 305-315. http://dx.doi.org/10.1007/s13205-013-0154-0

Prabhavathi RM, Parsad MP, Jayaramu M. 2016. Studies on qualitative and quantitative phytochemical analysis of *Cissus quadlangularis*. Advances in Applied Science Research **7(4)**, 11-17.

Prathibha KA, Siddalingeshwara KG. 2013. Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescence* as rhizobacteria on seed quality of sorghum. International Journal of Current Microbiology and Applied Sciences **2(3),** 11-18.

Rahman AHM, Parvin MIA. 2014. Study of medicinal uses of Fabaceae family at Rajshahi, Bangladesh. Research in Plant Sciences **2(1)**, 6-8. http://dx.doi.org/10.12691/plant-2-1-2

Raaijmakers JM, Weller DM. 2002. Exploiting genotypic diversity of 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp.: characterization of superior root-colonizing *P. fluorescens* strain Q8r1-96. Applied and Environmental Microbiology **56,** 2545-2554. http://dx.doi.org/10.1128/AEM.67.6.2545-2554.2001

Rajalakshmi K, Banu N. 2014. Extraction of chlorophyll from medicinal plants. International Journal of Chemical and Pharmaceutical Research **4(11),** 209-212.

Rayavarapu VGB, Padmavathi T. 2016. *Bacillus* sp. as potential plant growth promoting rhizobacteria. International Journal of Advancements in Life Sciences **9(1),** 29-36.

Silva-Beltrán NP, Ruiz-Cruz S, Cira-Chávez LA, Estrada-Alvarado MI, Ornelas-Paz JJ, López-Mata MA, Del-Toro-Sánchez CL, Ayala-Zavala JF, Márquez-Ríos E. 2015. Total phenolic, flavonoid, tomatine, and tomatidine contents and antioxidant and antimicrobial activities of extracts of tomato plant. International Journal of Analytical Chemistry **2015**, 1-10. http://dx.doi.org/10.1155/2015/284071

Yadav AN, Verna P, Singh B, Chauahan VS, Suman A, Saxena AK. 2017. Plant growth promoting bacteria: biodiversity and multifunctional attributes for sustainable agriculture. Advances in Biotechnology and Microbiology **5(5),** 1-16. http://dx.doi.org/10.19080/AIBM.2017.05.555671