for the sustainability of agriculture. Nutritionally chickpea grain is an important source of protein (12.4-31.5%) in vegetarian diet and has become more important to mitigate the problem of protein energy malnutrition (Akibode and Maredia, 2011). Other than having high protein content, it is also a rich source of carbohydrates (52.4–70.9%), minerals (including iron, calcium, zinc, phosphorous, calcium, magnesium and manganese) and β-carotene (Awasthi et al., 1991).

Global yields of chickpea has been stagnant (0.5 and 1.0 t ha−1) for the last 50 years in spite of adopting conventional breeding and molecular approaches and extensively using synthetic fertilizers and pesticides (FAOSTAT, 2017). Major constraints in increasing production of chickpea are poor soils, inadequate moisture, harsh climatic conditions, weeds, insect, pests, pathogens and inadequate or even no fertilizer supply (Khan et al., 1989; Aslam et al., 1996). Bacteria present in root nodules of legumes are mainly species of Rhizobium (*Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium* and *Sinorhizobium*). Rhizobacteria that benefit plant growth by producing plant growth regulators, enhancing the nutrient availability, inducing root exudation and controlling phytopathogens are termed as PGP bacteria (Kloepper and Schroth, 1980).

Rhizobium in addition to other bacteria termed as plant growth-promoting Rhizobacteria (PGPR) can be applied together as biofertilizers as an alternative to chemicals to promote plant growth and crop yield and control the soil and seed-borne pathogens (Deshwal et al., 2011 Beneduz et al., 2012, Gupta et al., 2015). Hence, screening and selection of proper rhizobial strains is important for biological nitrogen fixation. Keeping in view the importance of Rhizobia in legume plants as well as in non-legume plants, the present study was undertaken to shed some light on different morphological and biochemical properties of Rhizobial isolates and to screen some efficient rhizobial isolates based on their plant growth promoting activities, from the root nodules of chickpea plants.

2. MATERIALS AND METHODS

2.1 Sample collection and isolation of root nodule bacteria

The *Rhizobium* isolates were isolated from the root nodules of *Cicer arietinum (Chickpea)* collected from the agricultural field's sites at different locations of Paghman district, Kabul state, Afghanistan. Chickpea plants were uprooted carefully and loosely adhered soil was removed by gentle shaking. These were brought in laboratory without any delay. The nodules along with roots were washed in running tap water until the removal of adhered soil particles. Large sized healthy, undamaged and pinkish nodules were detached from the roots and were selected for isolation of bacteria. The surface sterilization was done with 70% ethanol for 30 seconds, to break surface tension and to remove air bubbles from nodule tissues, followed by 30% hydrogen peroxide (H2O2) for 2 minutes and then rinsed thoroughly with sterilized distilled water (six times) in order to remove the chemicals.

Surface sterilized nodule was aseptically crushed with a sterilized glass rod in a test tube that contained 1 ml distilled water with 0.5 % NaCl. One loopful of the nodule suspension was streaked on Petri plates that contained yeast extract mannitol agar (YEMA) medium supplemented with 0.0025% (w/v) congo red as an indicator. The plates were sealed by parafilm to avoid contamination and incubated at 28°C for 48-72 hours. At the end of incubation period, the rhizobial colonies appeared white, translucent and elevated and mucilaginous whereas contaminations turned red. They were picked out using a sterilized inoculating loop and were further purified by streak plate method. The most prominent isolates were maintained on YEMA slants at 4°C in refrigerator for further characterization (Singh et al., 2008, Vincent, 1970).

2.2 Morphological characterization

The colony morphology of the bacteria (*rhizobium*) isolated from the root nodules was examined on YEMA plates after incubation of 72 hours at 28ºC, individual colony was characterized on the basis of colony- form, margin, elevation, colour, mucosity optical density and Gram stain reaction as per the method described by (Somasegaran and Hoben, 1994; Aneja, 2003).

2.3 Confirmatory Tests of Rhizobium

Four different confirmatory tests (Growth on YEMA with Congo red, Hoffer's alkaline Test, Keto-lactose Test and Growth on Glucose- peptone agar,) were performed to confirm the isolate as Rhizobia and to differentiate them from other contaminating microbes.

2.3.1 Growth on YEMA with Congo red

The purity of the rhizobial isolates was detected by addition of Congo red in YEMA medium. In general, Rhizobia absorb the dye weakly and produce white colonies, whereas many other bacteria including Agrobacteria, take up the dye strongly (Somasegaran et al., 1994).

2.3.2 Hoffer's alkaline Test

This test is based on the fact that Agrobacterium grows at higher pH level whereas Rhizobium unable to do so. A medium i.e. Hoffer's alkaline having high pH of 11.0 was used to screen isolated nodulated bacteria for this purpose. Bacteria were inoculated in above mentioned broth and incubated for 24-48 hours at 28±2°C (Hofer, 1935).

2.3.3 Keto-lactose Test

Keto-lactose test widely used to differentiate Rhizobia from other contaminating bacteria (Bernaertz and Daley, 1963). Keto-lactose agar medium (Lactose 10 g/L, KH2PO4 0. 5 g/L, MgSO4. 7H2O 0.2 g/L, NaCl 0. 1 g/L, Yeast extract 1 g/L, Agar 15 g/L, pH adjusted to 6.8) was poured into the sterilized Petri dishes and allowed to solidify. Actively grown rhizobial isolates were streaked on the Keto-lactose agar medium and incubated for 2-3 days. The plates were flooded with Benedict's reagent and kept at room temperature for 1-2 hours. Absence of yellowish zones around the rhizobium colonies indicated the purity of the isolates.

2.3.4 Glucose peptone agar (GPA) test

The test was performed to check the ability of the isolates to utilize glucose as the sole carbon source. Glucose peptone agar medium contains Bromocresol purple indicator dye (glucose 40 g/L, peptone5 g/L, agar15 g/L, Bromocresol purple 100 mg/L pH 7.0) to differentiate rhizobia, which usually shows no growth or very poor growth on the media without altering the pH of the medium, contaminants like Agrobacteria, shows massive growth on the media with a distinct change in pH (Singh et al., 2008).

2.4 Biochemical characterization of Rhizobium

The pure isolates were grown in YEM broth (pH 7) on the orbital shaker at 150 rpm. The 48-72 h fresh cultures were used for different biochemical characteristics namely catalase test, oxidase test, starch hydrolysis test, citrate utilization test, nitrate reduction test, urease test, and gelatin liquefaction test following standard procedure (Somasegaran and Hoben, 1994; Aneja, 2003; Cappuccino and Sherman, 2005).

2.5 *In vitro* Screening of Multiple Plant Growth Promoting Activities of Rhizobium spp.

The isolated bacteria from the root nodules of chickpea were characterized for their PGP traits including indole acetic acid (IAA), siderophore production, hydrocyanic acid (HCN), ammonia production, phosphate solubilization (TCP) under *in vitro* conditions. The isolated bacteria were assayed qualitatively for indole acetic acid production by spot inoculating on nutrient agar medium amended with 5 mM L-tryptophan and after incubation for 24-48 h; the inoculated points were overlaid with 10mm-diameter nitrocellulose membrane (NCM) disk. After incubation, the NCM saturated with few drops of Salkowski reagent (1mL 0.5M $FeCL_3$, 50mL H_2SO_4) (Gordon and Weber, 1950). After two minutes, appearance of pink color was observed which was indicator of IAA production (Myron and Williams, 1989). Siderophore production of the isolates was carried out by spot inoculating test organism (5 µL inoculum, 1×10^8 CFU mL−1) on chrome Azurol S agar plates and incubated at 30 °C for 2-3 days in dark.

Appearance of yellow to orange halo around the bacterial colonies was considered as positive for siderophore production (Schwyn and Neiland, 1987). Phosphate solubilizing ability was carried out by spot inoculating the isolated bacterial cultures on NBRIP media (Glucose 10g, Ca_3 $(PO4)_2$ 5g, $MgCl{\cdot}6H_2O$ 5g, KCl 0.2g, $(NH4)_2SO_4$ 0.1g, $MgSO_4{\cdot}7H_2O$ 0.25 g, Agar 15g, distilled water 1lt). The formation of halo zones around the colonies was considered as positive for phosphate solubilization (Mehta and Nautiyal, 2001).

Bacterial isolates were tested for the production of ammonia in peptone water broth as per (Joseph et al., 2007). Peptone broth tubes were inoculated with freshly grown cultures and incubated for 4 days at 30 °C and 120 rpm on an incubator shaker. After incubation, few drops of Nessler's reagent were added to each tube. Development of deep yellow to

brown color is a positive test for ammonia. For qualitative estimation of HCN, all the isolates were streaked on nutrient agar plate supplemented with 4.4 % glycine. A whatman filter paper no. 1 soaked in a solution of 2% Na_2CO3 in 0.5% picric acid was placed between base and lid of petri plate and incubated at 28 ± 2 °C in inverted position for 96 h and observed for color change from yellow to orange brown as described (Bakker and Schipper, 1987).

2.6 *In vitro* antagonistic activity against *Rhizoctonia solani*

Antagonistic activity against *Rhizoctonia solani* was detected by the dual culture technique method. Soil borne plant pathogenic fungi *Rhizoctonia solani* was grown on potato dextrose agar (PDA) media. A 5 mm diameter plug of fungal mycelium was cut from an actively growing fungal culture and placed on the center of the Petri plate containing potato dextrose agar. A loopful of exponentially grown culture of each isolates were streaked in a straight line on one edge of a 90 mm diameter petri plate and the distance between the fungus and the test culture was kept at 2 cm, and the plates were incubated at 28°C for 4-7 days (Rabindran et al., 1996). Inhibition radial growth of test fungus was observed daily. Culture plates with the test fungus served as control. In each case three (3) replicates were taken. The diameters of the colonies were measured after five days and average values compared with control were taken as a measure of fungitoxicity. Growth inhibition (%) of test fungus was determined by using the formula quoted (Pani and Patra, 1997).

$$\text{Growth Inhibition Percentage} = \frac{\text{Control} - \text{Test} \times 100}{\text{Control}}$$

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Bacteria

Rhizobium is a Gram-negative bacterium which has the capability to fix atmospheric nitrogen. In the present study a total of 7 isolates of rhizobia were successfully isolated from the root nodules of *Cicer arietinum* (Chickpea) collected from the agricultural field's sites at different locations of Paghman district, Kabul state, Afghanistan. All the isolates showed maximum growth on YEMA medium at pH 7.0 after incubation for 48-72 h at 28°C. Colonies of Rhizobial sp. were found to be round, creamy white, raised, opaque, some translucent and produced mucous when grown on YEMA plates. Similar result was reported (Vincent, 1970; Holt et al., 1994). Microscopic examination revealed that the isolates were Gram negative and rod in shape. The results of the morphological characteristics of the bacterial isolates are represented in Table 1 and Fig 1A, 1B, 1C, 1D. Similar results were also reported by various workers (Deora and Singhal, 2010; Gauri et al., 2011; Deshwal and Chaubey, 2014; Gachande and Khansole, 2011). From the above observations we could conclude that all the bacterial isolates were rhizobium spp.

Table 1: Morphological Characterization of root nodule bacteria grown on YEMA at 28°C.

Bacterial Isolates	Colony Form	Colony Margin	Colony Elevation	Colony Colour	Colony Texture	Optical density	Cell shape	Gram staining	Suspect Organism
AIQ-1	Circular	Entire	Raised	White	Mucoid	Opaque	Rod	+ve	Rhizobium
AIQ-2	Circular	Entire	Raised	Creamy	Mucoid	Translucent	Rod	-ve	Rhizobium
AIQ-3	Circular	Entire	Raised	Creamy	Mucoid	Translucent	Rod	-ve	Rhizobium
AIQ-4	Circular	Entire	Raised	Milky white	Mucoid	Opaque	Rod	-ve	Rhizobium
AIQ-5	Circular	Entire	Raised	Milky white	Mucoid	Opaque	Rod	-ve	Rhizobium
AIQ-6	Circular	Entire	Raised	Milky white	Mucoid	Opaque	Rod	-ve	Rhizobium
AIQ-7	Circular	Entire	Raised	Creamy	Mucoid	Opaque	Rod	-ve	Rhizobium

3.2 Confirmatory Tests of Rhizobium

For confirmation of all the isolates as rhizobium spp., all the 7 rhizobial spp. were screened for different confirmatory tests using different media viz. Congo red test, growth in Hofer's alkaline broth, ketolactose test, and glucose peptone agar test (Table 2). The colonies did not absorb the Congo red color and such nature, differentiate Rhizobium from Agrobacterium. All the rhizobium isolates showed the negative results on Hofer's alkaline medium (Deshwal and Chaubey, 2014; Trinick et al., 1982).

Normally rhizobium cannot grow in Hofer's medium; the results are also supported by studies (Deka and Azad, 2006). In Keto-lactose test, no yellow zone was observed around the colonies after the addition of Benedict's reagent which is the characteristic of rhizobium and the same results were observed (Deshwal and Chaubey, 2014). In GPA test all the isolates showed no growth on GPA medium that indicated the features of rhizobia. Regarding the growth in glucose peptone agar, Vincent et al., reported that rhizobia showed either no growth or grew very poorly on GPA media. From the above observations we could conclude that all the bacterial isolates were rhizobium spp.

Table 2: Conformity tests for differentiation of Rhizobium from Agrobacterium and other contaminants.

Bacterial Isolates	Congo red test	Growth on Hoffer's alkaline medium	Production of ketolactose test	Growth on glucose peptone agar
AIQ-1	Na	-	-	-
AIQ-2	Na	-	-	-
AIQ-3	Na	-	-	-
AIQ-4	Na	-	-	-
AIQ-5	Na	-	-	-
AIQ-6	Na	-	-	-
AIQ-7	Na	-	-	-

Here, Na, non-absorbing.

3.3 Biochemical characterization of Rhizobium

The results of biochemical characteristics of bacterial isolates are represented in Table 3. All the isolates were catalase and oxidase positive as confirmed by liberation of effervescence of oxygen around the bacterial colonies and change in colour of oxidase discs, respectively (Figure 1E). Some researchers also observed bubble formation around bacterial colonies (Mahana et al., 2000; Datta et al., 2015). All the isolates showed positive results for starch hydrolysis except (AIQ-5- and AIQ-6). Iodine and starch make a complex of blue color, while iodine does not react with any product of starch degradation and hence no color is formed in such cases. When the inoculated plates were flooded with iodine solution, clear zones around the colonies were observed while blue color appears on no growth areas. The same results match with those who observed that rhizobium isolates have capability to use starch (Figure 1F) (De Oliveira et al., 2007).

Most of the isolates showed negative results for citrate utilization test and gelatin liquefaction test. Negative gelatinase activity is a feature of rhizobium. Negative gelatinase activity of rhizobium was also observed (Hunter et al., 2007). In the nitrate reduction test, a red color change on the addition of sulphanilic acid and α-naphthylamine indicates a positive test (Cappucino and Sherman, 1992). In the *in vitro* examination, all the isolates were found to be positive for nitrate reduction (Kumari et al., 2010; Graham and Parker, 1964; Salve and Gangawanae, 1992). A change in color of the media from yellow to deep pink as the pH becomes higher indicates the production of ammonia due to the urease enzyme secreted by the incubated isolates which is a positive reaction for the test. In our study, all isolates showed a positive test for urease. Similar observation was reported (Gauri et al., 2011).

Table 3: Biochemical Characteristics of the root nodule bacteria

Bacterial Isolates	Cat	Oxid	Star	Cit	NR	Gel	Urease
AIQ-1	+	+	+	+	+	-	+
AIQ-2	+	+	+	-	+	-	+
AIQ-3	+	+	+	-	+	-	+
AIQ-4	+	+	+	-	+	-	+
AIQ-5	+	+	-	-	+	-	+
AIQ-6	+	+	-	-	+	-	+
AIQ-7	+	+	+	-	+	-	+

Key: (+) Positive, (-) Negative, Cat: Catalase, Oxid: Oxidase, Star: Starch hydrolysis, Cit: Citrate utilization, NR: Nitrate reduction, Gel: Gelatinase.

3.4 Plant Growth Promoting (PGP) Traits of the Test Isolates

The bacterial isolates were screened for multiple plant growth promoting activities which are represented in the table 4. The bacterial isolates were screened for plant growth promoting traits IAA, i.e. indole-3-acetic acid is considered to be the best categorized auxin found in plants. IAA is known to enhance cell elongation, cell division and differentiation in plants (Singhet al., 2013). Out of seven Rhizobial isolates, five were able to produce IAA in this analysis. AIQ-2, AIQ-3 and AIQ-4 showed high intensity (+++) of pink colour and AIQ-5 and AIQ-7 showed moderate (++) intensity of pink colour whereas the isolate AIQ-1 and AIQ-6 showed negative activity for IAA production (Figure 1 G). Microorganisms also enhance plant growth by scavenging available iron (Fe3+), which involves secretion of high affinity, low molecular weight iron chelating ligands called siderophores (Anitha and Kumudini, 2014).

Siderophores also play an important role in the biocontrol of some soil-borne plant diseases caused by several pathogens. Because siderophores sequester the limited supply of iron in the rhizosphere, they limit its availability to pathogens and ultimately suppress their growth (Schroth et al., 1984). Out of the seven rhizobial isolates, five isolates were able to produce siderophores. Further, out of five isolates AIQ-3, AIQ-4 and AIQ-5 exhibited strong (+++) siderophore production, and AIQ-2 showed moderate activity (++) whereas the isolate AIQ-6 and AIQ-7 showed negative activity for siderophore production (Figure 1K). All the isolates were able to produce ammonia. Further, out of seven isolates, AIQ-2 AIQ-3, AIQ-4, and AIQ-5 exhibited strong (+++) ammonia production and AIQ-7 produced moderately (++) whereas the remaining two isolates viz., AIQ-1 and AIQ-6 showed slight activity (+) for ammonia production (Figure 1 H).

Hydrocyanic acid (HCN) synthesized by some rhizobacteria inhibits diseases in plant and thereby increasing the bio control mechanism (Schippers, 1990). Out of the seven rhizobial isolates, five isolates were able to produce HCN. Further, out of five isolates AIQ-3 and AIQ-4 exhibited strong (+++) HCN production, and AIQ-2, AIQ-5 and AIQ-7 showed moderate activity (++) whereas the remaining two isolates namely AIQ-1 and AIQ-6 showed no activity (-) for HCN production (Figure 1 I). After nitrogen, phosphorus (P) is the most limiting nutrient for plant growth. Rhizobia, including *R. leguminosarum, R. meliloti, M. mediterraneum, Bradyrhizobium* sp. and *B. japonicu* are the potential P solubilizers. These bacteria synthesize low molecular organic acids which acts on inorganic phosphorous. Out of the seven rhizobial isolates, five isolates were able to solubilize phosphate on NBRIP media containing Tri calcium phosphate. Further out of five rhizobial isolates AIQ-3 and AIQ-4 recorded the highest solubilization zone (+++) and AIQ-2, AIQ-5 and AIQ-7 showed slight (+) solubilization zone (Figure 1J).

Table 4: *In vitro* screening of root nodule bacterial for PGPR traits

Bacterial Isolates	IAA	SID	TCP	AMM	HCN
AIQ-1	-	+	-	+	-
AIQ-2	+++	++	+	+++	++
AIQ-3	+++	+++	+++	+++	+++
AIQ-4	+++	+++	+++	+++	+++
AIQ-5	++	+++	+	+++	++
AIQ-6	-	-	-	+	-
AIQ-7	++	-	+	++	++

Legend (+++; Good activity, ++; average activity, +; slight activity, -; no activity) IAA: Indole acetic acid, AMM: Ammonia production, TCP: Tri calcium phosphate solubilization, HCN: hydrocyanic acid, SID: Siderophore.

3.5 In vitro antagonistic activity against Rhizoctonia solani

Bacteria belonging to the group of rhizobia are of considerable scientific and economic interest because of their ability to fix atmospheric nitrogen in leguminous plants. Moreover, along with nitrogen fixation efficiency of rhizobia, it also has a good potential of use as biological control agents against soil borne plant pathogens. Some researcher found, under field conditions, that *Sinorhizobium meliloti, Rhizobium leguminosarum* and *Bradyrhizobium japonicum* used either as seed dressing or as soil drench reduced infection of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium spp.,* in both leguminous and non-leguminous plants (Noreen et al., 2016; Ehteshamul-Haque and Ghaffar, 1993). In the present study none of the rhizobia showed complete growth inhibition of the test fungi but exhibited significant growth reduction.

Out of seven rhizobium isolates, 2 isolates showed inhibition potential against *Rhizoctonia solani*, viz. AIQ-3 (60.70%) and AIQ-4 (55.90%). Hence it can be inferred that the rhizobium isolates AIQ-3 and AIQ-4 could be considered for their bio control activity (Figure 1 L). The inhibition of fungal growth of the test fungi *in vitro* by certain of the rhizobia and formation of inhibition zones were presumably due to the metabolites released by the bacteria into the culture medium. These metabolites may include antibiotics and/or cell-wall degrading enzymes. Different studies have implicated antifungal secondary metabolites produced by rhizobium spp. in the control of plant diseases caused by pathogenic fungi (Siddiqui et al., 2000).

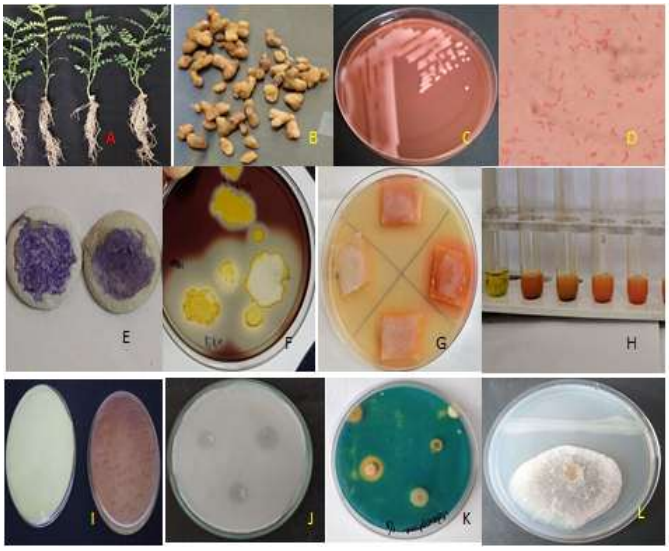

Figure 1: (A) Chickpea plants collected from agricultural fields; (B) Root nodules of chickpea; (C) Purified cultures of rhizobium on YEMA medium; (D) Gram staining of isolated root nodulated bacteria; (E) Oxidase test (F) Starch hydrolysis test; (G) IAA production; (H) Ammonia production; (I) Hydrocyanic acid production; (J) TCP solubilization; (K) Siderophore production; (L) Antifungal activity of isolates against *Rhizoctonia solani.*

4. CONCLUSION

To boost the crop yield and food production, farmers use synthetic nitrogen fertilizers which has unfavorable effects and hazardous to environment and human population. Growing awareness of this environmental damage has motivated the study of biological alternatives. The use of biofertilizers in preferences to chemical fertilizer is always welcome taking into consideration the suitability of agriculture. Thus, from the present study it can be concluded that the application of beneficial microbes devouring plant growth promoting traits will reduce the use of such chemical fertilizers to some extent thereby remediating the crop soil. In the present study the isolates were characterized based on their morphological and biochemical features, also the isolates were screened for different plant growth promoting activities. In future the isolates can be screened for different plant growth promoting traits and suitable PCR based genotypic techniques can be employed to confirm their identity at strain level and to predict the phylogenetic relationship of the isolates.

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REFERENCES

An, D.O., La, D.O., Js, A., Jaf, C., 2007. Rhizobia amylase production using various starchy substances as carbon substrates. Braz. J. Microbiol., 38, Pp. 208-216.

Aneja, K.R., 2003. Experiments in Microbiology, Plant Pathology and Biotechnology (4th edn). New Age International Publishers, New Delhi, India.

Anitha, G., Kumudini, B.S., 2014. Isolation and characterization of fluorescent pseudomonads and their effect on plant growth promotion. Journal of Environmental biology, 35, Pp. 627-634.

Antoun, H., Beauchamp, C.J., Goussard, N., Chabot, R., Lalande, R., 1998. Potential of Rhizobium and Bradyrhizobium species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus L.*). Plant and Soil, 204, Pp. 57–67.

Bakker, A.W., Schipperes, B., 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomoas spp. - mediated plant growth stimulation. Soil Biol. Biochem., 19, Pp. 451 - 457.

Bernaerts, M.J., Deley, J.A., 1963. A biochemical test for crown gall bacteria. Nature, 197, Pp. 406-07.

Cappuccino, J.C., Sherman, N., 1992. Microbiology: A Laboratory Manual, New York, Pp. 125-179.

Cappuccino, J.G., Sherman, N., 2005. Microbiology: A Laboratory Manual, 7th Ed, Pp. 161-204. Pearson, India

Datta, A., Singh, R.K., Kumar, S., 2015. Isolation, characterization and growth of Rhizobium strains under optimum conditions for effective bio-fertilizer production. Int. J. Pharm. Sci. Rev. Res., 32 (1), Pp. 199-208.

Deka, A.K., Azad, P., 2006. Isolation of Rhizobium strains: Cultural and Biochemical characteristics. Legumes Res., 29 (3), 209-212.

Deora, G.S., Singhal, K., 2010. Isolation, biochemical characterization and preparation of biofertilizers using Rhizobium strains for commercial use. Biosci. Biotech. Res. Comm., 3 (2), Pp. 132-136.

Deshwal, V.K., Chaubey, A., 2014. Isolation and characterization of Rhizobium leguminosarum from root nodule of Pisum sativum L. J. Acad. Indus. Res., 2 (8), Pp. 464-467.

Dilworth, M.J., Parker, C.A., 1969. Devolopment of the nitrogen fixing system in legumes. J. Theor. Biol., 25, Pp. 208-218.

Ehteshamul-Haque, S., Ghaffar, A., 1993. Use of rhizobia in the control of root rots diseases of sunflower, okra, soybean and mungbean. Journal of Phytopathology, 138, Pp. 157-163.

FAOSTAT, 2017. Statistical Database 2017. Available at ⟨http://faostat.fao.org⟩

Fatima, Z., Zia, M., Chaudhary, M.F., 2006. Effect of Rhizobium strains and phosphorus on growth of soybean (Glycine max) and survival of Rhizobium and P solubilizing bacteria. Pak. J. Bot., 38, Pp. 459-464.

Franche, C., Lindstrom, K., Elmerich, C., 2009. Nitrogen fixing bacteria associated with leguminous and non-leguminous plants. Plan Soil, 321, Pp. 35-59.

Gachande, B.D., Khansole, G.S., 2011. Morphological, cultural and biochemical characteristics of Rhizobium japonicum syn. and Bradyrhizobium japonicum of' soybean. Bioscience Discovery, 2 (1), Pp. 1-4.

Gauri, Singh, A., Bhatt, R., Pant, S., Bedi, M., Naglot, A., 2011. Characterization of Rhizobium isolated from root nodules of Trifolium alexandrinum. J. Agric. Technol., 7 (6), Pp. 1705-1723.

Gauri, Singh, A., Bhatt, R., Pant, S., Bedi, M., Naglot, A., 2011. Characterization of Rhizobium isolated from root nodules of *Trifolium alexandrinum*. J. Agric. Technol., 7 (6), Pp. 1705-1723.

Gordon, S.A., Weber, R.P., 1950. Colorimetric estimation of indoleacetic acid. Plant Physiol., 26, Pp. 192-195.

Graham, P.H., Parker, C.A., 1964. Diagnostic features in the characterization of root nodule bacteria of legumes. Plant Soil, 20, Pp. 383-396.

Hofer, A.W., 1935.Methods for distinguishing between legume bacteria and their most common contaminant. J. Am. Soc. Agron., 27, Pp. 228-230.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T., 1994. In: Bergey's manual of Determinative Bacteriology. Williams and Wilkins Press, Baltimore, U.S.A.

Hunter, W.J., Kuykendall, L.D., Manter, D.K., 2007. *Rhizobium selenireducens* sp. Nov.: A selenite-reducing alpha-proteobacteria isolated from a bioreactor. Curr Microbiol., 55, Pp. 455-460.

Joseph, B., Patra, R.R., Lawrence, R., 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (Cicer arietinum L.). Int. J. Plant Prod., 2, Pp. 141-152.

Kloepper, J.W., Leong, J., Teintze, M., Schroth, M.N., 1980. Enhanced plant growth by siderophores produced by plant-growth promoting Rhizobacteria. Nature, 286, Pp. 885-886.

Kumari, B.S., Ram, M.R., Mallaiah, K.V., 2010. Studies on nodulation, biochemical analysis and protein profiles of Rhizobium. Malay. J. Microbiol., 6 (2), Pp. 133-139.

Lee, J.Y., Song, S.H., 2007. Evaluation of groundwater quality in coastal areas. Implications for sustainable agriculture. Environmental Geology, 52, Pp. 1231-1242.

Mahana, S.K., Garg, R., Parvateesam, M., 2000. Cultural and Biochemical Characteristics of root nodule bacteria from induced mutants of Vigna mung L. Seed Pathology. Printwell publications, Jaipur, Pp. 417-421.

Mehta, S., Nautiyal, C.S., 2001. An Efficient Method for Qualitative Screening of Phosphate-Solubilizing Bacteria. Current Microbiology, 43, Pp. 51–56.

Myron, N.V., Williams, 1989. Metabolism of Tryptophan and Tryptophan Analogs by Rhizobium meliloti. Plant Physiol., 92, Pp. 1009-1013.

Pani, B.K., Patra, A.K., 1997. Utilization of some phytoextracts of S. rolfsii during paddy straw mushroom cultivation a new approach. Mushroom Res., 6 (1), Pp. 37-41.

Pani, B.K., Patra, A.K., 1997. Utilization of some phytoextracts of S. rolfsii during paddy straw mushroom cultivation a new approach. Mushroom Res., 6 (1), Pp. 37-41.

Rigby, D., Caceres, D., 2001. Organic farming and the sustainability of agricultural systems. Agricultural Systems, 68, Pp. 21-40.

Salve, P.B., Gangwanae, L.V., 1992. Rhizobium from wild legumes and nitrogen fixation in ground nut. In: Biofertilizers Technology Transfers Associated Publications Co., New Delhi, Pp. 95-100.

Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem., 160, Pp. 47-56.

Siddiqui, I.A., Ehteshamul-Haque, S., Zaki, M.J., Ghaffar, A., 2000. Greenhouse evaluation of rhizobia as bio-control agent of root infecting fungi in Okra. Acta Botanica, 53, Pp. 13-22.

Singh, Baljinder, Kaur, Ravneet, Singh, Kashmir. 2008. Characterization of Rhizobium strain isolated from the roots of *Trigonella foenamgraecum* (fenugreek). African Journal of Biotechnology, 7 (20), Pp. 3671 – 3676.

Somasegaran, P., Hoben, H.J., 1994. Handbook for rhizobia-methods in legume-rhizobium Technology, Springer-Verlag, New York.

Trinick, M.J., 1982. Biology. In Nitrogen Fixation (Broughton WJ). Clarendon Press, Oxford, 2, Pp. 76-146.

Vincent, J.M., 1970. A Manual for the Practical Study of Root-Nodule Bacteria. Blackwell Scientfic Publications, Oxford