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Research Article

OCCURRENCE, CHARACTERIZATION OF NATIVE RHIZOBIA AND NODULATION PATTERN, SOIL NUTRIENT STATUS GROWN IN GROUNDNUT OF TSUNAMI AFFECTED COASTAL AREA OF CUDDALORE DISTRICT OF TAMIL NADU

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ABSTRACT

The bacteria of the soil usually called Rhizobium have a considerable importance in agriculture because of their capacity to fix the atmospheric nitrogen in symbiosis with the plants of the family of legumes. The present research work, we have collected soil samples from thirty location of Tsunami affected soil of Cuddalore district. Total thirty soil samples were enumerate the total rhizobial population. The maximum population was recorded in periyapattu (8.70 X 103 / g-1 of soil) followed by other locations respectively. The root nodules were collected from 30 different locations of Cuddalore district. All the nodules were isolated of Bradyrhizoium on YEMA medium. Further these isolates were tested biochemical characters based on their morphological and biochemical test. All the thirty locations, the nodulation pattern was observed in Periyapattu followed by other location respectively. Further the physic-chemical properties of soil samples were analysed. Further these isolated rhizobial stains were screened their salt tolerant efficiency in different salt concentration level.

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INTRODUCTION

Soil salinity is a significant problem facing agricultural production in many areas and soil infertility in these areas is often due to the presence of large concentrations of salt. Most leguminous plants required neutral or slightly acidic soil for the growth, especially when they depend on symbiotic N_2 fixation and as well more sensitive to salinity than their rhizobial counterparts and consequently, the symbiosis being more sensitive to salt stress than free-living rhizobia.

Bacteria of family Rhizobiaceae are symbiotic and effectively convert atmospheric nitrogen which is utilized by the host. Rhizobiaceae family contains six genera namely., *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, *Azorhizobium* and *Bradyrhizobium* (Okazaki *et al*., 2004). Biofertilizer promotes plant growth and productivity has internationally been accepted as an alternative source of chemical fertilizer. Rhizobacteria effectively colonize plant root and increases plant growth by production of various plant growth hormones, P-solubilizing activity, N_2 fixation and biological control activity (Deshwal *et al.*, 2011). The alkaline soils have fertility problems due to poor physical properties which adversely affect the growth and the yield of crops and inoculation with *Rhizobium* and AMF biofertilizer is more effective for promoting growth of faba bean grown in alkaline soils than the individual treatment, reflecting the existence of synergistic relationships among the inoculants (Abd-Alla *et al.,* 2013). There are about 750 genera of legumes (Young and Haukka, 1996). Although most rhizobia are host specific, but it is also true that several different bacterial species are also isolated from a single legume species and it is only from limited hosts which have been examined as far as microsymbionts are concerned (Arora *et al.*, 2001). These rhizobia are characterized into two groups on the basis of growth rate. First group is fast grower rhizobia and second is slow grower rhizobia (Lohis and Hansen, 1921).

Deshwal *et al.* (2003) reported that the association of slow grower and fast growing rhizobia with *Arachis hypogaea* L. Both *Rhizobium* and *Agrobacterium* have placed in the family Rhizobiaceae in the order Eubacterials. Fred *et al.* (1932) observed that the *Agrobacterium* spp. too show colonies on YEMA medium indistinguishable from fast growing species of *Rhizobium*. Allen and Allen (1950) observed that YEMA medium containing congo red (1:400) is absorbed by *Agrobacterium*. On the other hand, *Bradyrhizobium* rapidly utilized hexose (galactose, gluconate, glucose and mannose) were able to lower the pH when supplied with any one of arabinose, galactose, glucose, mannose or xylose (pH 5-8) (Padmanabhan *et al.*, 1990). Rhizobia is characterized on the basis of biochemical tests. Gachande and Khansole (2011)

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isolated *Rhizobium joponicum* syn. and *Bradyrhizobium japonicum* from root nodules of Soy bean (*Glycine max* L.) on YEMA medium and its morphological, cultural and biochemical characteristics were studied. The aim of present study is to occurrence, isolation, characterization of native rhizobia and nodulation pattern and soil nutritional status of groundnut in Tsunami affected coastal area of Cuddalore district.

MATERIALS AND METHODS

Collection of plant

Root nodules were collected from young and healthy seedling of groundnut from farmer's field at different locations of Tsunami affected area of Cuddalore district of Tamilnadu.

Occurrence of native rhizobia in different soil samples (MPN) (Thornton, 1983)

Groundnut seeds were surface sterilized with 0.1% mercuric chloride and washed in sterile water for several times. The sterilized seeds were sown on nitrogen free plant nutrient agar plants in test tubes by 4 x 20 cm capacity. On germination, they were inoculated with suspensions (10-1, 10-2,... 10-10) of the soil samples. At regular intervals the moisture contents of the tubes was, checked. The plants were examined and observed for extent of nodulation. The tubes with nodules were recorded as positive tubes and those without nodules as negative tubes - based on this the most probable number (MPN) of *Bradyrhizobium* was calculated .

Table 1 Native population of R*hizobium* in groundnut fields of 30 different locations of Tsunami affected saline areas of Cuddalore district of Tamil Nadu

S.No	Locations	*Rhizobium* population 1 X 10^3 / g^{-1} of moisture free soil
1	Periyakuppam	0.94
2	Rasapettai	3.4
3	Nanamedu	2.88
4	Sithirapettai	0.8
5	Alapakkam	4.00
6	Devanampattinam	2.88
7	Sonankuppam	5.66
8	Ayyampettai	3.00
9	Periyapattu	8.7
10	M.G.R. Thittu	1.88
11	Mudasal odai	2.44
12	Kodiyampalaiyam	3.66
13	B. Mutulur	0.78
14	Parangipettai	6.00
15	Killi	5.44
16	Singarakuppam	3.88
17	Thillaividangan	2.00
18	Boondiyan kuppam	7.00
19	Pichavaram	5.00
20	Kavarapattu	2.44
21	Kanakkarapattu	1.22
22	Kothattai	3.22
23	Silambimangalam	5.00
024	Samiyarpettai	5.2
25	Manampadi	6.22
26	Jayankondapattinam	7.88
27	Vallampadugai	4.3
28	Keelaperambai	4.66
29	Melathirukazhiphalai	6.44
30	T.S. Pettai	4.00

Isolation of root nodulating Rhizobia

Groundnut plants were uprooted carefully so as to get intact are obtained. These were brought in laboratory without any delay. Healthy peanut nodules were detached from the root and further isolation of root nodulating rhizobia was carried out (Vincent, 1970). The detached root nodules were washed in tap water to remove the adhering soil particles from nodule surface. Nodules were dipped in 0.1% mercuric chloride ($HgCl_2$) solution for 30 sec and later washed successively ten times with sterilized distilled water to remove the traces of toxic $HgCl_2$. Surface sterilized nodules were transferred in test tube containing 5 mL sterilized distilled water. These nodules were crushed with the help of sterilized glass rod to obtain a milky suspension of bacteriods. These were streaked on YEMA containing congo red. The plates were sealed by parafilm to avoid contamination and incubated at 28±1^oC for 24-48 h. *Bradyrhizobium* or *Rhizobium* colonies were remained white, translucent, elevated and mucilaginous, after 24-72 h, where as contaminations turned red. The colony were picked up and transferred to YEMA slant for further characterization.

Biochemical tests of rhizobia

Biochemical tests such as gram staining, growth on congored, growth on glucose peptone agar (Kleczkowska *et al.*, 1968), ability to produce 3-ketolactase (Gaur *et al.*, 1973), growth on hofer's alkaline medium was done.

Physico-chemical properties of Groundnut soil
Determination of soil properties of groundnut soils Soil pH

Ten gram of soil samples was taken in a beaker, 2.5 ml of water was added and stirred at regular intervals for 20-30 minutes. The pH meter was switched on and allowed for five minutes to warm up. Zero control was adjusted so as to bring the indicator to zero. By using a standard buffer solution the required pH range (0-7 or 7- 14) was made. The temperature dial was adjusted to the temperature of test solution. Control was adjusted to zero. After rinsing and wiping of the electrodes they were dipped into the test soil solution. By adjusting the range switch to pH, the indicator reading was noted and pH value recorded.

Organic matter (Walkey and Black, 1934)

The 0.5 g of finely ground soil sample was transferred to a 500 ml conical flask. To this 10 ml of the 1N Potassium dichromate prepared by dissolving 49.04 g of pure crystals of Potassium dichromate in one liter of water with the help of pipette. The 20 ml of concentrated H_2SO_4 was added and the contents of the flask shaken for a minute and set on an asbestos pad for exactly half an hour. At the end of the period, 200 ml of distilled water, 10 ml of phosphoric acid, and one ml of the diphenlamine indicator (prepared by dissolving 0.5 g of diphenlamine in a mixture of 100 ml of conc. H_2SO_4 and 20 ml of water.

Total nitrogen (Kenny and Bremner, 1962)

It was estimated by converting the combined nitrogen in soil organic matter to ammoniacal form with concentrated sulphuric acid. Hundred mg samples were transferred into 50 ml pyrex microkheldahl flask. A quarter teaspoonful of digestion mixture

Table 2 Biochemical characterization of *Bradyrhizobium* isolates obtained from groundnut of Tsunami affected saline areas of Cuddalore district of Tamil Nadu

Name of the Bradyrhizobial isolates	Infectivity test	Gram staining	Growth on Congored	Growth on Glucose peptone agar	Growth on Ketolactose agar	Growth on Hofer's alkaline medium
GNBJ-1	+	-ve	NA	NG	NC	NG
GNBJ -2	+	-ve	NA	NG	NC	NG
GNBJ -3	+	-ve	NA	NG	NC	NG
GNBJ -4	+	-ve	NA	NG	NC	NG
GNBJ-5	+	-ve	NA	NG	NC	NG
GNBJ-6	+	-ve	NA	NG	NC	NG
GNBJ-7	+	-ve	NA	NG	NC	NG
GNBJ-8	+	-ve	NA	NG	NC	NG
GNBJ-9	+	-ve	NA	NG	NC	NG
GNBJ-10	+	-ve	NA	NG	NC	NG
GNBJ-11	+	-ve	NA	NG	NC	NG
GNBJ-12	+	-ve	NA	NG	NC	NG
GNBJ-13	+	-ve	NA	NG	NC	NG
GNBJ-4	+	-ve	NA	NG	NC	NG
GNBJ-15	+	-ve	NA	NG	NC	NG
GNBJ-16	+	-ve	NA	NG	NC	NG
GNBJ-17	+	-ve	NA	NG	NC	NG
GNBJ-18	+	-ve	NA	NG	NC	NG
GNBJ-19	+	-ve	NA	NG	NC	NG
GNBJ-20	+	-ve	NA	NG	NC	NG
GNBJ-21	+	-ve	NA	NG	NC	NG
GNBJ-22	+	-ve	NA	NG	NC	NG
GNBJ-23	+	-ve	NA	NG	NC	NG
GNBJ-24	+	-ve	NA	NG	NC	NG
GNBJ-25	+	-ve	NA	NG	NC	NG
GNBJ-26	+	-ve	NA	NG	NC	NG
GNBJ-27	+	-ve	NA	NG	NC	NG
GNBJ-28	+	-ve	NA	NG	NC	NG
GNBJ-29	+	-ve	NA	NG	NC	NG
GNBJ-30	+	-ve	NA	NG	NC	NG

-ve =Gram negative NA= No Absorbance NG=No Growth NC=No Colour change

Table 3 Survey on the Nodulation pattern of Groundnut grown in Tsunami affected saline areas of Cuddalore district of Tamil Nadu

S.No	Locations	No. of Green nodules (Plant^{-1})	No. of Pink Nodules (plant^{-1})	Total no of Nodules (plant^{-1})
1	Periyakuppam	2	12	14.00
2	Rasapettai	3	8	11.00
3	Nanamedu	5	8	13.00
4	Sithirapettai	12	15	27.00
5	Alapakkam	7	9	16.00
6	Devanampattinam	4	8	12.00
7	Sonankuppam	6	15	21.00
8	Ayyampettai	4	14	18.00
9	Periyapattu	12	22	33.00
10	M.G.R. Thittu	10	8	18.00
11	Mudasal odai	2	9	11.00
12	Kodiyampalaiyam	7	10	17.00
13	B. Mutulur	3	9	12.00
14	Parangipettai	8	6	14.00
15	Killi	7	12	19.00
16	Singarakuppam	6	8	14.00
17	Thillaividangan	4	7	11.00
18	Boondiyan kuppam	3	7	10.00
19	Pichavaram	5	7	12.00
20	Kavarapattu	4	6	10.00
21	Kanakkarapattu	3	8	11.00
22	Kothattai	4	10	14.00
23	Silambimangalam	6	9	15.00
24	Samiyarpettai	3	7	9.00
25	Manampadi	4	7	11.00
26	Jayankondapattinam	8	12	20.00
27	Vallampadugai	2	9	11.00
28	Keelaperambai	3	10	13.00
29	Melathirukazhiphalai	5	11	16.00
30	T.S. Pettai	5	7	12.00

Table 4 Physico-chemical properties of the soil samples collected from different locations of Cuddalore District of Tsunami affected saline areas of Tamil Nadu

S.No	Locations	Soil type	Soil pH	EC (dsm^{-1})	Organic carbon (%)	Availble N (kg ha^{-1})	Availble P (kg ha^{-1})	Available K (kg ha^{-1})
1	Periyakuppam	Sandy loam	7.45	0.47	0.44	82.00	12.00	132.00
2	Rasapettai	Sandy loam	7.60	0.52	0.68	76.38	14.20	160.00
3	Nanamedu	Sandy loam	8.20	0.41	0.60	80.16	15.30	145.27
4	Sithirapettai	Sandy loam	7.50	0.60	0.62	65.47	13.89	100.00
5	Alapakkam	Sandy loam	7.34	0.27	0.85	98.35	17.00	196.00
6	Devanampattinam	Sandy loam	8.36	0.35	0.72	72.30	14.00	185.25
7	Sonankuppam	Sandy loam	8.00	0.44	0.58	96.75	11.00	120.20
8	Ayyampettai	Sandy loam	7.76	0.66	0.75	105.18	16.00	170.45
9	Periyapattu	Sandy loam	7.95	0.52	0.84	60.20	11.05	175.40
10	M.G.R. Thittu	Sandy loam	7.54	0.37	0.72	90.85	15.10	178.25
11	Mudasal odai	Sandy loam	7.97	0.70	0.69	78.47	11.00	150.00
12	Kodiyampalaiyam	Sandy loam	8.05	0.68	0.50	100.00	10.50	160.00
13	B. Mutulur	Loamy sand	7.68	0.54	0.68	108.10	13.00	145.10
14	Parangipettai	Clay loam	7.85	0.49	0.71	92.56	12.00	98.00
15	Killi	Sandy loam	7.92	0.73	0.59	110.20	12.89	85.00
16	Singarakuppam	Sandy loam	7.87	0.84	0.68	88.00	12.00	165.70
17	Thillaividangan	Sandy loam	7.80	0.75	0.65	107.45	14.40	129.30
18	Boondiyan kuppam	Clay loam	7.20	0.66	0.80	103.17	13.00	200.60
19	Pichavaram	Clay loam	7.46	0.58	0.75	112.00	13.10	125.00
20	Kavarapattu	Clay loam	7.38	0.62	0.72	129.00	15.70	137.00
21	Kanakkarapattu	Clay loam	7.42	0.75	0.67	110.00	12.00	176.00
22	Kothattai	Sandy loam	7.58	0.82	0.78	104.18	12.85	164.00
23	Silambimangalam	Sandy loam	7.53	0.63	0.56	100.20	16.89	190.00
24	Samiyarpettai	Sandy loam	8.12	0.77	0.65	113.35	19.00	138.00
25	Manampadi	Clay loam	7.85	0.82	0.58	95.17	13.60	156.00
26	Jayankondapattinam	Sandy loam	7.81	0.75	0.50	124.35	16.89	138.00
27	Vallampadugai	Sandy loam	7.76	0.69	0.63	110.25	19.45	120.00
28	Keelaperambai	Clay loam	7.38	0.71	0.76	120.00	18.70	195.00
29	Melathirukazhiphalai	Sandy loam	7.47	0.75	0.58	79.10	17.00	140.00
30	T.S. Pettai	Sandy loam	7.60	0.64	0.72	85.28	15.30	155.40

of (10 parts of reagent grade potassium sulphate, 1 part of cupric sulphate and 0.1 part of selenium metal powder) and one ml of salicylic sulphuric acid with a pinch of sodium thiosulphate were introduced and the contents were slowly heated till frothing ceased and then heated strongly. Completion of digestion was indicated by solution turning bluish green.

After cooling about 15 ml of distilled water was added to flask and cooled, the contents were transferred into the distillation unit and 25 ml of the 40 per cent sodium hydroxide added and steam distilled into an excess of 0.1 N sulfuric acid (10 ml) containing 2 drops of methyl red indicator. Distillation was continued for 10 minutes. The contents were back titrated using. 0.1 N potassium hydroxide till the appearance of golden yellow color. Nitrogen in the sample was calculated using the factor l ml of 0.1 N sulfuric acid = 0.0014g of nitrogen.

Available phosphorus (Olsen et al., 1954)

Five gram of soil was taken in a conical flask and one teaspoonful of charcoal powder, 100 ml of sodium bicarbonate solution 0.5 M (prepared by dissolving 42 g of $NaHCO_3$ in 1000 ml distilled water and pH is adjusted to 8.5 with 10.20 per cent NaOH solution) was added to the soil and shaken for half an hour. This was filtered through Whatmann No.40 and 5 ml of filtrate was pipetted out into a 25 ml volumetric flasks and 5 ml of molybdate reagent (15 g of ammonium molybdate in 400 ml of distilled water, filtered and 400 ml of conc. HCl was added and made upto one litre) Was added, and 1 ml of dilute solution of stannous chloride was added and made upto 25 ml and the phosphorous was determined volumetrically.

Electrical conductivity (Jackson, 1973)

Conductivity cell was immersed into the soil water extract (prepared by adding 20 g of soil to 100 ml of distilled water,) the contents shaken, were kept undisturbed and the supernatant liquid filtered through the filter paper. The filtrate kept in a beaker, knob was rotated and dark segment to magic eye was observed till the maximum deflection of the dark segment is magic eye is *obtained to get the* null point. Dial reading was noted and the range of 'multiply' knob. Electrical conductance was multiplied by cell constant, temperature and correction factor to obtain the specific conductance in dsm^{-1}.

RESULTS AND DISCUSSION

Native rhizobium population in the groundnut field from Tsunami affected area of Cuddalore district

Native rhizobial population of the different soil samples was estimated by the most propable number (MPN) method. Among the 30 soil samples, the soil collected at Periyapattu possessed the maximum rhizobial population of (8.70 x 10^6 cell g^{-1}) number of cells followed by other locations respectively. The minimum population was recorded in sithirapettai of Cuddalore district (Table-1).

Isolation of Root nodulating rhizobia from groundnut

The root nodules were collected from 30 different locations of Cuddalore district. All the nodules were isolated of *Bradyrhizoium* on YEMA medium and the results were presented in Figure-1. These isolates were used for further biochemical characteristics.

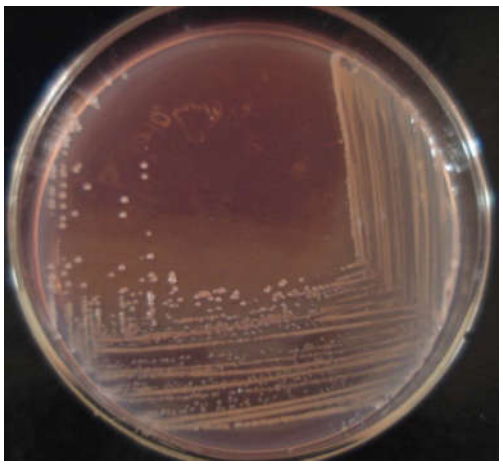

Fig-1 Isolated culture of *Bradyrhizobium japanicum* on YEMA medium

Biochemical Characterization of rhizobia

All the thirty *Bradyrhizobium* isolates were tested based on their biochemical characterization and identified. And the results were presented in Table-2.

Survey on the nodulation pattern of groundnut in different location of Cuddalore district

Thirty different location were selected in Tsunami affected area of Cuddalore district for the survey of nodulation pattern of Groundnut in rainfed areas in such a way that each and every sector of district is represented in the survey. The observation made on the natural nodulation are given in Table-3.

Physico chemical properties of soil samples collected from different locations of Cuddalore district

The thirty soil samples collected from different locations of Tsunami affected area of Cuddalore district were analysed such as pH, EC and NPK respectively in Table-4.

The occurance of native rhizobial population differ from different soil condition. In general saline soil contain low population when compared to normal soil. In our results the rhizobial population recorded average population in all the thirty soil samples. The similar results also reported by Bottomley and Maggard (1990).

In the present study, strains of root nodulating bacteria were isolated from the root nodules of an important fodder legume groundnut growing in different location of Tsunami affected area in Cuddalore district. All the thirty isolates were obtained Bradyrhizobium strains. similarly, Similarly, Shahzad *et al.* (2012) isolated *Rhizobium* from root nodules of Alfalfa (*Medico sativa*) plant and characterized on the basis of various biochemical tests.

Microscopic examination revealed that the isolates were rod shaped and gram negative in nature (Singh and Singh, 2008). In our result all the thirty rhizobia isolates were identified based on their morphological and biochemical characters. All the strains were identified as Bradyrhizobium japanicum. Similarly Gauri et al. (2011) characterization of *Rhizobium* isolated from root nodules of *Trifolium alexandrinum*.

Previously, Sadowsky *et al.* (1983) mentioned that fast-growing soybean rhizobia were positive for catalase, urease, oxidase, nitrate reductase, tolerated 2% NaCl, capable to grow at pH 9.5 and fermented L-arabinose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, L-rhamnose and D-xylose. Similarly, Singh and Singh. (2008) also characterized *Rhizobium* strains on the basis of biochemical tests. *Rhizobium* is symbiotic bacteria which form nodule in leguminous plant.

Salinity is a severe problem in many regions of the world which changes physico-chemical characteristics of soil. In salt-affected soil pH inhibits water and nutrient uptake although there is sufficient quantity of them in soil (Bor et al., 2003).

In our result, all the thirty soil sample were analyzed physic-chmical properties the Ph ranged from 7.20 to 8.36 , Ec ranged from0.27 to 0.82 dsm^{-1} and organic carbon ranged from 0.44 to 84 % respectively. The three major nutrient such as nitrogen, phosphorus and potassium were ranged from 60.20 to 124.35 kg ha^{-1}, 10.50 to 18.70 kg ha^{-1} and 85.00 to 200.60 kg ha^{-1} respectively. Soil organic carbon is a key resource owing to its ameliorative effect on nutrient supply, detoxification of harmful soil constituents, moisture and nutrient retention and its role in soil structure formation. Organic carbon content (Table-4). The results also reported by Siderius (1992). This indicates low N releases from the organic matter sources, since nitrogen content of soils is usually positively, correlated with organic matter content, since over 90% of the nitrogen found in soils is in organic form (Brady, 1990). There is generally a decreasing trend in available P with increasing depths in all the three pedons. The low organic matter content coupled with the highly acidic conditions of these soils may explain the low available–P levels in the soils (Munns & Franco, 1982). The low levels of available pottassium observed are attributable to the low organic matter levels and pH of these three pedons (Munns & Franco, 1982)

CONCLUSION

The results from this study showed that the native rhizobia population isolated in saline soil recorded average population and it can able to survive and grow in salinity area. The root nodulation pattern observed the good nodule in Tsunami affected soil. The available N,P, K also recorded average. Further the isolated rhizobial stains were screened their salt tolerant efficiency in different salt concentration level.

References

Abd-Alla, M.H., El-Enany, A.W., Nafady, N.A., Khalaf, D.M. and Morsy, F.M. 2013. Synergistic interaction of

Rhizobium leguminosarum bv. *viciae* and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. *Microbiol. Res.* S0944-5013(13): 117-121.

Allen, E.K. and Allen, O.A. 1950. Biochemical and symbiotics properties of the rhizobia. *Bacteriol. Rev.* 14: 273-330.

Arora, N.K., Kang, S.C. and Maheshwari, D.K. 2001. Isolation of siderophore producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. *Curr. Sci.* 81: 673-677.

Bor, M, F.Ozdenir and I. Turkan, Plant Sci., 2003, 164, 77-84.

Bottomley, P.J. and Maggard, S.P. (1990). Determination of viability within serotypes of a soil population of *Rhizobium leguminosarum* bv. *trifolii*. Appl. Environ. Microbiol. 56:533-540.

Brady N. C. (1990). The Nature and Properties of Soils, 10th edn. Collier Macmillan Publishers, London, UK. pp. 83–97.

Deshwal, V.K., Pandey, P., Kang, S.C. and Maheshwari, D.K. 2003. Rhizobia as a biological control agent against soil borne plant pathogenic fungi. *Ind. J. Exp. Biol.* 41: 1160-1164.

Deshwal, V.K., Vig, K., Amisha, D.M., Yadav, P., Bhattacharya, D. and Verma, M. 2011. Synergistic effects of the inoculation with plant growth-promoting *Rhizobium* and *Pseudomonas* on the performance of Mucuna. *Ann. Forestry*. 19(1): 13-20.

Elkan, G.H. 1992. Taxonomy of the rhizobia. *Can. J. Microbiol.* 38: 446-450.

Fred, E.B., Baldwin, I.L. and McCoy, E. 1932. Root nodule bacteria and leguminous plants. University of Wisconsin Studies in Science No.5. University of Wisconsin, Madison.

Gachande, B.D. and Khansole, G.S. 2011. Morphological, cultural and biochemical characteristics of *Rhizobium japonicum* syn. and *Bradyrhizobium japonicum* of' soybean. *Biosci. Discov.* 2(1): 1-4.

Gaur, Y.D., Sen, A.N. and Subba Rao, N.S. 1973. Usefulness of limitation of ketolactose test to distinguish *Agrobacterium* from *Rhizobium*. *Curr. Sci.* 42: 545-546.

Gauri.L, Singh AK, Bhatt RP, Pant S, Bedi MK, Naglot A. Characterization of Rhizobium isolated from root nodules of *Trifolium alexandrinum*. Journal of Agricultural Technology 2011;6:1705-23.

Jackson, M. C. 1973. Soil chemical analysis. Practice Hall of India Pvt. Ltd., New Delhi.

Kenny, D. R. and J. M. Bremner 1970. Determination of nitrogen by Microkjeldanl method. *J. Aqric. Sci*., 55:11 - 33.

Kleczkowska, J., Nutman, P.S., Skinner, F.A. and Vincent, J.M. 1968. The identification and classification of *Rhizobium*. In, *Identification Methods of Microbiologists,* Part B, (eds. Fibbs B. M. & Shapton D. A., London). pp.51-65.

Löhis, F. and Hansen, R. 1921. Nodulating bacteria of leguminous plant. *J. Agric. Res*. 20: 543-556.

Munns D. N. and Franco A. A. (1982). Soil constraints on legume production. In Biological Nitrogen Fixation Technology for Tropical Agriculture (P. Graham and S. C. Harris, ed.), pp. 133–152. CIAT, California.

Okazaki, S., Nukui, N., Sugawara, M. and Minamisawa, K. 2004. Rhizobial strategies to enhance symbiotic Interactions: Rhizobiotoxine and 1-Aminocyclopropane -1-Carboxylate deaminase. *Microb. Environ*. 19(2): 99-111.

Olsen, S. R., C. V. Cole, R S. Wataube and L. A. Dean 1954. Estimation of available phosphorus in soils by extraction with sodium carbonate. U. S. Dept. Agri. Circ., 939.

Sadowsky, M.J., Keyser, H.H. and Bohlool, B.B. 1983. Biochemical characterization of fast- and slow-growing rhizobia that nodulate soybeans. *Int. J. Syst. Bacteriol.* 33(4): 716-722.

Shahzad, F., Shafee, M., Abbas, F., Babar, S., Tariq, M.M. and Ahmad, Z. 2012. Isolation and biochemical characterization of *Rhizobium meliloti* from root nodules of Alfalfa (*Medico sativa). J. Animal Plant Sci.* 22(2): 522-524.

Siderius W. (1992). Soil derived land qualities SOL. 48. Soil Science Division, Department of Land Resources and Urban Science, International Institute of Aerospace Survey and Earth Sciences, The Netherlands. pp. 37–84.

Singh, B., Kaur, R. and Singh, K. 2008. Characterization of *Rhizobium* strain isolated from the roots of *Trigonella foenumgraecum* (fenugreek). *Afr. J. Biotechnol*. 7(20): 3671-3676.

Thornton, F. E. and C. B. Davey, 1983. Acid tolerance of *Rhizobium trifolii* in culture medium. *Soil Sci. Am J.,* 47 : 496-501.

Vincent, J.M. 1970. A manual for the practical study of root nodule bacteria. IBP Hand book No. 15, Blackwell publication, oxford, U.K.

Walkley, A. and I. A. Black, 1934. An examination of the Degtjarett method for determining soil organic matter and a proposed modification of the chronic acid titration method. *Soil Sci.,* 34 :29-38.

Young, J.A.W. and Haukka, K. 1996. Diversity and phylogeny of rhizobia. *New. Phytol*. 133: 87-94.

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