Research Article

Isolation, Biochemical Characterization of *Rhizobium* sps SN01 Strain from Root Nodules of *Mimosa pudica* and their Impact on Agriculture Crops

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ABSTRACT

The present study deals with the isolation of *Rhizobium* bacteria from root nodules of *Mimosa pudica*, and to check its efficiency as bio-fertilizer in improving plant growth. *Rhizobium* sps SN01 was isolated from root nodules of *Mimosa pudica* and confirmed to be *Rhizobium* bacteria through following biochemical tests of indole, methyl red, Voges-Proskauer and citrate utilization (IMViC), urease, hydrogen sulphide, carbohydrate fermentation tests. *Rhizobium* sps SN01 was analysed for antibiotic sensitivity test towards three different antibiotics such as Azithromycin, Amoxicillin and Cephalosporin. To check the efficiency, seeds of legume crops like cow peas, peas, and fenugreek were treated with *Rhizobium* sps, SN01, sowed in soil and coco-peat to study its germination rate and effectiveness in plant growth resulting in root length, shoot length, protein content and chlorophyll content. Impact of *Rhizobium* sps SN01 bacteria in soil and coco-peat quality and improvement was also focused by analyzing micro and macro nutrients responsible for its quality to improve plant growth.

Keywords: *Rhizobium* sps SN01, *Mymosa pudica*, Bio-fertilizer, Legume crops, Cow peas, Soil, Coco-peat.

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INTRODUCTION

Bio-fertilizers are actually the natural mini- fertilizers which are responsible for providing safer plant nutrition and increasing the soil fertility improvement through natural processes.[1]

A bio-fertilizer is a substance containing living microorganisms which was applied to seeds, plant surfaces or soil, colonize the inner part of the plant or the rhizosphere, and also promote growth parameters by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers are cost-effective and ecofriendly in nature. The use of bio-fertilizer improves soil fertility by fixing atmospheric nitrogen, solubilising insoluble phosphates and producing plant growth-promoting substances in the soil. It also promotes nodulation efficiency and increases yield o crops by around 16–60%.[2] Nitrogen is the essential component which serves as the building blocks of proteins and nucleic acids and it is abundantly found in the earth's atmosphere but it cannot be utilized by plants because of the inert nature due to the presence of triple bonds between the nitrogen atoms. So for the nitrogen to be used by plants it must be fixed or converted to the form of ammonium or nitrite ions. There are certain microorganisms which are capable of converting the atmospheric nitrogen into ammonia or nitrite ions by a process known as nitrogen fixation. These

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nitrogen fixation can be done by certain soil microbes symbiotically as well as non-symbiotically.[1,3]

Many species of leguminous plants form a symbiotic association with various bacteria which are collectively named as "rhizobia". The rhizobia fix inert atmospheric nitrogen (N_2) into biologically useful forms within legume root nodules in a process called "Biological N_2 fixation" (BNF).[4] This symbiotic association is the largest natural source of the N cycle to sustain natural systems.[5] Nitrogen fixing bacteria popularly known as *Rhizobium* and it has been found to be having a greatest bio-activity in fixing this atmospheric nitrogen for plants and also increasing the soil fertility.[6] The aim of the current study is to isolate, characterize the *Rhizobium* bacteria from root nodules of *Mimosa pudica* and to check its impact on agriculture crops as bio-fertilizer in various leguminous plants.

MATERIALS AND METHODS

Isolation of *Rhizobium* bacteria from root nodules of *Mimosa pudica*

Root nodules of *Mimosa pudica* were freshly collected in sample collecting pouches and brought to the laboratory within 24 hr for the isolation of *Rhizobium* bacteria. Root nodules were cleansed thoroughly with tap water, followed by ethanol, distilled water and hydrogen peroxide to remove the adhering contaminants. Series of inoculum obtained from root nodules of *Mimosa pudica* and it was inoculated onto Selective Yeast Extract Mannitol Agar (YEMA) (Containing g/lt of Mannitol-10g; Magnesium Sulphate – 0.2g; Sodium Chloride-0.10g; Potassium di-hydrogen phosphate-0.50g; Calcium chloride-0.20g; ferric chloride-0.01g; Yeast extract-1gm; Congo red – 0.025g: Agar-10gm) and incubated at 35°C for 48 hr.[5,7]

Biochemical Characterization of *Rhizobium* sps

Isolates obtained from root nodule of *Mimosa pudica* were subjected to few of the biochemical tests, such as Gram's staining, catalase test, oxidase test, IMViC tests, urease test, hydrogen sulphide test, starch hydrolysis test, carbohydrate fermentation test (Glucose and Lactose) and antibiotic sensitivity test.[8]

Seed Treatment of Legume Crops with *Rhizobium* sps

The *Rhizobium* sps isolated from *Mimosa pudica* were batch cultured using yeast extract mannitol agar broth (YEMB), after the completion of incubation period, the bacterial load produced was separated by filtration and centrifugation and used as a bio-fertilizer on agriculture legume crops like, fenugreek, peas and cowpea. The above said seeds of legume crops were collected, brought into laboratory, processed by surface sterilization using tap water followed by hydrogen peroxide and distilled water to remove the contaminants adhering to the surface of seeds,. Later seeds were treated with the *Rhizobium* sps and sowed in soil and coco-peat to know the germinate rate. The test was repeated for the other batch of seeds without treating with *Rhizobium* sps and maintained as control.[9-10]

Evaluation of germination rate, protein and chlorophyll content in the legume crops

The treated and untreated legume crops were evaluated based on germination rate, protein content and chlorophyll content periodically to check the effect of *Rhizobium* sps.

Germination rate of plants in treated batch and untreated batch were studied by measuring its root length and shoot length every 5 days of interval for up to 30 days.[11]

Protein content was determined as described by Lowry's method[12] for the duration of 50 days study with interval of 10 days in each batch of crops and the total chlorophyll content was determined by the determination of chlorophyll A and B as described by Patel *et al*.[13]

Impact of *Rhizobium* sps on nutritional factors present in soil and coco- peat required for plant growth

The nutritional factors like pH, conductivity, organic carbon, organic matter, organic nitrogen, calcium, magnesium, sodium, potassium, nitrate, sulphate, phosphate and micronutrients like iron, zinc, nickel, lead, copper were studied in soil and coco-peat analysed as described by Gomare *et al.,*[9] with slight modification, in order to check *Rhizobium* sps contribution in enhancement of soil quality and coco-peat quality.[14]

RESULTS

Isolation of *Rhizobium* Bacteria from Root Nodules of *Mimosa pudica*

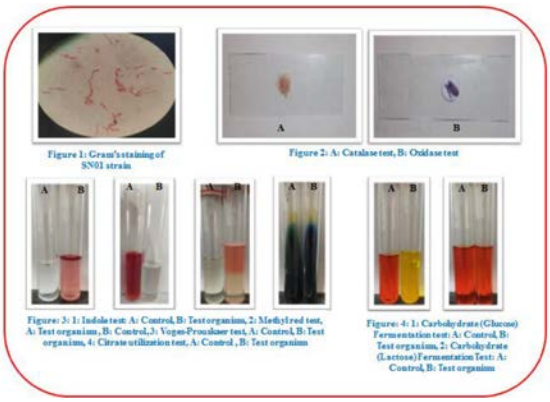
Rhizobium sps, was isolated on yeast extract mannitol agar (YEMA), distinct colonies similar to *Rhizobium* sps confirmed by colony characters like circular shape, semi-translucent, raised, whitish in color and designated as SN01 species number. The culture strain was sub-cultured and stored at 4°C for further work.

Biochemical characterization of *Rhizobium* sps

The results of biochemical tests are as tabulated in the Table 1 and images of tests are represented in Figures 1 to 5. Biochemical characterizations tests of SN01 showed gram negative rod shaped structure in

Table 1: Results of SN01 strain towards biochemical tests.

Sl. No.	Biochemical tests	Results
1.	Gram's Staining	Negative
2.	Shape	Rod
3.	Catalase test	Positive
4.	Oxidase test	Positive
5.	Indole production test	Positive
6.	Methyl red test	Positive
7.	Voges Proskauer test	Positive
8.	Citrate utilization test	Positive
9.	Hydrogen sulfide production test	Negative
10.	Carbohydrate fermentation test	
a.	Glucose	Positive
b.	Lactose	Negative
11.	Urease test	Positive
12.	Starch hydrolysis test	Positive



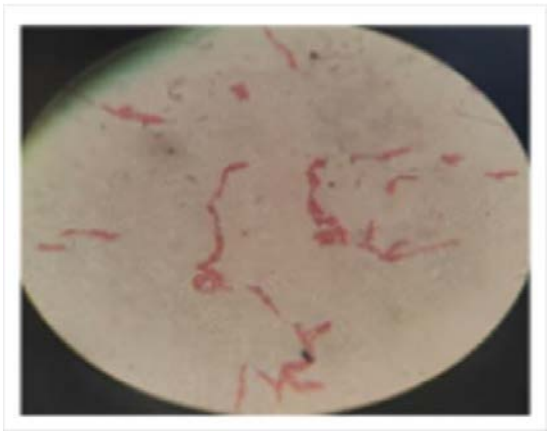


Figure 1: Gram's staining of SN01 stain.

Figure 2: Catalase test, B: Oxidase test

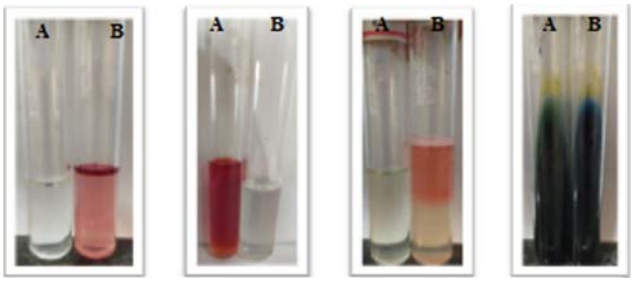


Figure 3: 1 Indole test, A: Control, B: Test organism, 2: Methylred test, A: Test organism, B: Control, 3: Voges-Prouskaer test, A: Control, B: Test organism, 4: Citrate utilization test, A: Control, B: Test organism.

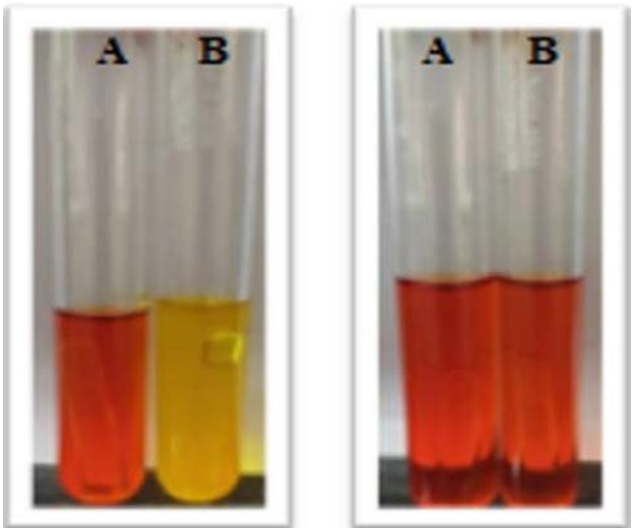


Figure 4: 1: Carbohydrate (Glucose) Fermentation test: A: Control, B: Test organism, 2: Carbohydrate (Lactose) Fermentation test: A: Control, B: Test organism

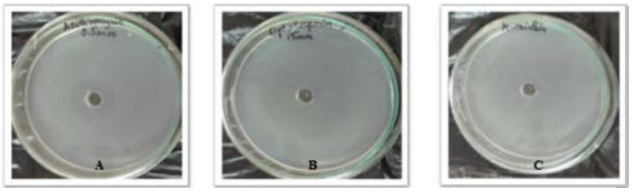


Figure 5: Antibiotic sensitivity test A: Azithromycin-5mm, B: Cephalosporin-15mm, C: Amoxicillin-Resistant.

Table 2: Effectiveness of *Rhizobium* sps SN01 in increasing germination rate at 15th day.

Potting Mix with Coco-peat								
Legume Crop ↓	Root Length (cm)		Shoot Length (cm)		Germination Rate (%)		Vigor Index	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Cow Pea	15	08	25	18	98	90	2465	1628
Fenugreek	10	8.5	15	10	96	86	1450	868.5
Peas	15.3	10	20.2	15	98	88	1994.9	1330
Potting Mix with Soil								
Legume Crop ↓	Root Length in cm		Shoot Length in cm		Germination Rate (%)		Vigor Index	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Cow Pea	12.4	08	22.6	18.8	98	88	2227.2	1662.4
Fenugreek	8	07	10	09.2	98	86	988	798.2
Peas	14	10.1	18.5	12.4	98	88	1827	1101.3

Table 3: Effectiveness of *Rhizobium* sps SN01 in increasing protein and chlorophyll content at 15th day.

Potting Mix →	Coco-peat				Soil			
Legume Crop ↓	Protein Content (mg/g)		Chlorophyll Content (mg/g)		Protein Content (mg/g)		Chlorophyll Content (mg/g)	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Cow Pea	59.5	52.0	0.64	0.48	40.0	39.5	0.43	0.6
Fenugreek	54.0	42.0	0.4	0.3	34.0	13.5	0.29	0.21
Peas	39.0	30.5	0.51	0.41	49.5	38.0	0.5	0.46

Gram's staining studies, positive result was showed by strain SN01 for catalase test, oxidase test, IMViC tests, urease test and starch hydrolysis test, in carbohydrate fermentation test strain SN01 showed positive result for glucose and negative result for lactose. Negative result was recorded for triple sugar iron agar test by SN01 strain. Antibiotic sensitivity test for antibiotics amoxicillin, cephalosporin, azithromycin was tested, strain SN01 showed 05mm and 15mm of inhibition towards azithromycin and cephalosporin respectively, whereas strain SN01 was resistant for amoxicillin.

Seed treatment of legume crops with *Rhizobium* sps SN01

Healthy seeds of cow peas, fenugreek, peas were selected for the germination study, the seeds were treated with culture of *Rhizobium* sps SN01 and sowed in two set of potting trays, and the other set was control which was untreated with *Rhizobium* bacteria, two sterile potting mixes, soil and coco-peat were used in the study.

Evaluation of Germination Rate, protein and chlorophyll Content in the Legume Crops

Rhizobium sps SN01 effectiveness in improving plant germination rate and plants major character like protein and chlorophyll content were studied for a period of 30 days. Germination rate and its vigor index was studied by checking root and shoot length of treated and untreated legume crops, Shoot and root length was more in treated plants compared to untreated plants, out of peas, cowpea and fenugreek plants, cowpeas in both soil and coco-peat showed higher vigor index. The results are as tabulated in Table 2, 3 and image in Figure 6. The Protein and chlorophyll content was measured in the plants, it was found to be treated plants have more protein and chlorophyll content compared untreated legume crops in both potting mix.

Impact of *Rhizobium* sps on nutritional factors present in soil and coco-peat required for plant growth

Nutritional factors in potting mixes play significant role in maintaining its quality and it also improves plant growth, So *Rhizobium* sps SN01 activity towards increasing nutritional factors were studied, major and minor nutrients in soil and coco-peat before treatment and after treatment were analysed, from the study it was noticed that *Rhizobium* sps SN01 was responsible in increasing the required nutritional factor in both soil and coco-peat. The results of the study are represented in Table 4.

Figure 6: Germination rate in legume crops, A: Cow pea, B: Fenugreek, C: Peas.

Table 4: Impact of *Rhizobium* sps *SN01* in increasing nutritional elements.

Nutritional Elements	Soil		Coco-peat	
	Treated	Untreated	Treated	Untreated
PH	6.8	6.5	5.5	5.2
Conductivity (µs/cm)	204	198	494	406
Organic Carbon (%)	1.0	0.8	8.7	08
Organic Matter (%)	1.7	1.0	15	11
Organic Nitrogen (mg/kg)	0.28	0.2	30.1	25.2
Calcium (mg/kg)	20	16	50	36
Magnesium (mg/kg)	10	08	10	08
Total Nitrogen (mg/kg)	4.8	1.2	39	30.8
Sulphate (mg/kg)	408	398	188	115
Sodium (mg/kg)	22	18	60	48
Potassium (mg/kg)	90	82	120	86
Phosphate (mg/kg)	10.2	08	16	12
Lead (mg/kg)	31	25	10	4
Copper (mg/kg)	19	10	11	6
Iron (mg/kg)	1748	1563	1555	1089
Nickel (mg/kg)	15	10	05	0.9
Zinc (mg/kg)	38	28	33	22

DISCUSSION

Enrichment of soil nutrients by nitrogen fixing bacteria in leguminous plants as a symbiotic relationship has been known history and natural system. Scientific investigation on this symbiotic relationship of plants and microbes was reported from 19th century and it evidenced the presence of microbial diversity in legume root nodules in that bacteria's were predominant and responsible for fixing atmospheric nitrogen.[3] *Rhizobium* spp. considered well known bacteria for the symbiotic relationshion with various plants to fix the nitrogen as a primary source. Such types of bacteria are infecting the leguminous plant roots, leading to establish the lump formation or cystic nodules where the occurrence of synthesis of nitrogen fixation.

Isolation and characterization of *Rhizobium* strain assessed for various studies, in that, the biochemical test conducted strain SN01 was confirmed to be a species of *Rhizobium* by the characters like, shape, size, media, pH, temperature, substrate specificity, etc. Hence the strain was further designated as *Rhizobium* sps SN01 with the result remarks. The results obtained for the test organism was similar to the work of Kumar *et al.*[15] and Purwaningsih *et al.*[16]

The work of Patel *et al.*[13] on soyabean with *Rhizobium* sps and other bio-fertilizers in accordance with germination rate, vigor index, protein and chlorophyll content proved that *Rhizobium* is an effective bio-fertilizer in improving and maintaining plant growth. Ranjbar *et al.*[11] proved the effectiveness of *Rhizobium leguminosarum* on *Pisum satvium* in increasing seed yield rate and various physical properties. Hence these similar reports are evidence for our work to present that *Rhizobium* sps SN01 strain plays a vital role in improving plant growth.

Nutrition in potting mixes play significant role in maintaining its quality and it also improves plant growth, So *Rhizobium* sps SN01 activity towards increasing nutritional factors were studied, major and minor nutrients in soil and coco-peat before treatment and after treatment were increased all the parameters such as pH (6.8 & 5.5), conductivity (204 & 494 µs/cm), organic carbon (1.0 & 8.7%), organic matter (1.7 & 15%), organic nitrogen (0.28 & 30.1 mg/kg), calcium (20 & 50 mg/kg), magnesium (10 & 10 mg/kg), total nitrogen (4.8 & 39 mg/kg), sulphate (408 & 188 mg/kg), sodium (22 & 60 mg/kg), potassium (90 & 120 mg/kg), phosphate (10.2 & 16), lead (31 & 10 mg/kg), copper (19 & 11 mg/kg), iron (1748 & 1555 mg/kg), nickel (15 & 5 mg/kg), zinc (38 & 33 mg/kg) compared tocontrol untreated parameters respectively. Gomare *et al.*[9] and Mia *et al.*[10] reported on rice and legume seeds respectively to check the ability of *Rhizobium* sps to help plant growth.

The present work also shows similar results and proves that *Rhizobium* sps SN01 is effectively improving germination rate of legume crops like cow peas, peas and fenugreek in soil and coco-peat conditions. Similar observation of Gomare *et al.*[9] proven that *Rhizobium* sps isolated from root nodules are responsible in enhancing soil characteristics before adding inoculum and after adding inoculum.

CONCLUSION

In the present study *Rhizobium* sps SN01 isolated from root nodules of *Mimosa pudica* was efficient in improving germination rate of legume crops like cow peas, peas and fenugreek in soil and coco-peat conditions, out of three crops *Rhizobium* sps SN01 was more responsible towards cowpeas in improving germination rate to 98% in soil and coco-peat, vigor index to 2465 in coco-peat and 2227.2 in soil, protein to 595mg/g in coco-peat and 400 mg/g in soil, chlorophyll to 0.64 mg/g in coco-peat and 0.43 mg/g in soil. *Rhizobium* sps SN01 influence in improving soil quality and coco-peat quality was also more effective, it was found that all the micro and macro nutrients required for plant growth was found to be significant in higher concentration compared to untreated soil and coco-peat. The present study concludes that potting mix coco-peat is more suitable for the growth of legume crops in the presence of *Rhizobium* sps SN01, in acquiring more yield and quality of all the three legume crops cow peas, peas and fenugreek.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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