ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

A COMPARATIVE STUDY OF *Rhizobium spp.* FROM VARIOUS LOCALITIES OF JALNA DISTRICT (M.S.)

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ABSTRACT

The soil is full of microorganisms (bacteria) which directly or indirectly helps plant in their growth and development. Such bacteria are known as plant growth promoting bacteria (PGPR). *Rhizobium* is one of the PGPR. It helps plants to fix atmospheric nitrogen into the form that the plants can use as nitrates and nitrites. *Rhizobium* symbiotically forms root nodules in legume plants and which makes legume rich in nitrogen. Now days, these symbiotic bacteria are used as biofertilier in plants other than legumes, which helps to replace use of chemical fertilizers. In this study, 12 different legume plant root nodules were collected from different localities. From these root nodules *Rhizobium* samples were isolated on *Rhizobium* mannitol agar. Rhizospheric soil samples were used for determining pH, water holding capacity and moisture content. Out of these collected 12 samples, strain 5, strain 6, strain 7 and strain 9 did not show resemblance with *Rhizobium* species. Morphological tests, biochemical tests of all stains were performed. *Rhizobium* is gram negative bacteria with rod shape having cell size approximately 0.07 to 1.2µm. Colony is circular, milky or yellowish average 3.38 mm in diameter which is translucent in appearance with entire margin. Biochemical tests were also performed which showed negative results for citrate while positive result for starch hydrolysis, catalase, urease, indole production and nitrate reduction. As *Rhizobium* can be used as bio fertilizer replacing commercially and economically harmful chemical fertilizers. So, its study is gaining importance in research.

Keywords: *Rhizobium* spp., PGPR, bioferilizer, Jalna district, biochemical tests.

Introduction-

Plant growth promoting rhizobacteria are certain group of rhizobacteria which have the mechanism to improve growth and health of plant. *Rhizobium* is one of the PGPR which is involved in biological nitrogen fixation. *Rhizobium* is the symbiotic nitrogen fixing bacteria which forms root nodules on the roots of legumes. It symbiotically and effectively converts atmospheric nitrogen which is utilized by the host. The microorganisms get attracted towards plants because of substances exuded by roots are essential for microbes (Sugiyama and Yazaki, 2012). Approximately 79% nitrogen is present in air but plants cannot use it directly (Kumari

ISSN: 2278-4632
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and Sinha 2011). Burdass (2002) confirmed that *Rhizobium* is the most well-known species that acts as primary symbiotic nitrogen fixer. *Rhizobium* as an essential microorganism in the growth and development of legumes (Agah *et al.,* 2013) *Rhizobium* is soil bacteria having unique ability to infect root hairs of legumes and induce formation of root nodules. *Rhizobium* is nonsporous, aerobic and motile bacteria. As because of *Rhizobium*-legume symbiosis, legume has rich supply of nitrogen, it is nutritionally important sources of protein and dietary fiber. (Annor *et al*., 2014), (Jahreis *et al*., 2015).

The association of *Rhizobium* with leguminous plants is superior to other nitrogen fixing systems with respect to nitrogen fixing potential and adaptation to severe conditions (Zahran, 1999). *Rhizobium* is tolerant to extreme conditions of salinity, alkalinity, acidity, drought, fertilizers, metal toxicity etc. in soil conditions. *Rhizobium* need more attention as it replaces chemical fertilizers and potentially reduce the risk of environmental pollution. *Rhizobium* is always a point of attraction for researchers because it can be applied as a biofertilizer.

Jalana district is one of the seventh districts of Aurangabad Administrative region in Martathwada region. Physioghrahically it has part of Western Ghats, Satmala hill range, Ajanta hill range and Godavari plain. Climatic conditions in Jalana district are dry and tropical with hot summer and mild winter with moderate rainfall. Main River in district is Godavari and Purna and its tributaries. Jalana district covers eight talukas as Jalana, Bhokhardhan, Jafrabad, Badnapur, Ambad, Ghansawangi, Partur and Mantha. Here, soil is black cotton soil on which principle cultivating plants are cotton, cereals, pulses, jowar and wheat. Cotton is major cash crop in district. Major pulses are Tur (*Cajanus cajan*), Mung (*Vigna radiata*), Gram (*Cicer arietinum*) and Udid (*Vigna mungo*). Out of total existing industries in district, about 15% are Agro-based industries. So, in present study, different *Rhizobium* species from different localities are done

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from different talukas of district.

Material and method-

Different legume plants as pea, groundnut, tur, gram, mung were collected from 12 different localities of Jalana district. From these plants, healthy and pink colored root nodules were collected. Also, the rhizospheric soil was brought for determination of soil pH on pH meter. While the water holding capacity and moisture content were recorded in observation table 01. The fresh root nodules were washed under tap water and surface sterilized with 70% ethanol and mercuric chloride and again washed with sterile water (Pawar *et al*., 2014), (Deshwal and Chaubey, 2014). Isolation was done by crushing the root nodules in between two glass slides which helps to release bacteria and transferred to *Rhizobium* mannitol agar for growth. Confirmation of bacteria was done by relevant biochemical tests which were performed along with motility and Gram-staining (Phalke *et al*., 2017). Colonies were formed within 24-48 hrs. with translucent elevations. The biochemical characterization was carried out through Starch hydrolysis, Catalase, Indole Production, Urease, Protease, Citrate and Nitrate Reduction tests.

Result and Discussion-

The values of pH, water holding capacity and moisture content was recorded in Observation table no. 01. Average pH value of soil in study area is 6.80, average water holding capacity is 51.56% and average moisture content of soil is 32.2%. Maximum soil pH was recorded in Khedgaon while minimum in Patoda. Maximum water holding capacity and moisture content was recorded in Chappar i.e. 53.97% and 33.97%while minimum was recorded in Chincholi i.e. 48.34% and 31.14%. The cells of *Rhizobium* are gram negative, rod shaped size varies from 0.07 to 1.2 µm. Colonies are yellow colored, circular with approximately 3.38mm in

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diameter appearing translucent, milky with entire margin. Out of 12 collected bacterial samples, 4 samples did not show similar morphological characters with *Rhizobium.* Results were recorded in Observation Table 02. This was confirmed by biochemical tests and its results were reported in Observation Table 03. Similar results regarding cell morphology and colony morphology were reported by Singha in 2013, Pawar *et al.,* in 2013, Deshwal and Chaubey in 2013.

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Table 01: Collection of leguminous plants from different localities of Jalana district

Sr. No.	Location	pH	Water Holding Capacity of Soil(%)	Moisture content (%)	Crop
1	Umari Tq. Jalana, Dist. Jalana	6.49	50.13	31.84	*Vigna mungo* (Udid)
2	Antarwala Tq. Jalana, Dist. Jalana	7.1	53.03	31.38	*Cajanus cajan* (Tur)
3	Ambad Tq. Ambad, Dist. Jalana	7.03	52.97	33.1	*Cicer arietinum* (Gram)
4	Alamgaon Tq. Ambad, Dist. Jalana	7.16	51.14	32.79	*Vigna radiata* (Mung)
5	Khedgaon Tq. Ambad, Dist. Jalna	7.2	50.97	32.24	*Cajanus cajan* (Tur)
6	Dhakefal Tq. Ghansavangi, Dist. Jalana	6.49	51.24	31.49	*Vigna radiata* (Mung)
7	Chappar Tq. Jafrabad, Dist. Jalana	6.58	53.97	33.97	*Cicer arietinum* (Gram)
8	Koregaon Tq. Partur, Dist. Jalana	6.87	51.64	31.94	*Vigna radiata* (Mung)
9	Patoda Tq. Partur, Dist. Jalana	6.34	50.14	32.76	*Cajanus cajan* (Tur)
10	Khadakwadi Tq. Badnapur, Dist. Jalana	7.12	52.71	32.49	*Cicer arietinum* (Gram)
11	Chincholi Tq. Bhokhardan, Dist. Jalana	6.49	48.34	31.14	*Vigna radiata* (Mung)
12	Malegaon Tq. Mantha, Dist. Jalana	6.79	52.48	31.53	*Vigna mungo* (Udid)

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Table 02: Morphological characters of Bacterial strains collected from root nodules

Strain	Cell Morphology			Colony Morphology				
	Gram's Staining	Cell Size	Cell Shape	Color	Shape	Size	Appearance	Margins
Strain 1	-ve	0.7 to 1.04 µm	Rod	Yellow	Circular	3.2 mm	Translucent	Entire
Strain 2	-ve	0.7 to 1.02 µm	Large Rod	Whitish Yellow	Circular	3.6 mm	Milky	Entire
Strain 3	-ve	0.8 to 1.2 µm	Large Rod	Yellow	Circular	3.1 mm	Translucent	Entire
Strain 4	-ve	0.9 to 1.1µm	Rod	Yellow	Circular	3.3 mm	Translucent	Entire
Strain 5	-ve	0.3 to 0.7 µm	Rod	Creamy White	Circular	2.1 mm	Opaque	Entire
Strain 6	-ve	1.4 to 3.0µm	Rod	Red	Circular	4.3 mm	Translucent	Entire
Strain 7	-ve	0.8 to 1.04 µm	Rod	Red	Circular	4.9 mm	Translucent	Entire
Strain 8	-ve	1.5 to 2.2 µm	Large Rod	Yellow	Circular	3.7 mm	Translucent	Entire
Strain 9	-ve	1.01 to 1.05 µm	Rod	White	Round	2.4 mm	Milky and Translucent	Entire
Strain 10	-ve	0.09 to 1.05 µm	Rod	Yellow	Circular	3.2 mm	Translucent	Entire
Strain 11	-ve	0.9 to 1 µm	Rod	Yellow	Circular	3.6mm	Translucent	Entire
Strain 12	-ve	0.8 to 1.2 µm	Rod	Yellow	Circular	3.4mm	Translucent	Entire

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Table 03: Biochemical tests of bacterial strains collected from different localities

Strain/ Test	Starch Hydrolysis	Catalase	Urease	Citrate	Indole production	Nitrate Reduction
Strain 1	+	+	+	-	+	+
Strain 2	+	+	+	-	+	+
Strain 3	+	+	+	-	+	+
Strain 4	+	+	+	-	+	+
Strain 5	-	-	+	+	-	+
Strain 6	-	+	+	+	-	-
Strain 7	+	-	+	+	-	+
Strain 8	+	+	+	-	+	+
Strain 9	-	-	-	+	-	+
Strain 10	+	+	+	-	+	+
Strain 11	+	+	+	-	+	+
Strain 12	+	+	+	-	+	+

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