

RHIZOBIUM BIOFERTILIZER TECHNOLOGY FOR LEGUMES OF MAHARASHTRA

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Background

Rhizobium is a soil habitat Gram-negative bacterium, which can able to colonize the legume roots and fixes atmospheric nitrogen symbiotically. There is symbiotic association between plant and bacteria, initiated when bacteria in the soil attach to root hairs. This highly specific attachment process is mediated by plant proteins, the lectins that bind the bacteria to the surface of the root hairs and then penetrated by the microbes. The infected root cells divide and form a nitrogen fixing nodule which provides the anaerobic environment necessary for nitrogen fixation.

Pulse legumes are attributed to their ability to biologically fix nitrogen in symbiosis with certain types of bacteria (e.g. *Rhizobium*, *Bradyrhizobium*). These bacteria are able to convert atmospheric nitrogen into nitrogen compounds to the tune of 72 to 350 kg of nitrogen per ha per year. To improve the food and nutritional security the food legume has a major role. The dried seeds of legume have ability to be stored for long periods without compromising their nutritional value, thereby, increased food availability till the new arrivals. The legume growers have both the options of self- consumption and also cash crops to fetch income.

In addition to their nutritive value, by virtue of broad genetic diversity in food legumes and climate resilience to sustain well in adverse weather situations. Inclusion of legumes in dominated cereal-based cropping system increased the yield of subsequent cereal crops and reduce the fertilizer cost too resulting decrease in cost of production and increase profitability.

A rhizobium biofertilizer technology is developed under ICAR All India Network Project on Soil Biodiversity Biofertilizers (AINP SBB) at the VNMKV, Parbhani. The technical bulletin highlights production of rhizobium biofertilizer, quality and field evaluation. The rhizobium biofertilizer improves crop yield by 10-28 %. The product is commercially produced and marketed to farmers. During 2017-20 revenue generated was Rs 21.41 lakhs. The technology benefitted 4232 farmers. The technology has been transferred to one industry (M/s Microbia Solution Pvt.Ltd.Hingoli, Maharashtra) and marketed through 4 KVKs.

Procedure of biofertilizer production

Isolation of *Rhizobium*

a) Assessment of nodules

1. Wash the roots, free off adhering dirt
2. Cut 10 nodules randomly and note the colour of the juice coming out from nodules on crushing, if pink in colour it means it is effective, otherwise not.
3. Record the percentage of effective and ineffective nodules.
4. Record the percentage of nodules of main and lateral roots.
5. Note the colour, size, shape, abundance and location of the characteristics nodules.



b) Examination of nodules for bacteroids

1. Sterilize the nodules with 0.1 per cent acidified mercuric chloride for 2 minutes and wash in sterile water.
2. Crush the nodule in a drop of water on a clean slide, mix well with a loop.
3. Transfer several loop-full to another clean slide.
4. Dry and fix the slide on gentle heat and stain with rose bengal stain for 5 minutes.
5. Identify bacteroids having irregular shapes and an uneven distribution of protoplasmic contents.



c) Isolation of *Rhizobium* from nodules

1. Wash the soil from the root nodules and carefully cut a nodule from the root, leaving a small portion of the root attached.
2. Place the nodule in petri plate containing 0.1 per cent acidified HgCl_2 (1 ml conc. HCl/L) and keep it immersed for 5 minutes.
3. Use sterile forceps to transfer the nodule to a petri plate containing sterile water. Wash at least six times and place in 95 per cent ethyl alcohol for 3 minutes. Remove to a petri plate containing sterile water and rinse the nodule twice.
4. Add 1 ml of sterile water to each of six numbered sterile petri plates. Crush these sterilized nodules with forceps in petri plate No. 1. Mix the nodular tissue with water.
5. Transfer one loop full of the suspension to plate 2 and dilute by mixing with 1.0 ml of water. Repeat the loop dilution procedure for plate's No. 3, 4, 5 and 6.
6. Pour plates (No. 2 - 6) with Congo red mannitol agar and incubate the plates at 28°C for 5-7 days.
7. After incubation examine the plates, note typical *Rhizobium* colonies, raised, moist with round edges, later changing to opaque white. Stain with carbolcrythrosin (5 minutes) and compare the bacteria with those found in the nodule



Large Scale Production of Liquid *Rhizobium* Biofertilizers

Liquid *Rhizobium* biofertilizers production technology includes isolation or procurement of bacterial strains for required purpose, selection of suitable effective strain, preparation of mother culture, broth preparation, addition of cell protectants and their mixing, followed by packaging, storage and dispatch. The *Rhizobium* strains most adopted and found suitable in Marathwada region are produced at biofertilizer production unit, Vasantnao Naik Krishi Vidyapeeth, Parbhani.

Steps to be followed in preparation of liquid *Rhizobium* biofertilizers

1. Preparation of mother or starter cultures

Starter cultures of selected strains are obtained after ascertaining their performance in laboratory, pot culture experiments and at field levels. The pure culture of efficient strain of particular microorganism is grown on respective agar medium on slant and maintained in the laboratory. A loopful of inoculum from the slant is transferred in a 250 ml capacity conical flask containing liquid medium, keep the conical flask on rotary shaker for at least 72 hrs depending whether they are fast growing or slow growing. The content of these flasks usually attain a load of 10^6 - 10^7 cells per ml called mother culture or starter culture. This mother culture is further multiplied in larger flasks called as broth preparation.

2. Preparation of broth cultures

Prepare liquid medium for *Rhizobium*. Distribute equal quantity in big conical flasks (1000 ml). Sterilize it in autoclave for half an hour at 15 lbs pressure. After sterilization each flask containing suitable broth is inoculated with the mother culture in 1:5 proportions aseptically under laminar flow. Keep the flasks on rotary shaker for 72 hours or in sterile fermenter until the viable count per ml reaches to 10^{12} - 10^{14} cells. The broths become thicker in consistency. This broth culture with population of at least 10^{12} cells per ml should be stored at suitable temperature and condition.



3. Preparation of liquid *Rhizobium* biofertilizer with cell protectant

To prepare the liquid *Rhizobium* biofertilizers from the above prepared broth, all the ingredients required should be mixed thoroughly under laminar flow to avoid the contamination.

Ingredients

Sr.No.	Component	Ingredients / 100 lit
A)	Liquid broth	40 lit
B)	Cell protectants	Mix the following in 25 liters sterile water
	Trehalose	75 g
	Arabinose	100 g
	Fe-EDTA	75 g
	Polyvinyl pyrrolidone	1000 g
C)	Glycerol	8000 mL

* Mix A+B+C as per above and make the volume 100 liters with sterile water

4. Filling and packaging of bottles

After preparation of liquid *Rhizobium* biofertilizer as mentioned above the requisite quantity as per need should be filled in the sterile auto-lock high-density polyethylene (HDPE) plastic bottles under laminar flow to avoid contamination.



Dosage of liquid *Rhizobium* Biofertilizer in different crops

Recommended liquid biofertilizers, their application methods and quantity to be used for different crops is given follows:

Crop	Recommended Biofertilizer	Application method	Quantity to be used
Green gram, Black gram, Pigeon pea and Chickpea	<i>Rhizobium</i>	Seed treatment	100 ml 10 kg ⁻¹ seed
Soybean and Groundnut	<i>Rhizobium</i>	Seed treatment	50 ml 10 kg ⁻¹ seed

Results of effect of *Rhizobium* biofertilizers on various legume crop yield

Crops	Treatments	Yield (kg ha ⁻¹)		Per cent increase in yield
		Uninoculated	Inoculated	
Soybean	Seed treatment of liquid <i>Rhizobium</i> biofertilizer + RDF	1714	2182	27
Green gram	Seed treatment of liquid <i>Rhizobium</i> biofertilizer+ RDF	646	850	24
Black gram	Seed treatment of liquid <i>Rhizobium</i> biofertilizer + RDF	517	572	10
Chickpea	Seed treatment of liquid <i>Rhizobium</i> biofertilizer + RDF	1408	1752	24
Pigeon pea	Seed treatment of liquid <i>Rhizobium</i> biofertilizer + RDF	1284	1790	28
Groundnut	Seed treatment of liquid <i>Rhizobium</i> biofertilizer + RDF	1567	1917	23

Evaluation of biofertilizer : Front line demonstration



Liquid biofertilizer produced, marketed, revenue generated

Year	Biofertilizers marketed (L)	Revenue generated (Rs.)
2017-18	2442	9,15,750/-
2018-19	1529	5,73,375/-
2019-20	1740	6,52,500/-

Technology Transferred

Industry	Krishi Vigyan Kendra (KVK)
M/s Microbia Solution Pvt. Ltd. Hingoli (Maharashtra)	<p>Seed treatment of liquid <i>Rhizobium</i> biofertilizer for legumes and pulses @ 100 ml 10 kg⁻¹ seed (black gram, green gram, pigeon pea and chickpea) and @ 50 ml 10 kg⁻¹ seed (soybean and groundnut) is transferred to KVKs of University jurisdiction.</p> <p>Address of KVKs</p> <ol style="list-style-type: none"> 1. Krishi Vigyan Kendra, Aurangabad (M.S.) email – pckvkmkv@gmail.com Contact No. (0240) 2376558 2. Krishi Vigyan Kendra, Badnapur, Dist. Jalna (M.S.) email - kvk.jalna2@gmail.com Contact No. (02482) 261021 3. Krishi Vigyan Kendra, Khamgaon, Dist. Beed (M.S.) email - kvkmkv@rediffmail.com Contact No. 7588677583 4. Krishi Vigyan Kendra, Tuljapur, Dist. Osmanabad (M.S.) email – kvktuljapur@gmail.com Contact No. (02471) 295548

Trainings Organized and Beneficiaries

Year	No of farmers benefitted
2017-18	1762
2018-19	1120
2019-20	1350



Plate. *Rhizobium* Liquid Biofertilizers for Sale

The information given in the document is based on the experiments carried out at the AINP-SBB centre, Department of soil science and Agricultural Chemistry, VNMKV, Parbhani, Maharashtra. For training, demonstration and other enquiries please contact the centre.