

**Research Article**

**Effectiveness of Native Rhizobium on Nodulation Soybean  
(*GLYCINE MAX. L*) with Different Carriers**

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**ABSTRACT:**

A locally isolated strain of Rhizobia (SR5) of soybean from Marathwada region of Maharashtra state was assessed for its efficacy to be use as inoculants. Various carriers were inoculated with this strain in the pot trials. The assessment was done by counting the number of nodules per plant and increase in weight of plant. The newly isolated strain SR5 worked better with jaggery slurry medium in soybean.

**Keywords:** *Local specific Rhizobia SR5, Soybean carrier*

**[I] INTRODUCTION**

Biological nitrogen fixation is an important aspect of nutrition in crop production throughout the world. The process saves a huge sum of money which otherwise would have to be pumped agricultural productivity to consistently sustain an increase in world population. Among various symbiotic association of Biological Nitrogen fixation, the Rhizome legume association is the most common in both temperate and tropical climates. *Rhizobia* play a great role in nature as they are the greatest N<sub>2</sub> fixers, particularly in leguminous plants and leguminous plants are the predominant source of supplying protein to the population of developing countries like India [1]. Looking to the need of location specific strain of *Rhizobia* to increase yield of pulse and other

leguminous plants the investigations were concentrated on isolation and identifying these local strains for effective nodulation. IBP world catalogue of *Rhizobium* listed about 3000 *Rhizobium* strains from legume in different countries [2]. The performance of these locally isolated strains of *Rhizobia* depends up on the soil, Competitor microbes and other Rhizosperic factors. In the present study most efficient local strain of *Rhizobia* SR5 from Marathwada region of Maharashtra state was assessed for its Nodulation capacity per plants, Soybean is cultivated on a large scale now a days in this region and carrier based inoculants are widely used in the recent times. However, various factors on efficacy of this strain are already studied [3].

This paper describes the performance of the locally isolated strains of *Rhizobium* with different carriers on Soybean nodulation and increase in dry weight.

## [II] MATERIALS AND METHODS

### 2.1. Isolation and maintenance of *Rhizobium*

The Soybean seeds were collected from local market. The strain *Rhizobium* SR5 isolated from Chudawa, Parbhani Dist. of Maharashtra state of Maharashtra. *Rhizobium* were isolated and maintained on YEMA medium either by sub-culturing at frequent intervals or as lyophilized cultured kept at 5 °C this system maximizes genetic variation and contamination. The culture was also dried on proclain beads over a desiccant in screw cap bottles. The authenticity check was carried out regularly their effectiveness on nodulation capacity and dry matter of soybean was tasted. In earlier studies, strain *Rhizobium* SR5 from Chudawa village of Parbhani district of Maharashtra, India was found very much effective. Their capacity to modulate along with some carrier such as lime pelleting jaggery, lignite sawdust, and sterilized cow dung powder was tested by usual method. The number of nodules and dry matter of plants along with different inoculants were evaluated in a pot culture experiment for three years 2011-2014

### 2.2. Screening and culturing of *Rhizobium*

1) CRYEMA Test: 2.5 ml of Congo red dye was mixed with a one liter of YEMA medium to prepare CRYMA medium. Bacterial colonies on the YEMA medium were streaked on the CRYMA medium. Bacterial colonies on the YEMA medium were streaked on the CRYMA medium and the petriplates were incubated at 28 ±2 °C for 5-7 days. *Rhizobium* cells form white circular, entire convex colonies. The white colonies were picked up to produce *Rhizobium* inoculants.

2) Microscopic observation: Bacterial cells in the CRYMA medium were stained with carbol fuchsin and visualized under a compound microscope. This dye stains the β

polyhydroxybutrate granules. These colonies were picked up to establish *Rhizobium* inoculants.

3) Glucose peptone Agar test (GPA Test): *Rhizobium* colonies were streaked on YEMA medium and a master plate was made. Colonies in the master plates were transferred to GPA medium by replacing plating. Those colonies in the master plates fail to grow GPA medium belong to *Rhizobium*. This test was confirmative test the purity of *Rhizobium* colonies.

**2.3. Inoculation.** The surface sterilized seeds were used for inoculation. The seeds were dried in shade and sown in earthen pots of respective treatments. These pots were watered with an interval of two days or on when required. After 15 days of sowing the thinning was done and five plants were maintained in each pot.

The observations were recorded for number of nodules and dry matter. Plants were uprooted carefully washed and number of nodules per plant was recorded. The nitrogen content was determined by micro Kjeldahl method. To identify the best method of inoculants, a pot culture experiment was conducted using different method of inoculation. The strain SR5 which was found superior was used. Medium type of soil was sterilized in autoclave at 30 lbs for two hours and used in the experiment. The seeds were surface sterilized by treating with 0.1% mercuric chloride.

**2.4. Carriers:** The Soybean seeds were inoculated using following carriers

1. Lime pelleting: The seeds were inoculated with *Rhizobium* species coated with lime to form pellets.
2. *Rhizobium* culture and jaggery: Seeds were dipped in the slurry of jaggery and *Rhizobium* broth and dried in shade and use for sowing.
3. *Rhizobium* culture alone: Broth culture of *Rhizobium* was diluted with sterile distilled water and the seeds were soaked in it. The seeds were air dried in shade and used for sowing.
4. *Rhizobium* culture and jaggery with saw dust /cow dung /lignite saw dust was grinded fine powder of 0.01 mm size. The powder was

mixed with the *Rhizobium* broth culture separately. After through mixed the powder was dried in shade under aseptic conditions and was used separately for inoculation the seeds were soaked with sterile water, dried in shade and were sown in pots. The observation was recorded on nodule number and dry weight of whole plant on 40 days of sowing. The plants were grown on 40 days in uniform condition along with different inoculants. After 40 days the number of nodule per plant was recorded and the dry matter of plant was estimated by usual method. The experiment was done in three sets each and for three consecutive years i.e. 2011-2014.

Data were subjected to Analysis of variance (ANOVA) depending up on experimental design following described [4].

### [III] RESULTS AND DISCUSSION

The effect of *Rhizobium* SR5 inoculation on nodulation of Soybean by using various carriers is presented in table No.1. the result inoculated that the local *Rhizobium* strain SR5 performed well when jaggery was used as carrier and the number

of nodule was significantly higher than other carriers used. The trend in results recorded during 2011-2014 was similar. The effect of the *Rhizobium* on weight of Soybean by using various carriers is presented in the table No.2. The result showed that the local *Rhizobium* strain SR5 performed well when jaggery was used as carrier. The dry weight recorded was significantly higher than other carrier used. The trend in the results the trend in the results recorded during three consecutive years was similar.

Thus the results showed that this local strain seem to be very effective in Soybean when it is used for inoculation along with the jaggery. The process of infection of plants by *Rhizobia* is very much competitive. The jaggery slurry method was more successful for inoculation, because in some plants the invention of bacteria requires synthesis of important exo- polysaccharides [5]. Further investigation on local strains of bacteria and determining biodiversity of microbes is essential so the *Rhizobia* can effectively use as Biofertilizers [6].

**Table No. 1.** Effect of carrier by strain *Rhizobium* SR5 on nodulation of Soybean

Sr No	Method of inoculation	Number of nodules per plant			Pooled analysis
		2011-12	2012-13	2013-14	
1	<i>Rhizobium</i> culture with Lime pelleting.	2.70	21.28	18.05	20.01
2	<i>Rhizobium</i> culture with Jaggery.	24.07	25.28	25.86	25.07
3	<i>Rhizobium</i> culture with Lignite.	20.86	21.65	21.47	21.32
4	<i>Rhizobium</i> culture with saw dust.	27.45	16.00	17.95	20.46
5	<i>Rhizobium</i> culture with sterilized cow dung powder	14.75	13.03	4.59	14.12
6	<i>Rhizobium</i> culture with saw dust and cow dung powder	13.58	11.05	11.83	12.15
7	Control	10.14	10.96	7.54	9.54
	S.E+ <sub>-</sub>	1.05	1.44	1.41	1.31
	CD at 5%	3.25	4.44	4.34	3.94

**Table No. 2.** Effect of carrier by strain *Rhizobium* SR5 on Dry weight of Soybean plant

Sr. No.	Method of inoculation	Dry weight of plant	Pooled analysis
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		2011-12	2012-13	2013-14	
1	<i>Rhizobium</i> culture with Lime pelleting	0.65	0.61	0.67	0.64
2	<i>Rhizobium</i> culture with Jaggery	0.73	0.63	0.70	0.68
3	<i>Rhizobium</i> culture with Lignite.	0.54	0.47	0.41	0.47
4	<i>Rhizobium</i> culture with saw dust.	0.52	0.53	0.51	0.52
5	<i>Rhizobium</i> culture with sterilized cow dung powder	0.41	0.34	0.40	0.38
6	<i>Rhizobium</i> culture with saw dust and cow dung powder	0.41	0.41	0.48	0.43
	Control	0.39	0.25.	0.20	0.28
7	S.E+ <sub>-</sub>	0.01	0.01	0.01	0.93
	CD at 5%	0.04	0.04	0.05	2.79

#### [V] CONCLUSION

In this study, the extent of nodulation in soybean and subsequently its effect on plant biomass was studied. The effect of inoculation was assessed based on different carries. This effect was studied in pot experiments and it was proved that all the carriers studied were effective but performance *Rhizobia* with jaggery as carrier was best as expressed by nodulation and biomass on soybean plants.

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