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Effect of *Bradyrhizobium* Broth on Growth of Root, Shoot and Nodule of Soybean

Manisha S. Lande*, K.V. Bawankule, R.D. Solanki and R.G. Aware

Department of Plant Pathology, College of Agriculture, Nagpur
Dr. PDKV Akola (MS), India

**Corresponding author*

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A B S T R A C T

A pot culture experiment was conducted in the year 2012-2013 to test the performance of glycerol based inoculant of *Bradyrhizobium* on growth of soybean using variety JS-335, at Plant Pathology Department, College of Agriculture, Nagpur. Experiment was laid out in Completely Randomized Design (CRD) with eight treatments and three replications. During experiment, root and shoot length as well as number of nodules were measured at 15, 30 and 45 days after inoculation. Among all treatments, maximum root and shoot length was recorded in T2, i.e., 35 ml glycerol + 65 ml *Bradyrhizobium* broth (23.8 cm and 17.93 cm/ plant) respectively. However, maximum nodulation was recorded in T3, i.e., 35 ml glycerol + 65 *Bradyrhizobium* broth (11.00). All the treatments showed a significant difference over untreated plot.

Introduction

Soybean (*Glycine max* L. Merill), family Leguminaceae is unique crop, versatile nutritional attribute yielding in both oil and protein. It is also known as "Golden Bean". The speedy growth in soybean cultivation has placed India in world map of soybean. India ranks fifth in the world, in area and production after USA, Brazil, China and Argentina. India is considered as a secondary center of demonstration of soybean. Soybean ranks first among oil seed crops in the world. However, it has manifold importance in agriculture, medicinal and industrial sector. It has high nutritive value, contains 40% protein, 20% oil, 30% carbohydrate and 5% fiber. It also contains vitamin A, B, C, D, E and other essential amino acids. Soybean is an excellent food available at the most economic rate. It is recommended in diabetes, stomach, heart and kidney disease.

Recent day's *Rhizobium* biofertilizers are widely used by farmers to increase legume crop yield. Biofertilizer can not be totally replace conventional chemical fertilizers but for most of crop and soil conditions up to 20 per cent of nitrogen requirement can be met through biofertilizer, which can be the best

supplement for chemical fertilizer. It may help to reduce the cost of chemical fertilizer and avoid the soil problems.

Rhizobium belongs to bacterial group and classical example of symbiotic nitrogen fixation. Among the different BNF processes, legume *Rhizobium* symbiosis is the most effective means of nitrogen addition to terrestrial ecosystem. The bacteria infect the legume root and form root nodule within which they reduce molecular nitrogen to ammonia which is reutilized by plant to produce valuable proteins, vitamins and other nitrogen containing compounds. The site of symbiosis is within the root nodules. It has been estimated that 40-250 Kg N/ha/year is fixed. But the effectiveness of biofertilizer as a nutrient input in crop production depend on efficiency of microbial strains i.e., their shelf life in a carrier and soil and weather parameters during crop growth, Although peat, charcoal are suitable as a carrier of biofertilizer. There is scope for development of better carrier to improve effectiveness of biofertilizer agents for longer shelf life.

Liquid biofertilizer is increasingly available in the market as one of the alternative to powder based biofertilizer. The shelf life of liquid biofertilizer is two to four years. The application of liquid formulation in the field is also very simple and easy. Liquid formulation contains special cell protectant or substance that encourages formation of resting spores or cysts. It contains special nutrients that ensure longer shelf life, better survival on seed and soil and tolerance to adverse conditions, to protect the microorganism from harmful environmental factors as the target site (field) increasing persistence.

The powder carrier based biofertilizer are used since a long time. The traditional nitrogen fixing biofertilizer have suffered from problems of short shelf life, instability to ambient temperature and laborious large scale application. Whereas liquid inoculants could be produced with minimum labour, space and energy and also the quantity of inoculum required is less compared to carrier based formulations. It is easier for farmers to handle. Liquid biofertilizer formulation could be considered as one potential strategy for improving the shelf life of biofertilizer. Unlike solid carrier based biofertilizers, liquid formulations allow the manufacturer to include sufficient amount of nutrients, cell protectant and inducers responsible for cell/spore/cyst formation to ensure prolonged shelf life.

In present study *Bradyrhizobium japonicum* was selected as test organism. It is slow growing bacterium of family *Rhizobiaceae.* Inoculation of soybean seed with *Bradyrhizobium* can fix 60 to 80 kg N/ha and subsequently increase the nodulation, shoot length of plant, plant height, number of pods and seed yield over control. Solid carriers are difficult to process to consistent characteristics and may not appropriate using with planting equipment used on large scale of field operation (Singleton *et al.,* 2002). Liquid inoculants formulation is one solution to the problems associated with processing of solid carriers. The use of broth culture amended with substances that promote cells survival in the package and after application for seed.

Materials and Methods

An experiment was conducted in Plant Pathology Laboratory, College of Agriculture, Nagpur during the year 2012-2013. The seed of soybean (JS- 335) was obtained from Central Research Station, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Whereas, carrier material taken for study *viz*. glycerol, gum arabica, EDTA, PVP and lignite which were procured in ready condition (120 mesh).

Pure culture

Pure culture *of Bradyrhizobium* was collected from Plant Pathology Section, College of Agriculture, Nagpur. This culture was used as test culture in the present investigation. All glassware sterilized in hot air oven at 180^0 C for 1 hour. Culture media and distilled water was sterilized in autoclave at 15 lbs pressure for 15 minutes.

Preparation of yeast extract mannitol agar

For maintenance and purification of bacterial culture YEMA media was used. YEMA broth was used for mass multiplication of bacteria.

The medium was prepared by using the following ingredients.

K_2HPO_4		-0.5 g
$MgSo_4$-$7H_2o$	-	0.2g
Nacl	-	0.1g
Mannitol		-10g
Agar-Agar		-20g
Congo red		-2.5ml
Distilled water		-1000ml

Growth, maintenance and preservation of *Bradyrhizobium* culture

Pure culture of *Bradyrhizobium* was maintained on YEMA slants (Graham and Parker, 1967). Sub culturing and checking for purity was done once in two months when stored at 4^0 C temperatures.

Pot culture experiment

The investigation was conducted in pot culture by following Completely Randomized Design (C.R.D.) to test the performance of glycerol based inoculant of *Bradyrhizobium* on growth of soybean using variety JS-335.Soybeanseedwere treated with glycerol based *Bradyrhizobium* broth and *Rhizobium* liquid biofertilizer available in market. The treated seeds were sown in pots contains sterilized soil according to treatment details and observations on growth parameters of soybean such as root length, shoot length and root nodule germination percent was recorded.

For field work

Soybean seed @ 1 Kg was be treated as under

Treatments	Treatment details
T_1	15 ml glycerol + 85 ml *Bradyrhizobium* broth
T_2	25 ml glycerol +75 ml *Bradyrhizobium* broth
T_3	35 ml glycerol +65 ml *Bradyrhizobium* broth
T_4	25g Powder base inoculants
T_5	*Rhizobium* liquid biofertilizer(Market product-1)
T_6	*Rhizobium* liquid biofertilizer(Market product-2)
T_7	Untreated control

Experimental details

Duration	-2012-13
Design	-CRD
Treatments	-7
Replications	-3
Variety	-Soybean JS-335

Observation

1. Root length: Root length was recorded after 15, 30, 45 days.

2. Shoot length: Shoot length was recorded after 15, 30, 45 days.

3. Nodulation: Plants from each pot were dug out carefully without loss of nodule along with soil in the field. The plants were then

washed under tap water to remove soil adhering to root surface. The nodules were cut from the root carefully and their total number was recorded.

Towel paper method

Seed of soybean JS-335 was treated with different liquid inoculants at different concentration as per treatment details. Root length (cm), shoot length (cm), seedling vigour index and germination percentage were recorded by towel paper method.

The seedling vigour index was computed by using the following formula.

Vigour Index = germination(%) × [root length (cm) + shoot length (cm)]

The per cent seed germination was computed, based on number of seed germinated in a moistened paper towel where the hundred treated seed were placed and incubated at room temperature.

Analysis of data

The data of various experiments was analyzed using Complete Randomized Design. Analysis of variance, means were tested for significance and critical difference was used for comparison whenever the differences were found to the significant as indicated 'F' test (Gomez and Gomez, 1984)

Results and Discussion

Studies were undertaken on "Performance of Glycerol Based Inoculant of *Bradyrhizobium* Growth on Soybean" during the year 2012 in Completely Randomized Design (CRD) with eight treatments and three replications using the soybean variety JS-335. The results are presented in tables, photograph and figures are depicted in each head in this chapter.

Rhizobial population in glycerol carrier

Two substrates *viz*., glycerol and lignite powder mixed individually with broth of *Bradyrhizobium japonicum* at 10^7 cells/ml. The experiment was set for 180 days. The data obtained is presented in Table 1.

It was revealed from the data that there were significant differences in rhizobial population at all the intervals. Maximum population was attained with 10 ml glycerol (61.66 X 10^7 cell/ml.) and it was found significantly superior over all other treatment at 120 days in respect to other treatment next to 10 ml glycerol treatment maximum rhizobial population was noticed in 85 ml *Bradyrhizobium* broth + 15 ml glycerol (56.66 X10^7 cells/ml.) and found significantly superior over other treatments. And it was followed by T_3 (80 ml *Bradyrhizobium* broth + 20 ml glycerol), T_5 (70 ml *Bradyrhizobium* broth +30 ml glycerol), T_4 (75 ml *Bradyrhizobium* broth +25 ml glycerol), T_6 (65 ml *Bradyrhizobium* broth +35 ml glycerol) showed the rhizobial population 35x 10^7 cell/ml, 33.66x 10^7 cell/ml, 32.66 x10^7 cell/ml and 31.33 x10^7 cell/ml after 120 days, respectively. There is decrease in rhizobial population was observed after 120 days. But in treatment T_7 (25 g lignite + 75 ml broth) and T_8 (Control) noticed high rhizobial population after 60 days i.e. 27.00 x10^7 cell/ml, 25.33 x10^7 cell/ml respectively. This may be due to the more water holding of carrier material. These results are in accordance with earlier studies of scientist Lorda and Balatti (1996) showed that the more rapid growth in a balanced medium that used 10 ml glycerol as a substitute for mannitol. Sridhar *et al.* (2004) stated that higher population up to storage period of 180 days. Brahmaprakash (2011) showed lignite recorded maximum rhizobial colonies (27 X 10^6 cells/ml) at 60 days. Kandasamy and Prasad (1971) also reported that lignite was

better carrier material for rhizobial population.

Effect of carrier on the root length

The data in respect of root length are given in Table 2 plant root length were recorded at 15, 30, 45 DAS. It is seen from the data that root length was significantly affected by various treatments over control. The maximum root length (23.80 cm) was recorded after 45 days by 25 ml glycerol + 75 ml *Bradyrhizobium* broth treatment (T_2). Followed by (T_3) treatment, 35 ml glycerol + 65 ml *Bradyrhizobium* broth (23.63 cm) root length was recorded and it was found to be significantly superior over all other treatment. Followed by treatment, T_4 (25 g powder base inoculants), i.e. 21.00 cm and both the treatment T_1 (85 ml *Bradyrhizobium* broth + 15 ml glycerol) and T_5 (*Rhizobium* liquid biofertilizer (Market product-2) showed the same root length i.e. 20.16 cm at 45 DAS. The minimum root length was observed in treatment T_6 (*Rhizobium* liquid biofertilizer (Market product-2) followed by T_7 (Untreated) i.e., 19.96 and 15.78 cm respectively

Effect of carrier on the shoot length (cm)

As regards to shoot length, the data presented in Table 3 showed significant differences over control. The maximum shoot length was recorded in treatment T_2 (25 ml glycerol + 75 ml *Bradyrhizobium* broth) i.e., 18.16 cm/plant) as compared to all other treatment. Next best treatment T_3 (35 ml glycerol + 65 ml *Bradyrhizobium* broth), i.e. 17.93 cm/plant over all other remaining treatments. And further followed by T_5 (*Rhizobium* liquid biofertilizer (Market product-1), T_1 (15 ml glycerol + 85 ml *Bradyrhizobium* broth), T_4 (25g powder base inoculants), T_6 *(Rhizobium* liquid biofertilizer (Market Product-2) was recorded 17.86 cm, 17.36cm, 16.86cm, 15.96cm respectively, after 45 DAS. All the treatment increased the shoot length over control. Treatment T_8 (Control) was recorded minimum shoot length of plant 15.46 cm/ plant.

Effect of carrier on number of nodules per plant

A pot culture experiment was conducted to test the efficacy of the carrier materials on nodulation of soybean crop and the result are tabulated in Table 4. The Maximum nodulation was noticed in 35 ml glycerol +65 ml *Bradyrhizobium* broth (11.00 nodules/ plant) and it was significantly superior over all other treatment. The next treatment T_2 (75 ml *Bradyrhizobium* broth + 25 ml glycerol) was found significantly better over other treatment recorded 10.66 nodules/plant. Followed by T_4 (25 g powder base inoculants), T_5 (*Rhizobium* liquid biofertilizer Market product -1), T_6 (*Rhizobium* liquid biofertilizer (Market product -2), T_1 (85ml *Bradyrhizobium* broth +15 ml glycerol), recorded no. of nodules per plant i.e. 10.36 nodules/plant, 9.66 nodules/plant, 8.33 nodules/plant and 6.33 nodules/plant after 45 days. Seed inoculation with *Rhizobium* increased the nodulation. These findings are in agreement with the reports Kurundkar *et al*. (1991) and Chore and Shastri (1991). Benifical effect of carrier on nodulation was earlier reported by Jadhav *et al*. (1988).

Effect of various treatment on root and shoot length by paper towel method

Seeds of soybean variety JS-335 were treated with glycerol and liquid based inoculants (Market products) as described in treatment and inoculated with *Bradyrhizobium japonicum* for recording shoot length, root length, germination and seedling vigour index by using paper towel method. There were significant differences noticed due to various

treatment and data presented in Table 5 reveals that maximum root and shoot length was recorded by (T_1) 15 ml glycerol +85 ml *Bradyrhizobium* broth (11.23 cm and (9.20 cm) followed by (T_4) powder base treatment noticed (10.33) root length, and (7.23) shoot length. Further followed by T_6 (*Rhizobium* liquid biofertilizer (Market product-2) with 9.66 cm root length 7.20 cm, shoot length, T_3 (35 ml glycerol +65 ml *Bradyrhizobium* broth), with 9.43 cm root length 6.13 cm shoot length, T_2 (25 ml glycerol + 75 ml *Bradyrhizobium* broth) with 9.30cm root length and 6.30 cm shoot length. Minimum root length T_7 (Control) recorded the root length 9.23 cm, shoot length 5.8 cm, and T_6 (*Rhizobium* liquid biofertilizer (Market product-2) was recorded root length 8.20 cm, and shoot length 6.53 cm. Adu and Misari (1989) reported improvement of shoot length due to the inoculation of *Rhizobium*.

The data presented in above Table showed that treatment T_2 (25 ml glycerol + 75 ml *Bradyrhizobium* broth) registered maximum germination percentage i.e. 79.3% which is significantly superior over all the other treatments. Next best treatment T_1 (15 ml glycerol + 85 ml *Bradyrhizobium* broth) registered i.e. 77.3%. And further followed by T_4 (25 g powder base inoculant) i.e. 76.6%, and treatment T_3 (35 ml glycerol +65 ml *Bradyrhizobium* broth) and T_5 (*Rhizobium* liquid biofertilizer (Market product-1) showed the same germination percentage with 74%. And the minimum germination percentage was recorded in treatment T_6 (*Rhizobium* liquid biofertilizer (Market product-2) i.e. 74% followed by T_7 (Control) with 72%. Penaranda *et al*. (1988) observed the enhancement of germination due to *Rhizobium* inoculation on soybean, mungbean and pea.

Table.1 Rhizobial population (x 10^7 cells/ml of carrier) in glycerol carrier

Tr. No.	Treatment	Months					
		I	**II**	**III**	**IV**	**V**	**VI**
T_1	90ml *Bradyrhizobium* broth +10 ml glycerol	34.33	42.33	48.33	61.66	52.00	**44.66**
T_2	85ml *Bradyrhizobium* broth+15ml glycerol	28.66	31.66	41.66	56.66	49.33	**40.33**
T_3	80ml *Bradyrhizobium*broth+20ml glycerol	24.66	26.33	30.33	35.00	32.33	**29.66**
T_4	75ml *Bradyrhizobium* broth+25ml glycerol	19.66	28.00	31.66	32.66	30.66	**26.00**
T_5	70ml *Bradyrhizobium* broth+30ml glycerol	19.33	21.66	29.66	33.66	32.00	**29.00**
T_6	65ml *Bradyrhizobium* broth+35ml glycerol	18.33	26.33	29.00	31.33	28.00	**24.66**
T_7	25g lignite+75ml *Bradyrhizobium* broth	26.00	27.00	24.33	20.66	18.00	**17.66**
T_8	Control	22.33	25.33	20.33	19.66	17.66	**17.33**
	F test	Sig.	Sig.	Sig.	Sig.	Sig.	**Sig.**
	SE $\pm$ (m)	1.39	1.25	1.49	1.38	1.48	**1.58**
	CD (P = 0.05)	**4.18**	**3.73**	**4.48**	**4.14**	**4.44**	**4.74**

Table.2 Effect of carrier on the root length (cm)

Tr.No.	Treatment	15 DAS	30 DAS	45 DAS
T_1	85ml *Bradyrhizobium* broth+15ml glycerol	5.20	11.70	**20.16**
T_2	25ml glycerol +75 ml *Bradyrhizobium* broth	7.90	16.06	**23.80**
T_3	35ml glycerol +65ml *Bradyrhizobium* broth	7.83	15.16	**23.63**
T_4	25g powder base inoculants	5.06	11.16	**21.00**
T_5	*Rhizobium* liquid biofertilizer (Market product-1)	5.26	13.80	**20.16**
T_6	*Rhizobium* liquid biofertilzer (Market product-2)	5.16	11.50	**19.96**
T_7	Control	5.03	8.16	**15.78**
	F test	Sig.	Sig.	**Sig.**
	SE $\pm$ (m)	0.14	0.25	**0.34**
	CD (P = 0.05)	**0.52**	**0.77**	**1.08**

Table.3 Effect on carrier on the shoot length (cm)

Tr.No.	Treatment	15 DAS	30 DAS	45 DAS
T_1	15ml glycerol +85ml *Bradyrhizobium* broth	12.83	14.9	**17.36**
T_2	25ml glycerol +75 ml *Bradyrhizobium* broth	14.9	17.16	**18.16**
T_3	35ml glycerol +65ml *Bradyrhizobium* broth	14.1	15.43	**17.93**
T_4	25g powder base inoculants	12.2	14.8	**16.86**
T_5	*Rhizobium* liquid biofertilizer (Market product-1)	13	15.2	**17.86**
T_6	*Rhizobium* liquid biofertilzer (Market product-2)	12.53	12.13	**15.96**
T_7	Control	11.70	13.76	**15.46**
	F test	Sig.	Sig.	**Sig.**
	SE $\pm$ (m)	0.60	0.16	**0.29**
	CD (P = 0.05)	**0.19**	**0.51**	**0.89**

Table.4 Effect of carrier on number of nodules per plant

Tr.No.	Treatment	No. of nodule/plant
T_1	85 ml *Bradyrhizobium* broth+15ml glycerol	**6.33**
T_2	75 ml *Bradyrhizobium* broth +25ml glycerol	**10.66**
T_3	65 ml *Bradyrhizobium* broth +35 ml glycerol	**11.00**
T_4	25g powder base inoculants	**10.36**
T_5	*Rhizobium* liquid biofertilizer (Market product-1)	**9.66**
T_6	*Rhizobium* liquid biofertilizer (Market product-2)	**8.33**
T_7	Control	**0.00**
	F test	**Sig.**
	SE $\pm$ (m)	**0.67**
	CD (P= 0.05)	**2.05**

Table.5 Effect of various treatment on root and shoot length by paper towel method

Tr.No.	Treatment	Root length (cm/pl.)	Shoot length (cm/pl.)	Germination	SVI
T_1	15ml glycerol+85ml *Bradyrhizobium* broth	11.23	9.20	77.3%	**1579**
T_2	25ml glycerol+75ml *Bradyrhizobium* broth	9.30	6.30	79.3%	**1237**
T_3	35ml glycerol+65 ml *Bradyrhizobium* broth	9.43	6.13	75%	**1167**
T_4	25g powder base inoculants	10.33	7.23	76.6%	**1344**
T_5	*Rhizobium* liquid biofertilizer (Market product-1)	8.20	6.53	75%	**1104**
T_6	*Rhizobium* liquid biofertilizer (Market product-2)	9.66	7.20	74%	**1247**
T_7	Control	9.23	5.8	72%	**1082**
	F test	Sig.	Sig.	Sig	-
	SE $\pm$ (m)	0.1	0.10		-
	CD (P= 0.05)	**0.30**	**0.34**	-	-

The results recorded in the above Table reveals that the seedling vigour index of soybean increased significantly in treatment T_1 (15 ml glycerol + 85 ml *Bradyrhizobium* broth) i.e. increased by 1579 over uninoculated treatment. The remaining

treatments i.e. T_4 (25 g powder base inocualant), T_6 (*Rhizobium* liquid biofertilizer (Market product-2), T_2 (25 ml glycerol + 75 ml *Bradyrhizobium* broth), T_3 (35 ml glycerol + 65 ml *Bradyrhizobium* broth), T_5 (*Rhizobium* liquid biofertilizer (Market product-1) which gave 1344, 1247, 1237, 1167 and 1104 respectively. The minimum seedling vigour index was recorded in uninoculated control i.e., 1082.

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