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SHELF LIFE OF *BRADYRHIZOBIUM* ON DIFFERENT LIQUID BASED INOCULANTS AND PERFORMANCE ON GROWTH AND YIELD OF SOYBEAN

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Abstract: The present investigation shelf life studies of *Bradyrhizobium* by using different carriers Saline solution, glycerol, carboxymethyl cellulose, soybean oil, paddy bran oil, mineral oil, lignite powder mixed with 90, 80, 60 and 75 ml *Bradyrhizobium* broth combination. Shelf life studies indicated significant differences in rhizobial colonies. In field experiment, among all the treatments maximum root length were recorded @ 30 and 45 DAS in T_1 i.e. 10 ml saline solution +90 ml broth (24.77cm/plant, 36.93cm/plant) respectively. Maximum nodulation was recorded by 10 ml saline solution +90 ml *Bradyrhizobium* broth (13.00). Among 20g CMC + 80 ml *Bradyrhizobium* broth treatment recorded significantly higher number of nodules (11.36 nodules/ plant) as compared to other treatment. In paper towel method maximum germination percentage was recorded in 10ml saline solution + 90 ml *Bradyrhizobium* broth (86%) followed by 20g CMC + 80 ml *Bradyrhizobium* broth treatment (80.33%). However root and shoot length was maximum in 10ml saline solution + 90 ml *Bradyrhizobium* broth (11.30 cm) and (9.20 cm) respectively. The maximum seedling vigour index was 1756 by 10ml saline solution +90 ml *Bradyrhizobium* broth seed treatment.

Keywords: Soybean, Vigour index, Inoculants

Introduction:

Soybean (*Glycine max* (L.) Merill) owning to high potential plays an important role in boosting oilseed production in the country. Soybean is an important global crop belongs to the family *Leguminaceae*. The powder carrier based biofertilizers are used since a long time. The traditional nitrogen fixing biofertilizers have suffered from the 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. Also the quantity of inoculum required for application is less as compared to carrier based formulations. It is easier for farmers to handle and apply liquid biofertilizer's formulation which could be considered as one potential strategy for improving the shelf life of biofertilizer. Unlike solid carrier based biofertilizers, liquid formulations allows the manufacturer to include sufficient amount of nutrients, cell protectant and inducers responsible for cell/spore/cyst formation to ensure prolonged shelf life. As being leguminous crop soybean helps in soil enrichment with the atmospherically fixed nitrogen symbiotically available to the succeeding crop. It is estimated that the on an average 20 to 40kg nitrogen is fixed symbiotically (Kunal and Poonam Sharma, 2011). In present study shelf life and performance of liquid based *Bradyrhizobium* is analysed with following objectives to study the shelf life of *Bradyrhizobium* on different liquid carriers and the symbiotic relationship on growth and yield of soybean

In the 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, 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 promotes cells survival in the package and after seed treatment. Therefore, liquid inoculants are formed which have been developed to solve the problems associated with processing of solid carriers.

Material and Methods:

An experiment entitled "Shelf life of *Bradyrhizobium* on different liquid based inoculants and performance on growth and yield of soybean" was conducted in Plant Pathology Laboratory, College of Agriculture, Nagpur during the year 2015-2016. The details of material used and the methods adopted during the course of investigation are included in this chapter given under the following heads. The seed of soybean (JS-335) was obtained from Central Research Station, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Carrier material taken for study viz. saline solution, glycerol, CMC, soybean oil, paddy bran oil, mineral oil, lignite which were procured in ready condition (120 mesh). Seven carriers were tested as carrier material for *Bradyrhizobium,* they were sterilized at 15 lbs pressure for 3 hrs in an autoclave.

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 temperature. *Bradyrhizobium japonicum* broth at 10^7 cells/ml was inoculated with glycerol and lignite. The p^H of lignite was adjusted to 6.5 to 7 by using calcium carbonate. The samples were drawn at monthly interval up to 180 days to study the shelf life. The treatment details were as follow-

Treatment No.	Treatment details
T_1	10ml saline solution + 90 ml *Bradyrhizobium* broth
T_2	10ml glycerol+ 90 ml *Bradyrhizobium* broth
T_3	20 g CMC + 80 ml *Bradyrhizobium* broth
T_4	40ml soybean oil + 60 ml *Bradyrhizobium* broth
T_5	40ml paddybran oil + 60ml *Bradyrhizobium* broth
T_6	25 ml mineral oil + 75 ml *Bradyrhizobium* broth
T_7	Powder base (lignite) 200g + 100 ml *Bradyrhizobium* broth
T_8	Untreated control

Inoculation of carriers with *Bradyrhizobium* inoculants

Five day old isolated broth culture of *Bradyrhizobium japonicum* was used for inoculation so as to adjust the moisture level of carrier to 50% of water holding capacity of the carrier. The carriers after mixing with culture of *Bradyrhizobium japonicum* were cured at 20-25° C for 48 hrs.

In sterilized test tubes with the help of sterilized distilled water blank of 9 ml capacity were prepared. One gram of sample was taken from individual sample into 1st number of water blank to make 1:10 dilution to obtain uniform suspension. Dilution was vigorously shaken mechanically. Soil particles were allowed to settle down. One ml suspension transfer into second blank with the help of sterilized pipette and shake vigorously from 2nd upto 7th. One ml suspension was transferred to sterilized petriplate containing YEMA medium, 10^{-6} dilution was used for estimating rhizobial population in all the treatments.

Inoculation of plate

The sterilized melted medium was poured in previously sterilized petri plates. In each plate 20 ml of medium was poured and rotated with hands for uniform distribution of suspension and allowed to settle down. The plates were incubated at room temperature 28$\pm$ 2^{0}C. Shelf life was studied by serial dilution method at monthly interval

Results and Discussion:

Studies were undertaken on "Shelf life of *Bradyrhizobium* on different liquid based inoculants and performance on growth and yield of soybean" during the year 2015 in Randomized Block Design (RBD) 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 liquid based carrier

Six carriers viz., saline solution, glycerol, carboxy methyl cellulose, soybean oil, paddy bran oil, mineral oil and lignite powder were 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 saline solution (60.33 X 10^7 cell/ml.) and it was found significantly superior over all other treatment at 120 days in respect to other treatment.

Table-1: Rhizobial population (x 10^7 cells/ml of carrier) in liquid carriers

Tr. No.	Treatment	Months					
		I (30 days)	II (60 days)	III (90 days)	IV (120 days)	V (150 days)	VI (180 days)
T_1	Saline solution (10%)	38.96	42.06	48.15	60.33	56.46	45.48
T_2	Glycerol (10%)	26.03	33..65	35.83	47.33	42.95	38.80
T_3	Carboxy methyl cellulose (20%)	31.55	37.10	43.76	55.63	53.58	42.75
T_4	Soybean oil (40%)	19.23	25.71	26.74	36.17	33.28	32.40
T_5	Paddy bran oil (40%)	21.84	24.28	25.36	33.35	30.48	21.74
T_6	Mineral oil (25%)	17.18	19.79	27.44	32.62	32.85	21.51
T_7	Lignite (200%)	24.22	26.97	31.44	29.94	25.73	21.41
T_8	Control	21.28	25.32	26.56	30.71	26.03	18.50
	F test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	SE ± (m)	1.65	1.67	1.69	1.66	1.85	1.74
	CD (P = 0.01)	6.98	7.05	7.14	7.01	7.81	7.33

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

Seeds of soybean variety JS-335 were treated with liquid based inoculants 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 2 reveals that maximum root and shoot length was recorded by (T_1) 10 per cent saline solution (11.30 cm) and (9.20 cm) followed by (T_3) 20 per cent carboxy methyl cellulose treatment noticed (10.33 cm) root length, and (8.33 cm) shoot length.

The data presented in table 2 showed that treatment T_1 (10 per cent saline solution) was registered maximum germination percentage i.e. 86 percent which is significantly superior over all the other treatments. Next best treatment T_3 (20 per cent carboxy methyl cellulose) was registered i.e. 80.33 per cent.

The results recorded in the table 2 reveals that the seedling vigour index of soybean increased significantly in treatment T_1 (10 per cent saline solution) i.e. increased by 1756 over uninoculated treatment.

Table-2: Effect of various treatments on root and shoot length of soybean by paper towel method

Tr. No.	Treatment	Root length (cm/pl.)	Shoot length (cm/pl.)	Germination (%)	Seedling vigour index
T_1	Saline solution (10%)	11.30	9.2	86	1756
T_2	Glycerol (10%)	9.43	7.16	76	1286
T_3	Carboxy methyl cellulose (20%)	10.33	8.33	80.33	1498
T_4	Soybean oil (40%)	8.20	6.41	69	1064
T_5	Paddy bran oil (40%)	7.80	6.56	70.66	1046
T_6	Mineral oil (25%)	8.12	5.9	59	887
T_7	Lignite (200%)	8.72	6.59	53	895
T_8	Control	8.62	5.8	53.66	806
F test		Sig.	Sig.	Sig.	-
SE ± (m)		0.52	0.40		-
CD (P=0.01)		2.17	1.68	-	-

Effect of carrier on the root length

The data in respect of root length are given in table 3 plant root length were recorded at 30, 45 DAS. It is seen from the data that root length was significantly affected by various treatments over control. The maximum root length (36.93 cm) was recorded after 45 DAS by 10 per cent saline solution (T_1). Followed by (T_3) treatment 20 per cent carboxy methyl cellulose (35.73 cm) root length was recorded. and it was found to be significantly superior over all other treatment followed by treatment respectively.

Table-3: Effect of carrier on the root length (cm)

Tr. No.	Treatment	30 DAS (cm)	45 DAS (cm)
T_1	Saline solution (10%)	24.77	36.93
T_2	Glycerol (10%)	21.50	33.40
T_3	Carboxy methyl cellulose (20%)	22.79	35.73
T_4	Soybean oil (40%)	19.59	30.23
T_5	Paddy bran oil (40%)	19.65	31.40
T_6	Mineral oil (25%)	17.71	27.03
T_7	Lignite (200%)	18.86	28.40
T_8	Control	16.87	23.70
F test		Sig.	Sig.
SE ± (m)		1.26	2.19
CD (P = 0.05)		3.82	6.66

Effect of carrier on number of nodules, Pod number & 1000 grain weight.

A field 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 10 per cent saline solution (13 nodules/ plant) and it was significantly superior over all other treatment. Maximum number of pods 55.74 was recorded by treatment T_1 (10 per cent saline solution) which was significantly superior over another treatment. Maximum seed weight 165.66 gm was recorded by treatment T_1 (10 per cent saline solution) which was significantly superior over another treatment

Table-4: Effect of carrier on number of nodules per plant

Tr. No.	Treatment	Nodule Number / plant	Pods number per plant	1000 grain weight (g)
T_1	Saline solution (10%)	13.0	55.74	165.66
T_2	Glycerol (10%)	10.66	48.73	137.66
T_3	Carboxy methyl cellulose (20%)	11.36	53.59	147.66
T_4	Soybean oil (40%)	7.48	41.63	143
T_5	Paddy bran oil (40%)	6.55	37.12	124.66
T_6	Mineral oil (25%)	7.89	39.61	120.33
T_7	Lignite (200%)	8.33	46.21	121
T_8	Control	6.33	36.56	112.66
F test		Sig.		
SE ± (m)		0.54		
CD (P = 0.05)		1.66		

Effects of carriers on grain yield of soybean:

It is seen in Table 5 that grain yield was maximum in T_1 (10 per cent saline solution) i.e.1470.67kg per ha then it was followed by T_3 (20 per cent carboxy methyl cellulose) and T_2 (10 per cent glycerol) i.e. 1267.67kg per ha and 1336.67kg per ha respectively. It was seen from the data that there was significant difference between all the treatments and saline solution was the superior one among all the treatments.

Table-5. Effect of Carriers on grain yield of soybean

Tr. No.	Treatment	grain yield (kg/ha)
T_1	Saline solution (10%)	1470.67
T_2	Glycerol (10%)	1267.67
T_3	Carboxy methyl cellulose (20%)	1336.67
T_4	Soybean oil (40%)	1105.67
T_5	Paddy bran oil (40%)	1046.67
T_6	Mineral oil (25%)	1086.67
T_7	Lignite (200%)	1091.67
T_8	Control	962
F test		Sig.
SE ± (m)		49.18
CD (P = 0.05)		149.18

Conclusions:

Lignite, glycerol, carboxy methyl cellulose,saline solution, mineral oil,soybean oil and paddy bran oi are extensively used as carrier for rhizobia. The present investigation was undertaken on "Shelf life of Bradyrhizobium on different liquid based inoculants and Performance on growth and yield of soybean" during 2015. The observation on shelf life of rhizobia with saline solution 60.33x 10^7 cells/ml, seed germination percentage 86 per cent, root length 11.30 cm/pl and shoot length 9.20 cm/pl using paper towel method and nodulation i.e. 13 nodules/plant, root length, number of pods, 1000 grain weight were recorded. Similarly different carriers were also tested for shelf life of *Rhizobium* The maximum seedling vigour index 1756 in 10 per cent saline solution.

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