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Plant Growth Promotional Activity of Newly Developed Formulation of *Azospirillum* on Maize

Geeta Goudar*, G. Sreenivasulu, Basamma Kumbar and H. Nagaraj

Department of Agricultural Microbiology, College of Agriculture, Vijayapura, UAS, Dharwad, Karnataka, India
**Corresponding author*

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The research work was undertaken to develop solid and liquid based *Azospirillum* formulations using different additives. It was observed that different additives in the formulation supported the growth of *Azospirillum* in the formulations. Survivability of *Azospirillum* formulation in solid/liquid formulations were analyzed by storing them under ambient and refrigerated conditions. Population was estimated at 30, 60, 90, 120 and 180 days after inoculation. Maximum numbers of colonies were observed in liquid based formulations (85 x10^9 cfu/ml). In general the population of *Azospirillum* was more in the formulations which were stored under refrigerated condition compared to room temperature. The developed formulations were tested on maize for their plant growth promotional activity under pot culture condition. The liquid based *Azospirillum* formulation 1 @ 5 ml/kg seed performed better followed by liquid based *Azospirillum* formulation 2 @ 5 ml/kg seed. Among the solid talc based formulations, the formulation 3 @ 500 g/ha performed better.

Introduction

Biofertilizers are products of selected beneficial and live microorganisms, which help to improve plant growth and productivity mainly through supply of plant nutrients. Biofertilizers are also known as microbial inoculants or bioinoculants. Biofertilizers play an important role to improve soil fertility and to help plant growth by increasing the number and biological activity of desired microorganisms in the root environment (Sivasakthivelan and Saranraj, 2013). Death of the organisms in the inoculated seeds is one of the important factors contributing the failure of inoculation response in field condition. Research conducted on the inoculant production and formulation technologies is limited. A break through is needed in the inoculation technology to improve the shelf life and field efficacy of biofertilizer in India to make them commercially viable and acceptable to farmers. The present review was focused on different formulations of *Azospirillum*.

A technical concentrate of an organism that has been achieved by a particular process is called as formulation. There are varieties of formulation *viz.*, liquid and solid. The carrier based bioinoculants generally suffer from shorter shelf life, poor quality, high

contamination and low field performance. One more problem in inoculants technology is the survival of micro-organisms during storage and several parameters such as culture medium, physiological state of the microorganisms when harvested, the process of dehydrates, rate of drying, the temperature, storage and water activity (Aw) of the inoculums have an influence on their shelf life. So, studies to increase the shelf life of inoculants or finding alternate formulations for carrier based inoculants are important (Chunchun *et al.*, 1998).

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 (Sivasaktivelan and Saranraj, 2003).

Liquid biofertilizers contains desired microorganisms and their nutrients, special cell protectants or substances that encourage formation of resting spores for longer shelf life and tolerance to adverse conditions.

So the present study was conducted to improve the shelf life and quality parameters of *Azospirillum* by the addition of suitable additives and also developing new solid and liquid formulations and their role in plant growth promotion under pot culture condition.

Materials and Methods

Culture collection

The *Azospirillum* strain ACD-15 was obtained from the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad.

Preparation of formulations

Three types each of solid and liquid based formulations of *Azospirillum* were developed using different additives as given below.

Nutrient broth was used as a basal medium for solid (talc) based and liquid based formulation with appropriate concentrations of additives.

Solid (Talc based) formulations of *Azospirillum*

Formulation 1 was prepared by inoculating one ml of log phase culture of *Azospirillum* in the broth with different additives like Tween 80 (stabilizer), Glycerol (osmo-protectent), Trehalose (stabilizer), Sorbic acid (preservative), Potassium sorbate (antifungal agent) and flasks were incubated at room temperature for seven days (Table 1).

Formulation 2 was prepared by inoculating one ml of log phase culture of *Azospirillum* in the broth with different additives like Triton X 100 (dispersant), Poly Ethylene Glycol (PEG) (osmo-protectent), Disodium hydrogen phosphate (dispersant), Sorbic acid (preservative), Potassium sorbate (antifungal agent) and flasks were incubated at room temperature for seven days (Table 1).

Formulation 3 was prepared by inoculating one ml of log phase culture of *Azospirillum* in the broth with different additives like Tween 20 (dispersant), Poly Vinyl Alcohol (PVA) (osmo-protectent), Citric acid (stabilizer), Sorbic acid (preservative), Potassium sorbate (antifungal agent) and flasks were incubated at room temperature for seven days (Table 1). After incubation, the population of *A. brasilense* was estimated and culture was mixed with talc powder at 1:3 proportions. These formulations were dried and packed in polypropylene bags.

Liquid formulations of *Azospirillum*

Nutrient broth was used as a basal medium for talc based and liquid based formulation with appropriate concentrations of additives.

Formulation 1 was prepared by inoculating one ml of log phase culture of *Azospirillum* in the broth with different additives like Tween 80 (dispersant), Glycerol (thickner), Xanthan gum (suspending agent), Sorbic acid (preservative), Potassium sorbate (antifungal agent) and flasks were incubated at room temperature for seven days (Table 2).

Formulation 2 was prepared by inoculating one ml of log phase culture of *Azospirillum* in the broth with different additives like Tween 20 (dispersant), Poly Ethylene Glycol (PEG) (thickner), Skimmed milk (suspending agent), Sorbic acid (preservative), Potassium sorbate (antifungal agent) and flasks were incubated at room temperature for seven days (Table 2).

Formulation 3 was prepared by inoculating one ml of log phase culture of *Azospirillum* in the broth with different additives like Triton X-100 (dispersant), Carboxy methyl cellulose (CMC) (thickner), Bentonite powder (suspending agent), Sorbic acid (preservative), Potassium sorbate (antifungal agent) and flasks were incubated at room temperature for seven days (Table 2).

Survivability of *Azospirillum* in Solid/ Liquid formulations

To study the survivability of *Azospirillum* in different formulations, the developed solid and liquid formulations and one standard formulation were stored under ambient and refrigerated condition.

Three replications were maintained for each treatment. At monthly interval, the viable counts were determined upto180 days.

Plant growth promotional activity of *Azospirillum* formulations in Maize under pot culture condition

Preparation of pots

Red soil collected from the nearby fields of Vijayapura was mixed with sand and farm yard manure (4:1:1) and filled in to pots of 90 cm diameter at the rate of 6 kg per bag and were kept in a shade house.

Fertilizer application

The recommended dose of fertilizer for maize is 25:50:0 NPK kg/ha. Calculated quantities of urea, single super phosphate and muriate of potash were applied on soil weight basis after sowing *Azospirillum* treated maize seeds. The observation was recorded for plant growth and yield parameters.

Results and Discussion

Survivability of *Azospirillum* in Solid/ Liquid formulations

Results on survivability of *Azospirillum* in solid and liquid formulations stored under ambient and refrigerated conditions for 30, 60, 90, 120, 150 and 180 days are presented in Table 3.

At ambient temperature

Maximum population of *Azospirillum* was observed in F5 – LBAF2 ($101x10^9$ cfu/ml) followed by F4 – LBAF1 ($91 x10^9$ cfu/ml) at 30 days after incubation. The population of 10^9 cfu/ml was maintained till 120 DAI.

There after there was a gradual decrease in the population. At 180 DAI, maximum population of $57x10^8$ cfu/ml was recorded in F5 – LBAF2 followed by F4 – LBAF1 ($53 x10^8$ cfu/ml). Least population was observed

in F7 – STBAF (existing) ($30x10^8$ cfu/ml) at 180 days after incubation.

At refrigerated condition

Population of 10^9 cfu/ml was maintained in all the formulations stored under refrigerated condition up to 180 DAI. Maximum population of $96x10^9$ cfu/ml was observed in F5 – LBAF2 followed by F4 – LBAF1 (85 $x10^9$ cfu/ml) at 30 DAI. Thereafter slight decrease in the population was noticed at 150 and 180 DAI.

In general, population of *Azospirillum* was maintained under refrigerated condition than the ambient temperature. Sudden decrease in the population was observed in those formulations which were stored under ambient temperature. Among two types of formulations, the liquid formulation supported more growth of organism.

For the preparation of formulations F4 – LBAF1, different additives like Tween 80, Glycerol, Xanthan gum and for F5 – LBAF2, the additives like Tween 20, PEG, Skimmed milk were used.

Various polymers such as PVP, PEG and Gum arabic have adhesive properties. They have sticky consistency, which may enhance cell adherence to seed, and their viscous nature may slow the drying process of the bioinoculants (Temprano *et al.,* 2002).

Similarly, Kumaresan and Reetha (2011) reported that, liquid *Azospirillum* bioinoculant formulated with Trehalose (10mM) promoted long term survival of *Azospirillum* followed by Glycerol (10 mM), Gum arabica (0.3%) and PVP (2%) and they supported 10^8 cells/ml up to 11 months of storage under ambient temperature (28°C to 32°C), whereas PEG (1%), PVA (0.5%) and control (lignite carrier) recorded the same population up to 8 months, 6 months and 5 months respectively. Liquid inoculants formulated with 2% Poly Vinyl Pyrollidone (PVP 30 K), 0.1% Carboxy Methyl Cellulose (CMC-high density) and 0.025% Tween 20 promoted long-term survival of *Bacillus megaterium* var. *phosphaticum*, *Azospirillum* and *Azotobacter* with population of 5.6 x 10^7, $1.9x10^8$ and $3.5x10^7$ cfu/ml, respectively after 480 days of formulation when stored at 30°C (Amalraj, 2013).

Higher survival of inoculants was recorded in liquid formulation up to a storage period of 3 month. Better survival of *P. striata* in Glycerol and Tween 20 for longer period was observed and it could be improved by the addition of Skimmed milk and controlled dehydration (Mugilan *et al.,* 2011). Among the different additives, 2.5 % of Glycerol and 2.5 % of Tween 20 was found to record a maximum population of 20.11 x 10^9 on the 3rd month of storage followed by 2% of Glycerol and 2% of Tween 20. Whereas uninoculated additives (control) recorded only 12 x 10^9 populations. Carrier based inoculants generally suffer from shortage shelf, poor quality, high contamination and low field performance. The liquid formulations of *Azospirillum* amended with Trehalose, Poly Vinyl Pyrollidine and Glycerol enhanced and maintained the population level at 10^8 cfu/ml up to 10 months of storage (Vendan and Thangaraju, 2006). The formulations shows better adherence and survival on seeds, roots of seedlings and in the rhizosphere soil than the solid carrier based *Azospirillum* inoculants.

Plant growth promotional activity of *Azospirillum* formulations in Maize

Result on effect of different *Azospirillum* formulations on plant height of maize were recorded at 30, 60 days after sowing and at harvest are presented in Table 4.

At 30 DAS, highest plant height of 48.2 cm was observed in T1-STBAF1@500 g/ha (48.2 cm) which is significantly superior over rest of the treatments. The treatments T10-LBAF2@5ml/kg seeds (44.2 cm) and T12-LBAF3@5ml/kg seeds (44.20 cm) were significantly superior over rest of the treatments.

At 60 DAS, the highest plant height was recorded by the treatment T10-LBAF2@5ml/kg seeds (91.0 cm), which is significantly superior over all other treatments. The next highest plant height was noticed in the treatment T8-LBAF1@5ml/kg seeds (88.3 cm) which is on par with the treatments T5-STBAF3@500g/ha (86.6 cm) and T11- LBAF2 @ 3 ml/ kg seeds (86.30 cm).

Highest plant height at harvest was observed in T10-LBAF2@5ml/kg seeds (192.00 cm) which is significantly superior over all other treatments. The next highest plant height was observed in T8-LBAF1@5ml/kg seeds (187.3 cm) followed by T5-STBAF3@500g/ha (185.7 cm), whereas, least plant height was observed in T15-control (130.3cm). Results on effect of different *Azospirillum* formulations on shoot and root dry weight was recorded at 30, 60 days after sowing and at harvest are presented in Table 4.

At 30 DAS, highest shoot dry weight of 15.8 g was recorded by the treatment T10 – LBAF2@5ml/kg seeds which was significantly superior over rest of the treatments. The treatments T8 – LBAF1@5ml/kg seeds (13.7 g/plant), T5 – STBAF3 @ 500 g/ha (12.8g/plant) and T11-LBAF2 @ 3 ml/ kg seeds (12.5 g/plant) were on par with each other.

At 60 DAS, treatment T10 – LBAF2@5ml/kg seeds recorded highest shoot dry weight of 22.7 g/plant, which was significantly superior over rest of the treatments. The treatments T8 – LBAF1@5ml/kg seeds (19.7 g/plant) and T5 – STBAF 3 @ 500 g/ha (18.6 g/plant) were on par with each other.

Maximum shoot dry weight of 51.6 g/plant was recorded in T10 – LBAF2@5ml/kg seeds at harvest, which was significantly superior over rest of the treatments. The next highest shoot dry weight was recorded in T8 – LBAF1@5ml/kg seeds (48.1 g/plant) followed by T5– STBAF 3 @ 500 g/ha (45.8 g/plant). Least shoot dry weight was observed in T15 – control (32.0 g/plant).

Insignificant results were observed with respect to root dry weight of plant at 30 DAS.

At 60 DAS, highest root dry weight of 4.6 g was observed in T10– LBAF2@5ml/kg followed by T8 – LBAF1@5ml/kg seeds (4.2g/plant) which were on par with each other.

Maximum root dry weight of maize at harvest was found in T10 – LBAF2@5ml/kg seeds (5.9 g/plant) which was on par with T8 – LBAF1@5 ml/kg seeds (5.8g/plant) and T_5– STBAF 3 @ 500 g/ha (5.6g/plant). Least root dry weight was observed in T15 – control (3.2g/plant).

Result on the effect of different *Azospirillum* formulations on total dry matter weight was recorded at 30, 60 days after sowing and at harvest and are presented in Table 5.

At 30 DAS, the highest total dry matter weight of 17.6 g was recorded in T10– LBAF2@5ml/kg seeds which were significantly superior over rest of the treatments. The treatments T8– LBAF1@5ml/kg seeds (15.4 g/plant), T_5– STBAF3@500g/ha (14.5 g/plant) and T11-LBAF2 @ 3 ml/ kg seeds (14.1 g/plant) were on par with each other.

Table.1 Preparation of Solid (Talc based) formulations of *Azospirillum*

Ingredients		**Composition (% w/w or v/w)**			
Filler	Talc	**Existing formulation**	**Formulation 1**	**Formulation 2**	**Formulation 3**
Filler	Talc	100	95.0	95.0	95.0
Dispersant	Tween 80	--	3.0	--	--
	Triton X 100	--	--	3.0	--
	Tween 20	--	--	--	3.0
Osmo-protectant	Glycerol	--	1.0	--	--
	Poly Ethylene Glycol (PEG)	--	--	1.0	--
	Poly Vinyl Alcohol (PVA)	--	--	--	1.0
Stabilizer	Trehalose	--	0.6	--	--
	Disodium hydrogen phosphate	--	--	0.6	--
	Citric acid	--	--	--	0.6
Preservative	Sorbic acid	--	0.2	0.2	0.2
Antifungal agent	Potassium sorbate	--	0.2	0.2	0.2

Table.2 Preparation of Liquid formulations of *Azospirillum*

Ingredients		**Composition (% w/w or v/w)**		
Filler	Talc	**Formulation 1**	**Formulation 2**	**Formulation 3**
Filler	Culture broth	95.0	95.0	95.0
Dispersant	Tween 80	3.0	--	--
	Tween 20	--	3.0	--
	Triton X 100	--	--	3.0
Thickener	Glycerol	0.2	--	--
	Poly Ethylene Glycol (PEG)	--	0.2	--
	Carboxyl methyl cellulose (CMC)	--	--	0.2
Suspending agent	Xanthan gum	1.4	--	--
	Skimmed milk powder	--	1.4	--
	Bentonite powder	--	--	1.4
Preservative	Sorbic acid	0.2	0.2	0.2
Antifungal agent	Potassium sorbate	0.2	0.2	0.2

Table.3 Population of *Azospirillum* in different formulations stored under ambient and refrigerated conditions

Treatments	Ambient condition						Refrigerated condition					
	30 DAI (x 10^9)	60 DAI (x 10^9)	90 DAI (x 10^9)	120 DAI (x 10^9)	150 DAI (x 10^8)	180 DAI (x 10^8)	30 DAI (x 10^9)	60 DAI (x 10^9)	90 DAI (x 10^9)	120 DAI (x 10^9)	150 DAI (x 10^9)	180 DAI (x 10^9)
F_1– STBAF1	73	62	47	40	50	35	78	71	64	60	55	50
F_2– STBAF2	81	71	56	50	55	40	78	74	68	62	57	52
F_3– STBAF3	87	77	60	52	58	53	81	78	72	68	60	58
F_4– LBAF1	91	79	63	58	62	57	85	81	75	70	65	60
F_5– LBAF2	101	84	70	60	57	42	96	92	85	78	67	62
F_6– LBAF3	78	68	55	50	42	35	76	70	63	68	60	54
F_7– STBAF (Existing)	69	54	45	40	38	30	66	62	55	50	42	35
S.Em±	1.90	0.9	1.07	0.67	0.63	1.03	0.86	0.82	0.89	1.00	0.78	0.49
CD (0.01)	5.49	2.75	3.27	2.05	1.94	3.16	2.62	2.49	2.70	3.05	2.40	1.49

*STBAF- Solid talc based *Azospirillum* formulations
**LBAF- Liquid based *Azospirillum* formulations
***DAI- days after inoculation

Table.4 Effect of different *Azospirillum* formulations on plant height (cm) and dry matter content (g/plant) of Maize

Treatments	Plant height (cm)			Shoot dry weight			Root dry weight		
	30 DAS*	60 DAS	At harvest	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest
T_1– STBAF 1 @ 500 g/ha	48.2	80.6	178.0	9.8	14.2	37.7	1.2	2.9	5.2
T_2– STBAF 1 @ 250 g/ha	39.9	78.8	174.3	7.9	11.2	34.7	1.1	2.6	4.6
T_3– STBAF 2 @ 500 g/ha	34.9	84.3	182.0	11.5	16.7	42.0	1.4	3.4	5.4
T_4 – STBAF 2 @ 250 g/ha	35.9	79.3	176.7	8.5	12.0	36.6	1.1	2.7	5.0
T_5– STBAF 3 @ 500 g/ha	40.1	86.6	185.7	12.8	18.6	45.8	1.6	4.0	5.7
T_6 –STBAF 3 @ 250 g/ha	37.9	81.3	180.7	10.5	15.0	38.4	1.4	3.2	5.3
T_7– STBAF (Existing) @ 500 g/ha	40.2	76.6	138.0	7.5	10.6	34.6	0.9	2.2	4.1
T_8 – LBAF1 @ 5 ml/ kg seeds	39.0	88.3	187.3	13.7	19.7	48.1	1.7	4.2	5.8
T_9 – LBAF1 @ 3 ml/ kg seeds	38.4	83.3	182.0	10.8	15.1	38.7	1.4	3.2	5.3
T_{10} – LBAF2 @ 5 ml/ kg seeds	44.2	91.0	192.0	15.8	22.7	51.6	1.9	4.6	5.9
T_{11}– LBAF2 @ 3 ml/ kg seeds	39.9	86.3	185.0	12.5	17.9	42.9	1.6	3.9	4.1
T_{12} – LBAF3 @ 5 ml/ kg seeds	44.2	76.0	157.0	7.3	10.3	34.6	0.9	2.2	3.9
T_{13}– LBAF3 @ 3 ml/ kg seeds	41.9	71.3	152.7	6.7	9.4	38.2	0.9	2.1	3.7
T_{14} – Culture broth	42.2	84.0	145.0	10.9	15.4	40.7	1.4	3.3	4.8
T_{15}– Control	28.5	61.3	130.3	4.9	7.0	32.0	0.7	1.7	3.2
S.Em±	0.58	0.91	0.74	0.51	0.85	0.48	0.07	0.12	0.07
CD (0.01)	1.67	2.51	1.51	1.47	2.31	1.32	NS	0.33	0.19

*DAS- days after sowing

Table.5 Effect of different *Azospirillum* formulations on total dry matter content (g/plant) and yield of Maize

Treatments	Total dry matter content (g/plant)			Cob wt (g/plant)
	30 DAS*	60 DAS	At harvest	
T_1– STBAF 1 @ 500 g/ha	11.1	17.1	42.97	129.8
T_2– STBAF 1 @ 250 g/ha	9.0	13.8	39.37	126.1
T_3– STBAF 2 @ 500 g/ha	12.9	20.0	47.43	136.6
T_4 – STBAF 2 @ 250 g/ha	9.6	14.7	41.59	127.6
T_5– STBAF 3 @ 500 g/ha	14.5	22.6	51.43	141.3
T_6 –STBAF 3 @ 250 g/ha	11.9	18.3	43.71	137.8
T_7– STBAF (Existing) @ 500 g/ha	8.4	12.8	38.67	124.1
T_8 – LBAF1 @ 5 ml/ kg seeds	15.4	24.0	53.90	144.5
T_9 – LBAF1 @ 3 ml/ kg seeds	12.2	18.4	43.93	135.8
T_{10} – LBAF2 @ 5 ml/ kg seeds	17.6	27.3	57.50	146.3
T_{11}– LBAF2 @ 3 ml/ kg seeds	14.1	21.8	47.10	126.1
T_{12} – LBAF3 @ 5 ml/ kg seeds	8.2	12.4	38.42	124.3
T_{13}– LBAF3 @ 3 ml/ kg seeds	7.6	11.5	41.90	122.3
T_{14} – Culture broth	12.2	18.7	45.56	131.5
T_{15}– Control	5.6	8.7	35.20	101.9
S.Em±	0.6	0.76	0.4963	0.5
CD (0.01)	1.7	2.14	1.371	1.3

*DAI- days after inoculation

The treatment T10– LBAF2@5ml/kg seeds recorded the highest total dry matter weight of 27.3 g, which was significantly superior over rest of the treatments. The treatments T8– LBAF1@5ml/kg seeds (24.0 g/plant) and T_5– STBAF3@500g/ha (22.6 g/plant) were on par with each other.

Maximum total dry matter content at harvest was found in T10– LBAF2@5ml/kg seeds (57.5 g/plant) which was significantly superior over rest of the treatments followed by T8– LBAF1@5ml/kg seeds (53.9 g/plant) and T_5– STBAF3@500g/ha (51.4 g/plant). Least total dry matter content was found in T15 – control (35.2 g/plant).

Highest cob weight after harvest was found in T10 – LBAF2@5ml/kg seeds (146.3 g/plant) followed by T8 – LBAF1@5ml/kg seeds (144.5 g/plant) and T_5– STBAF3@ 500 g/ha (141.3 g/plant). Least total dry matter content was found in T15 – control (101.9 g/plant).

Among the different formulations tested, liquid formulation showed the maximum inoculation effect compared to carrier based formulations.

The results are in conformity with the observations made by Mugilan *et al.,* (2011), who reported maximum germination percentage, maximum root length and shoot length of paddy with liquid inoculation of *P. striata* compared to carrier based inoculation. The increased effect of liquid formulation may be due to higher population of *P. striata*. This increase in root colonization as influenced by these cells would increase the density and length of root hairs, as well as the appearance and elongation rate of lateral roots, thus increasing surface area (Fallik *et al.,* 1994).

The bioinoculant treatment of liquid formulation of *Azospirillum lipoferum* (AUAz-1) + *Bacillus megaterium* (AU Ba-1) + *Pseudomonas fluorescens* (AU Ps-1) produced the highest recorded values in all growth and yield parameters of sunflower compared with carrier based and alginate bead formulations (Sivasakthivelan and Stella, 2012).

Inoculation of maize crops with an active strain of *A. brasilense* has a beneficial effect on maize vigour and yield under the identical climatic and soil conditions (Subramanian, 2014).

Inoculation of soil with *Azospirillum* promoted sheath elongation, root depth, fresh weight of roots, fresh and dry weight of shoots, total nitrogen and bacterial counts in soil. The results showed that wheat inoculated with *Azospirillum* had a higher growth, mineral and chlorophyll when the plants were not supplemented with nitrogen (Sayed *et al.,* 2015). Inoculation with *Azospirillum* may improve plant growth and yield by supplementing the growing plants with fixed nitrogen and growth promoting substances (Sumne, 1990).

This increase in yield is also attributed mainly to an improvement in root development, an increase in the rate of water and mineral uptake by roots, and to a lesser extent, biological N_2 fixation. *A. brasilense* shows both chemotaxis and chemokinesis in response to temporal gradients of different chemoeffectors, thereby increasing the chance of root-bacterial interactions. Phytohormones synthesized by *Azospirillum* influence the host root respiration rate, metabolism and root proliferation and hence better the mineral and water uptake in inoculated plants (Okon and Itzigsohn, 1995).

Azospirillum is one of the important biofertilizer, which is found to fix nitrogen in association with world's most staple food crops like rice, maize, sorghum, wheat and millets (Boddey and Dobereiner, 1988). Formulation of biofertilizer plays a vital role in helping to solve many problems in agricultural field and in making an organism effective in the field. However this must be achieved in a cost effective manner so that product has to survive commercially. Formulation comprises aids to preserving organisms and delivering them to their target fields and once-there to improve their activities (Sivashaktivelan and Saranraj, 2013). A technical concentrate of an organism

that has been achieved by a particular process is called as formulation. There are varieties of formulation both liquid and solid. The carrier based bioinoculants generally suffer from shorter shelf life, poor quality, high contamination and low field performance. Therefore, it is desirable that new inoculant formulations being developed where liquid inoculants play a significant role.

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