

Comparative Evaluation of Selected Formulations of a Microbial Consortium

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ABSTRACT

A Laboratory investigation was carried out to compare and to evaluate the effect of four different formulations viz., alginate based, fluid bed dryer based, lignite and liquid formulations on survivability of microbial inoculants as single, dual and triple inoculants. The highest survival of *Rhizobium* sp., \log_{10} 9.47 cells/ml, *Bacillus megaterium* \log_{10} 9.26 cells/ml, were recorded in liquid formulation whereas, *Pseudomonas fluorescens* recorded highest survival of \log_{10} 9.68 cells/ g in alginate formulation. Overall, Maximum viable cells and per cent survival were recorded in the liquid formulation amended with PVP-40 and glycerol at the rate of 2 per cent followed by alginate based, lignite and least was observed in fluid bed dryer based formulations.

Keywords: Alginate, fluid bed dryer, lignite, liquid formulation, consortium

ORGANIC farming has received a substantial attention from the past two decade because of awareness about the soil health among the individuals. This approach is constantly increasing year after year. Microbes are often referred as wheels of organic farming (Sreenivasa, 2012) because of their pivotal role in maintaining the soil health as well as making the nutrients available to the plants either by mineralization, solubilization, mobilization or by decomposition of organic residues. These microbial agents are preferred to use as consortium (mixture of more than one genus/species of microbe as an inoculant) in order to fulfill more than one purpose. Use of combination of *Azotobacter* sp., *Bacillus* sp., and *Pseudomonas* sp., will encourage the growth and development of agricultural crops more rigorously than that of using single inoculant alone (Brahmaprakash and Sahu, 2012). Several studies have been reported on various formulations from conventional methods to advanced techniques (Sahu and Brahmaprakash, 2016). Conventional formulations include, use of lignite (Saranya *et al.*, 2011), talc (Shilpa and Brahmaprakash, 2016), sheep manure (Sarhan *et al.*, 2011), vermicompost (Kumar and Singh, 2001). Modern methods include, tablet effervescent (Sneha and Brahmaprakash, 2017), alginate beads for entrapment (Hegde and Brahmaprakash, 1992) and fluid bed dryer based formulation (Sahu *et al.*, 2013). At this context, an investigation was undertaken on two of conventional

formulation (Lignite and liquid) and two of modern era formulation (Alginate based entrapment and fluid bed dryer based) for the development of microbial consortium (*Rhizobium* sp., *Bacillus megaterium*, *Pseudomonas fluorescens*) and their effect on survival of microbial inoculants.

MATERIAL AND METHODS

The inoculants used in the study were obtained from the Department of Agricultural Microbiology, University of Agricultural Sciences, Gandhi Krishi Vignan Kendra, Bengaluru-560 065.

Preparation of different formulations

Alginate based formulation was prepared by mixing the bacterial culture aseptically with 2 per cent (wt/vol) sodium alginate powder and stirred gently for 1 hr. Then the mixture was added drop wise with aid of 10 ml sterile syringe into sterilized 0.1M CaCl_2 at room temperature resulting in formation of beads in the CaCl_2 solution. The beads were maintained in the solution at room temperature for an additional 1-2 hr to obtain regular solid beads. Then the CaCl_2 solution was pumped out and the beads were washed twice with sterile distilled water. After repeated washing the beads were incubated in fresh respective media for further study.

Fluid Bed Dryer (FBD) based formulation was prepared by subjecting the inoculants mixture with

talc amended with 0.5 per cent of carboxy methyl cellulose (CMC) in hot air which have been allowed to flow upwards through a bed of solid particles at a velocity greater than settling velocity of particles.

Lignite obtained was sieved through 2 mm sieve to remove the inert matter and later it was moistened with 15 ml distilled water along with calcium hydroxide at the rate of 4 per cent was added to adjust the pH and then packets containing lignite were autoclaved at 121°C for 15 min. After 3 days, packets were mixed with respective inoculants and stored at room temperature.

Liquid medium was prepared for respective inoculants (Yeast Extract Mannitol Broth (YEMB), Pikovskaya's broth and king's B broth for *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens*, respectively) each medium were amended with PVP-40 and glycerol at the rate of 2 per cent.

Preparation of microbial consortium and survival study

Four different formulations were inoculated with inoculants in single, dual and triple combination and their survival was monitored at an interval of 0, 10,

20, 30, 60, 90, 120, 150, 180 days of storage at room temperature. Statistical analysis of the population study was done by using complete randomized block design and means were compared by the Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Survival of single, dual and triple inoculants in alginate based formulations

The data pertaining to survival study of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* in alginate formulation are presented in Table I.

Single inoculant of *Rhizobium* sp., in alginate based formulation was recorded \log_{10} 10.11 cfu/g of population at the initial day of storage and population of \log_{10} 9.56 cfu/g of alginate beads was observed at 180 days of storage. The inoculant, *Bacillus megaterium* was recorded \log_{10} 9.85 cfu/g of population in the beginning day of storage. Later, \log_{10} 9.24 cfu/g of population was observed at 180 days of storage in alginate based formulation whereas, the single inoculant of *Pseudomonas fluorescens* was

TABLE I
Survival of microbial inoculants consortium in alginate formulation

Inoculants		Population (\log_{10} cfu/ g)								
		Duration of the storage (days)								
		0	10	20	30	60	90	120	150	180
Inoculant-1	Rh	10.11 ^a	10.08 ^b	10.06 ^{bc}	9.98 ^d	9.91 ^d	9.78 ^c	9.70 ^c	9.65 ^c	9.56 ^b
Inoculant-2	Bm	9.85 ^c	9.84 ^e	9.80 ^{ef}	9.74 ^f	9.63 ^f	9.56 ^d	9.43 ^d	9.34 ^e	9.24 ^c
Inoculant-3	Pf	10.14 ^a	10.11 ^a	10.11 ^a	10.08 ^a	10.04 ^a	9.97 ^a	9.92 ^a	9.81 ^a	9.68 ^a
Inoculant-4 (Rh+Bm)	Rh	10.10 ^a	10.08 ^b	10.05 ^c	9.98 ^d	9.95 ^c	9.91 ^b	9.83 ^b	9.74 ^{ab}	9.62 ^{ab}
	Bm	9.91 ^b	9.88 ^d	9.81 ^e	9.71 ^g	9.62 ^{fg}	9.46 ^e	9.31 ^e	9.19 ^f	9.05 ^d
Inoculant-5 (Rh+Pf)	Rh	10.12 ^a	10.10 ^{ab}	10.07 ^{bc}	10.05 ^b	9.98 ^{bc}	9.94 ^{ab}	9.88 ^{ab}	9.80 ^a	9.65 ^{ab}
	Pf	10.14 ^a	10.09 ^{ab}	10.06 ^{bc}	10.02 ^c	9.96 ^{bc}	9.92 ^b	9.86 ^{ab}	9.71 ^{bc}	9.60 ^{ab}
Inoculant-6 (Bm+Pf)	Bm	9.94 ^b	9.91 ^c	9.89 ^d	9.84 ^e	9.79 ^e	9.74 ^c	9.65 ^c	9.40 ^{dc}	9.25 ^c
	Pf	10.12 ^a	10.09 ^{ab}	10.04 ^c	10.00 ^{cd}	9.96 ^{bc}	9.93 ^{ab}	9.87 ^{ab}	9.80 ^a	9.62 ^{ab}
Inoculant-7 (Rh+Bm+Pf)	Rh	10.12 ^a	10.10 ^{ab}	10.09 ^{ab}	10.05 ^b	9.99 ^b	9.93 ^{ab}	9.66 ^c	9.46 ^d	9.22 ^c
	Bm	9.90 ^b	9.82 ^e	9.77 ^f	9.69 ^g	9.59 ^g	9.44 ^e	9.29 ^e	9.01 ^g	8.59 ^e
	Pf	10.12 ^a	10.09 ^{ab}	10.07 ^{bc}	10.02 ^c	9.99 ^b	9.93 ^{ab}	9.89 ^{ab}	9.76 ^{ab}	9.63 ^{ab}

Note: Means with same superscript are statistically on par at $P \leq 0.01$ by DMRT

Rh: *Rhizobium* sp., Bm: *Bacillus megaterium*, Pf: *Pseudomonas fluorescens*

recorded \log_{10} 10.14 cfu/g of population at the initial day of storage at the end of 180 days (\log_{10} 9.68 cfu/g). The population was recorded in alginate based formulations

Dual inoculant of *Rhizobium* sp. and *Bacillus megaterium* in alginate recorded the population of \log_{10} 10.10 cfu/g and \log_{10} 9.91 cfu/g, respectively in initially, The population of *Rhizobium* sp. started declining after 30 days (\log_{10} 9.98 cfu/g) whereas, the population of *Bacillus megaterium* started declining 90 days (\log_{10} 9.46 cfu/g). At the end of 180 days storage of *Rhizobium* sp. \log_{10} 9.62 cfu/g and *Bacillus megaterium* \log_{10} 9.05 cfu/g was recorded in alginate based formulation and the dual inoculants of *Rhizobium* sp. and *Pseudomonas fluorescens* recorded the population of \log_{10} 10.12 cfu/g and \log_{10} 10.14 cfu/g, respectively. Both the population of *Rhizobium* sp. and *Pseudomonas fluorescens* showed the stabilization till the end of 180 days (\log_{10} 9.65 cfu/g and \log_{10} 9.60 cfu/g, respectively) during the storage in alginate based formulation whereas, the alginate based formulation of *Bacillus megaterium* and *Pseudomonas fluorescens* recorded was \log_{10} 9.94 cfu/g and \log_{10} 10.12 cfu/g, respectively in initial population. The population of *Bacillus megaterium* started declining after 30 days (\log_{10} 9.84 cfu/g) whereas, the population of *Pseudomonas fluorescens* started declining after 60 days (\log_{10} 9.96 cfu/g). At the end of 180 days, storage of *Bacillus megaterium* \log_{10} 9.25 cfu/g and *Pseudomonas fluorescens* \log_{10} 9.62 cfu/g was recorded.

Triple inoculants of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* recorded \log_{10} 10.12 cfu/g, \log_{10} 9.90 cfu/g and \log_{10} 10.12 cfu/g, respectively in initial days of population. The population of *Rhizobium* sp. and *Bacillus megaterium* started declining after 60 days (\log_{10} 9.99 cfu/g and \log_{10} 9.59 cfu/g) whereas, *Pseudomonas fluorescens* maintained optimum growth till the end of 180 days. At the end of 180 days storage of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* recorded \log_{10} 9.22 cfu/g, \log_{10} 8.59 cfu/g and \log_{10} 9.63 cfu/g in alginate based formulation.

Since, the mixture of microbial inoculants and sodium alginate is allowed to react with calcium chloride resulted in formation of solid beads which can be stored for longer period. Usually, this kind of entrapment is done for enzymes to slow down its activity. The similar technique was used in the present study to ensure the longer shelf life within the beads by entrapping the microbial cells to restrict its metabolic activity tenaciously. The similar observations were made by Archana (2011).

Survival of single, dual and triple inoculants in fluid bed dryer based formulation

The data pertaining to survival study of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* in fluid bed dryer based formulation are presented in Table II.

The single inoculants of *Rhizobium* sp., in fluid bed dryer based formulation recorded \log_{10} 6.76 cfu/g of population at the initial day of storage. Subsequently, the population started to decline after 120 days (\log_{10} 5.89 cfu/g). The population of \log_{10} 5.00 cfu/g was recorded by *Rhizobium* sp. at 180 days of storage in fluid bed dryer based formulation.

The fluid bed dryer formulation of *Bacillus megaterium* recorded \log_{10} 7.08 cfu/ml of population at the initial day of storage. Later, the population at 60 days (\log_{10} 6.87 cfu/ml), 90 days (\log_{10} 6.81 cfu/ml) was recorded. Later, the population started to decline gradually after 120 days (\log_{10} 6.67 cfu/ml), 150 days (\log_{10} 6.52 cfu/ml) and the population of \log_{10} 6.38 cfu/ml was observed at 180 days of storage in liquid based formulation.

Pseudomonas fluorescens in fluid bed dryer formulation recorded \log_{10} 6.95 cfu/g of population at the initial day of storage, subsequently, the population started to decline after 90 days (\log_{10} 6.73 cfu/g). The population was recorded at 150 days \log_{10} 6.49 cfu/g and \log_{10} 6.25 cfu/g of population was observed at 180 days of storage in fluid bed dryer based formulation.

The fluid bed dryer based formulation of *Rhizobium* sp. and *Bacillus megaterium* recorded the population of \log_{10} 6.63 cfu/g and \log_{10} 7.07 cfu/g, respectively in the initial days of storage. At 60 days,

the populations of *Rhizobium* sp. and *Bacillus megaterium* started showing declination (\log_{10} 6.34 cfu/g and \log_{10} 6.81 cfu/g, respectively). Both the population of *Rhizobium* sp. and *Bacillus megaterium* at the end of 180 days showed \log_{10} 5.20 cfu/g and \log_{10} 6.05 cfu/g, respectively during the storage in fluid bed dryer based formulation.

The fluid bed dryer based formulation of *Rhizobium* sp. and *Pseudomonas fluorescens* recorded the population of \log_{10} 6.63 cfu/g and \log_{10} 6.96 cfu/g, respectively in the initial day of storage, after 60 days, the population of *Rhizobium* sp. started showing declination (\log_{10} 6.39 cfu/g) and after 150 days the population of *Pseudomonas fluorescens* (\log_{10} 6.35 cfu/g) started showing declination. Both the population of *Rhizobium* sp. and *Pseudomonas fluorescens* were recorded \log_{10} 5.40 cfu/g and \log_{10} 6.12 cfu/g, respectively at the end of 180 days of storage in fluid bed dryer based formulation.

The fluid bed dryer based formulation of *Bacillus megaterium* and *Pseudomonas fluorescens* recorded

the population of \log_{10} 7.07 cfu/g and \log_{10} 6.94 cfu/g, respectively in the initial day of storage. At 90 days, the population of *Bacillus megaterium* and *Pseudomonas fluorescens* started showing declination (\log_{10} 6.82 cfu/g and \log_{10} 6.61 cfu/g, respectively). Both the population of *Bacillus megaterium* and *Pseudomonas fluorescens* at the end of 180 days showed \log_{10} 6.24 cfu/g and \log_{10} 6.09 cfu/g, respectively during the storage in fluid bed dryer based formulation.

The fluid bed dryer based formulation of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* recorded population of \log_{10} 6.67 cfu/g, \log_{10} 7.06 cfu/g and \log_{10} 7.05 cfu/g, respectively in initial population. After 90 days of storage the population of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* started showing declination (\log_{10} 6.29 cfu/g, \log_{10} 6.89 cfu/g and \log_{10} 6.78 cfu/g, respectively). At the end of 180 days, the population of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* recorded population of \log_{10} 5.40 cfu/g, \log_{10} 6.45 cfu/g and \log_{10}

TABLE II
Survival of microbial inoculants consortium in fluid bed dryer based formulation

Inoculants		Population (\log_{10} cfu/ g)								
		Duration of the storage (days)								
		0	10	20	30	60	90	120	150	180
Inoculant-1	Rh	6.76 ^c	6.71 ^f	6.67 ^d	6.58 ^f	6.38 ^g	6.24 ^f	5.89 ^g	5.40 ^f	5.00 ^g
Inoculant-2	Bm	7.08 ^a	7.05 ^b	7.02 ^a	6.94 ^{ab}	6.87 ^{bc}	6.81 ^b	6.67 ^{bc}	6.52 ^{ab}	6.38 ^{ab}
Inoculant-3	Pf	6.95 ^b	6.90 ^e	6.90 ^c	6.85 ^{de}	6.80 ^d	6.73 ^{cd}	6.62 ^{bc}	6.49 ^{a-d}	6.25 ^{bc}
Inoculant-4 (Rh+Bm)	Rh	6.63 ^e	6.60 ^g	6.57 ^e	6.51 ^g	6.34 ^g	6.17 ^g	6.02 ^f	5.73 ^e	5.20 ^f
	Bm	7.07 ^a	7.01 ^c	6.97 ^b	6.89 ^{cd}	6.81 ^{cd}	6.71 ^d	6.59 ^{cd}	6.32 ^{cd}	6.05 ^d
Inoculant-5 (Rh+Pf)	Rh	6.63 ^e	6.58 ^g	6.52 ^f	6.53 ^g	6.39 ^g	6.24 ^f	6.11 ^f	5.73 ^e	5.40 ^e
	Pf	6.96 ^b	6.94 ^d	6.90 ^c	6.84 ^e	6.76 ^{de}	6.63 ^e	6.51 ^{de}	6.35 ^{bcd}	6.12 ^{cd}
Inoculant-6	Bm	7.07 ^a	7.08 ^a	7.03 ^a	6.95 ^{ab}	6.88 ^{ab}	6.82 ^b	6.67 ^{bc}	6.50 ^{abc}	6.24 ^{bc}
	Pf	6.94 ^b	6.91 ^e	6.89 ^c	6.81 ^e	6.73 ^e	6.61 ^e	6.49 ^e	6.30 ^d	6.09 ^{cd}
Inoculant-7 (Rh+Bm+Pf)	Rh	6.67 ^d	6.60 ^g	6.58 ^e	6.55 ^{fg}	6.47 ^f	6.29 ^f	6.09 ^f	5.92 ^e	5.40 ^e
	Bm	7.06 ^a	7.05 ^b	7.02 ^a	6.98 ^a	6.94 ^a	6.89 ^a	6.81 ^a	6.67 ^a	6.45 ^a
	Pf	7.05 ^a	7.04 ^b	7.00 ^{ab}	6.93 ^{bc}	6.88 ^{ab}	6.78 ^{bc}	6.69 ^b	6.43 ^{bcd}	6.19 ^{cd}

Note: Means with same superscript are statistically on par at $P \leq 0.01$ by DMRT

Rh: *Rhizobium* sp., Bm: *Bacillus megaterium*, Pf: *Pseudomonas fluorescens*

6.19 cfu/g, respectively during the storage in fluid bed dryer based formulation.

Highest survival was observed in *Bacillus megaterium* \log_{10} 6.45 cells/g in triple inoculant followed by *Pseudomonas fluorescens* \log_{10} 6.25 cells / g and least was recorded in *Rhizobium* sp., \log_{10} 5.40 cells/g (Table II) after 180 days of the survival study.

The current study implies that, due to hot air circulation during the development of FBD based microbial inoculants, it is very difficult to maintain high cell density as a result low population count obtain from the initial stage itself. Interestingly, *Bacillus megaterium* \log_{10} 6.45 cells/ g thrived well, this is due to production of endospore. Similar kind of observation were made by Lavanyaet. al. (2015).

Survival of single, dual and triple inoculants in lignite formulation

The data pertaining to survival study of *Rhizobium* sp., *Bacillus megaterium* and

Pseudomonas fluorescens in lignite formulation are presented in Table III.

The lignite formulation of *Rhizobium* sp. recorded \log_{10} 9.91 cfu/g of population at the initial day of storage. Later, the population was found stabilized form even after 90 days (\log_{10} 9.71 cfu/g), 120 days (\log_{10} 9.51 cfu/g), 150 days (\log_{10} 9.19 cfu/g) and the population of \log_{10} 8.82 cfu/g was observed at 180 days of storage in lignite formulation.

The lignite formulation of *Bacillus megaterium* recorded \log_{10} 9.81 cfu/g of population at the initial day of storage. Later, the population started to decline gradually after 90 days (\log_{10} 9.35 cfu/g), 120 days (\log_{10} 9.21 cfu/g), 150 days (\log_{10} 9.07 cfu/g). The population of \log_{10} 8.75 cfu/g was observed at 180 days of storage in lignite formulation.

The lignite formulation of *Pseudomonas fluorescens* recorded \log_{10} 9.96 cfu/g of population at the initial day of storage. Later, the population started to decline gradually after 90 days (\log_{10} 9.62 cfu/g),

TABLE III
Survival of microbial inoculants consortium in lignite formulation

Inoculants		Population (\log_{10} cfu/ g)								
		Duration of the storage (days)								
		0	10	20	30	60	90	120	150	180
Inoculant-1	Rh	9.91 ^b	9.92 ^{ab}	9.90 ^{ab}	9.84 ^a	9.79 ^a	9.71 ^a	9.51 ^{ab}	9.19 ^{bc}	8.82 ^b
Inoculant-2	Bm	9.81 ^{de}	9.79 ^f	9.76 ^e	9.64 ^e	9.55 ^c	9.35 ^d	9.21 ^{de}	9.07 ^{cd}	8.75 ^b
Inoculant-3	Pf	9.96 ^a	9.94 ^a	9.87 ^{bc}	9.81 ^{ab}	9.71 ^b	9.62 ^b	9.44 ^{bc}	9.19 ^{bc}	8.58 ^{bc}
Inoculant-4 (Rh+Bm)	Rh	9.88 ^{bc}	9.81 ^{ef}	9.72 ^f	9.63 ^e	9.53 ^c	9.33 ^{de}	9.11 ^e	8.78 ^e	8.20 ^{d e f}
	Bm	9.77 ^e	9.73 ^g	9.61 ^g	9.53 ^f	9.40 ^e	9.29 ^{de}	9.13 ^e	8.86 ^{de}	8.32 ^{cde}
Inoculant-5 (Rh+Pf)	Rh	9.91 ^b	9.89 ^{bc}	9.81 ^d	9.73 ^d	9.52 ^{cd}	9.36 ^d	9.14 ^e	8.90 ^{de}	8.42 ^{cd}
	Pf	9.97 ^a	9.96 ^a	9.87 ^{bc}	9.78 ^{bcd}	9.71 ^b	9.52 ^c	9.23 ^{de}	9.06 ^{cd}	8.86 ^b
Inoculant-6 (Bm+Pf)	Bm	9.89 ^b	9.87 ^{cd}	9.79 ^{de}	9.75 ^{cd}	9.46 ^{de}	9.32 ^{de}	9.13 ^e	8.81 ^e	8.00 ^f
	Pf	9.97 ^a	9.94 ^a	9.92 ^a	9.85 ^a	9.83 ^a	9.75 ^a	9.63 ^a	9.34 ^{ab}	8.86 ^b
Inoculant-7 (Rh+Bm+Pf)	Rh	9.90 ^b	9.89 ^c	9.85 ^c	9.80 ^{abc}	9.71 ^b	9.57 ^{bc}	9.31 ^{cd}	9.05 ^{cd}	8.84 ^b
	Bm	9.84 ^{cd}	9.84 ^{de}	9.71 ^f	9.60 ^e	9.42 ^e	9.27 ^e	8.94 ^f	8.49 ^f	8.10 ^{ef}
	Pf	9.96 ^a	9.92 ^{ab}	9.89 ^{ab}	9.81 ^{ab}	9.72 ^b	9.61 ^b	9.53 ^{ab}	9.42 ^a	9.29 ^a

Note: Means with same superscript are statistically on par at $P \leq 0.01$ by DMRT

Rh: *Rhizobium* sp., Bm: *Bacillus megaterium*, Pf: *Pseudomonas fluorescens*

120 days (\log_{10} 9.44 cfu/g), 150 days (\log_{10} 9.19 cfu/g) and the population of \log_{10} 8.58 cfu/g was observed at 180 days of storage in lignite formulation.

The lignite formulation of *Rhizobium* sp. and *Bacillus megaterium* recorded the population of \log_{10} 9.88 cfu/g and \log_{10} 9.77 cfu/g, respectively at the initial day of storage. Later, the population started to decline gradually after 90 days (\log_{10} 9.33 cfu/g and \log_{10} 9.29 cfu/g, respectively), the population of \log_{10} 8.20 cfu/g and \log_{10} 8.32 cfu/g recorded by *Rhizobium* sp. and *Bacillus megaterium*, respectively on 180 days of storage in lignite formulation.

The lignite formulation of *Rhizobium* sp. and *Pseudomonas fluorescens* recorded the population of \log_{10} 9.91 cfu/g and \log_{10} 9.97 cfu/g, respectively at the initial day of storage. Later, the population started to decline gradually after 90 days (\log_{10} 9.36 cfu/g and \log_{10} 9.52 cfu/g, respectively). The population of \log_{10} 8.42 cfu/g and \log_{10} 8.86 cfu/g recorded by *Rhizobium* sp. and *Pseudomonas fluorescens*, respectively after 180 days of storage in lignite formulation.

The lignite formulation of *Bacillus megaterium* and *Pseudomonas fluorescens* recorded the population of \log_{10} 9.89 cfu/g and \log_{10} 9.97 cfu/g, respectively at the initial day of storage. Later, the population of *Bacillus megaterium* was started to declining gradually after 60 days (\log_{10} 9.36 cfu/g) and the population of *Pseudomonas fluorescens* started declining (\log_{10} 9.75 cfu/g) after 90 days. Population of \log_{10} 8.00 cfu/g and \log_{10} 8.86 cfu/g was recorded by *Bacillus megaterium* and *Pseudomonas fluorescens*, respectively after 180 days of storage in lignite formulation.

The lignite formulation of triple inoculants of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* recorded the population of \log_{10} 9.90 cfu/g, \log_{10} 9.84 cfu/g and \log_{10} 9.96 cfu/g, respectively at the initial day of storage. After 90 days, the population of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* started to decline gradually (\log_{10} 9.57 cfu/g, \log_{10} 9.27 cfu/g and \log_{10}

9.61 cfu/g). The population of \log_{10} 8.84 cfu/g, \log_{10} 8.10 and cfu/g \log_{10} 9.29 cfu/g was recorded by *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens*, respectively on 180 days of storage in lignite formulation.

Lignite is one of the most conventional carrier material which is being used to develop microbial inoculant formulation. In the current study, it serves as intermittent between liquid, alginate and fluid bed dryer based formulations mainly because of lack of adherence of cells in the carrier material.

Survival of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* single, dual and triple inoculants in liquid formulation

The data pertaining to survival study of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* in liquid formulation are presented in Table IV.

The liquid formulation of *Rhizobium* sp. recorded \log_{10} 10.21 cfu/ml of population at the initial day of storage. Later, the population started declining after 60 days (\log_{10} 10.01 cfu/ml), 90 days (\log_{10} 9.93 cfu/ml). The population of \log_{10} 9.47 cfu/ml was observed at 180 days of storage in liquid formulation.

The liquid formulation of *Bacillus megaterium* was recorded \log_{10} 9.96 cfu/ml at the initial day of storage. Later, the population was started to decline gradually after 90 days (\log_{10} 9.65 cfu/ml) 120 days (\log_{10} 9.54 cfu/ml), 150 days (\log_{10} 9.41 cfu/ml) and the population of \log_{10} 9.26 cfu/ml was observed at 180 days of storage in liquid formulation.

The liquid formulation of *Pseudomonas fluorescens* recorded \log_{10} 10.13 cfu/ml of population at the initial day of storage. Later, on consecutive days, 10 days (\log_{10} 10.12 cfu/ml), 20 days (\log_{10} 10.05 cfu/g), 30 days (\log_{10} 10.01 cfu/ml), 60 days (\log_{10} 9.90 cfu/ml), 90 days (\log_{10} 9.83 cfu/ml), 120 days (\log_{10} 9.69 cfu/ml), 150 days (\log_{10} 9.47 cfu/ml) till the end of 180 days (\log_{10} 9.45 cfu/ml) the population recorded was statistically on par with all the observations during the storage in liquid formulation.

The population in liquid formulation of *Rhizobium* sp. and *Bacillus megaterium* recorded was

TABLE IV
Survival of microbial inoculants consortium in liquid formulation

Inoculants		Population (log ₁₀ cfu/ g)								
		Duration of the storage (days)								
		0	10	20	30	60	90	120	150	180
Inoculant-1	Rh	10.21 ^a	10.21 ^a	10.14 ^a	10.10 ^a	10.01 ^{ab}	9.93 ^b	9.83 ^{bc}	9.60 ^{bc}	9.47 ^{ab}
Inoculant-2	Bm	9.96 ^{fg}	9.91 ^{ef}	9.86 ^d	9.83 ^e	9.75 ^e	9.65 ^d	9.54 ^e	9.41 ^{de}	9.26 ^{cde}
Inoculant-3	Pf	10.18 ^{ab}	10.14 ^b	10.13 ^a	10.14 ^a	10.08 ^a	10.03 ^a	9.98 ^a	9.89 ^a	9.55 ^a
Inoculant-4 (Rh+Bm)	Rh	10.13 ^{bcd}	10.12 ^b	10.05 ^b	10.01 ^{bc}	9.90 ^{cd}	9.83 ^c	9.69 ^d	9.47 ^{cd}	9.45 ^{abc}
	Bm	9.98 ^f	9.94 ^e	9.84 ^{de}	9.72 ^f	9.71 ^{ef}	9.63 ^d	9.51 ^e	9.35 ^{de}	9.10 ^e
Inoculant-5 (Rh+Pf)	Rh	10.17 ^{abc}	10.14 ^b	10.11 ^a	10.11 ^a	10.08 ^a	10.01 ^a	9.93 ^{ab}	9.70 ^b	9.45 ^{abc}
	Pf	10.05 ^e	9.99 ^d	9.95 ^c	9.94 ^{cd}	9.86 ^d	9.82 ^c	9.76 ^{cd}	9.67 ^b	9.47 ^{ab}
Inoculant-6 (Bm+Pf)	Bm	9.95 ^{fg}	9.87 ^f	9.79 ^{ef}	9.73 ^f	9.64 ^f	9.48 ^e	9.45 ^e	9.30 ^e	9.16 ^{de}
	Pf	10.08 ^{de}	10.02 ^{cd}	9.97 ^c	9.92 ^d	9.85 ^d	9.80 ^c	9.72 ^{cd}	9.42 ^{de}	9.22 ^{de}
Inoculant-7 (Rh+Bm+Pf)	Rh	10.11 ^d	10.15 ^b	10.11 ^a	10.08 ^{ab}	9.96 ^{bc}	9.79 ^c	9.66 ^d	9.31 ^e	9.16 ^{de}
	Bm	9.92 ^g	9.87 ^f	9.75 ^f	9.59 ^g	9.42 ^g	9.37 ^f	9.22 ^f	9.08 ^f	8.78 ^f
	Pf	10.12 ^{cd}	10.05 ^c	9.99 ^c	9.91 ^d	9.85 ^d	9.83 ^c	9.76 ^{cd}	9.66 ^b	9.35 ^{bcd}

Note: Means with same superscript are statistically on par at P ≤ 0.01 by DMRT

Rh: *Rhizobium* sp., Bm: *Bacillus megaterium*, Pf: *Pseudomonas fluorescens*

log₁₀ 10.13 cfu/ml and log₁₀ 9.98 cfu/ml, respectively. The populations of *Rhizobium* sp. and *Bacillus megaterium* started showing declination after 120 days (log₁₀ 9.69 cfu/ml and log₁₀ 9.51 cfu/ml, respectively). At the end of 180 days storage of *Rhizobium* sp. log₁₀ 9.45 cfu/ml and *Bacillus megaterium* log₁₀ 9.10 cfu/ml was recorded in liquid formulation.

The liquid formulation of *Rhizobium* sp. and *Pseudomonas fluorescens* recorded was log₁₀ 10.17 cfu/ml and log₁₀ 10.05 cfu/ml, respectively. The population of both the inoculants in the liquid formulation showed optimum growth and were statistically on par till the end of the 180 days of storage. At the end of 180 days storage of *Rhizobium* sp. log₁₀ 9.45 cfu/ml and *Bacillus megaterium* log₁₀ 9.47 cfu/ml was recorded in liquid formulation.

The population in liquid formulation of *Bacillus megaterium* and *Pseudomonas fluorescens* recorded was log₁₀ 9.95 cfu/ml and log₁₀ 10.08 cfu/ml, respectively. The populations of *Bacillus megaterium* and *Pseudomonas fluorescens* started showing declination after 60 days (log₁₀ 9.64 cfu/ml and log₁₀

9.85 cfu/ml, respectively). At the end of 180 days storage of *Bacillus megaterium* log₁₀ 9.16 cfu/ml and *Pseudomonas fluorescens* log₁₀ 9.22 cfu/ml was recorded in liquid formulation.

The population in liquid formulation of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* recorded was log₁₀ 10.11 cfu/ml, log₁₀ 9.92 and log₁₀ 10.12 cfu/ml, respectively. The populations of *Rhizobium* sp., *Bacillus megaterium* started showing declination after 90 days (log₁₀ 9.79 cfu/ml and log₁₀ 9.37 cfu/ml, respectively) whereas, the population of *Pseudomonas fluorescens* (log₁₀ 9.76 cfu/ml) started showing declination after 120 days of storage. At the end of 180 days of storage of *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* recorded population of log₁₀ 9.16 cfu/ml, log₁₀ 8.78 cfu/ml and log₁₀ 9.35 cfu/ml of respective population in liquid formulation.

Liquid formulation amended with PVP-40 and glycerol at the rate of 2 per cent serves as the best formulation among the rest of formulations mainly because of PVP-40 and glycerol acts as adjuvants and

making the nutrient sources for prolonged time. The concurrent studies were reported by Vithal, 2004.

The present study on comparison between the four different formulation of microbial consortium consisting of *Rhizobium* sp., *Bacillus megaterium*, *Pseudomonas fluorescens* in single, dual, triple combinations on their survival rate revealed that, the microbial inoculants thrived well in liquid formulations followed by alginate based, lignite and fluid bed dryer based formulation.

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