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Evaluation of Selected Organic Materials as Carriers for Agriculturally Important Microorganisms

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Abstract

An experiment was conducted to study the survival of microbial inoculants in organic materials as carriers. In this investigation six different organic materials were used as a carrier for agriculturally important microbial inoculants. The highest survival rate of *Bradyrhizobium japonicum* $\log_{10}$ 8.48 CFU / sg, *Pseudomonas fluorescens* $\log_{10}$ 8.20 cells / g, *Bacillus megaterium* $\log_{10}$ 7.78 cells / g and *Azotobacter chroococcum* $\log_{10}$ 7.89 cells / g were recorded in sterilized organic materials. Results revealed that the survival of microbial inoculants increased by addition of sterilized organic materials @ 10 per cent to talc was found more efficient than the unsterilized organic materials upto 90 days of storage period.

The value of biofertilizers has increased in an eco-conscious world and gaining increasing importance for their sustainable and holistic approach to maintain purity of the soil and enhance crop productivity. Biofertilizers efficiency mainly dependents on carrier material because carrier is used as medium to transfer live microorganisms from laboratory to plant rhizosphere. An ideal carrier material should be cost effective, contain non-toxic compounds and high organic content, easy to process, more than 50 per cent water holding capacity and available in bulk quantity (Mishra and Dahich, 2010; Bazilah *et al.,* 2011). Various organic materials such as FYM, vermicompost, compost etc. have been used as carrier materials for formulation of beneficial microbial inoculants. The advantage of use of organic based carrier materials increases survivability of the microbial inoculants. In this investigation, different organic based materials were used as carrier material for agriculturally important microbial inoculants.

The microbial inoculants used in the study were from the Department of Agricultural Microbiology, GKVK, UAS, Bengaluru. A loop full of *Bradyrhizobium japonicum, Azotobacter chroococcum, Bacillus megaterium* and *Pseudomonas fluorescens* from pure culture were aseptically transferred to 250 ml conical flasks containing 100 ml of the yeast extract mannitol broth, Waksman 77 broth, Pikovskaya's broth and King's B broth respectively and incubated for 5-6 days at 28° ± 2° C to obtain maximum growth. Slant cultures maintained at 4° C served as mother culture. The different organic materials like Vermicompost (VC), Poultry manure (PM), Sheep manure (SM), Urban compost (UC), Coco peat (CP) and Biochar (BC) were used in this study alone and in combination with talc as carrier for microbial inoculants. The collected organic materials were sieved through 2 mm sieve and shade dried for 4-5 days, to bring the moisture level up to 5 per cent, powdered and sieved through 90 ìm sieve. The processed samples were kept in polythene bags. The above mentioned organic materials were inoculated individually with the microbial inoculants such as *Bradyrhizobium japonicum*, *Azotobacter chroococcum, Bacillus megaterium* and *Pseudomonas fluorescens.* Microbial inoculants were inoculated to the sterilized and unsterilized organic materials. Survival study was monitored at an interval of 0, 10, 20, 30, 60 and 90 days of storage period at ambient temperature. Statistical analysis of population data from laboratory experiment was done by Two Factorial Complete Randomized Design.

Bradyrhizobium japonicum in Talc + 10 per cent Vermicompost formulation recorded $\log_{10}$ 7.78 CFU / g in sterilized condition and $\log_{10}$ 7.73 CFU / g in unsterilized condition at the beginning of storage and there was increase of population in both conditions upto 60 days. At 90 days unsterilized condition recorded $\log_{10}$ 7.96 CFU / g, and sterilized condition $\log_{10}$ 8.48 CFU / g (Table I).

TABLE I

Survival of Pseudomonas fluorescens in unsterilized and sterilized organic materials

Treatments	Population density (log_{10} CFU/ml) Duration of storage (days)																	
	0 days			10 days			20 days			30 days			60 days			90 days		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
T_1 : VC	7.25	7.82	7.54	7.46	8.12	7.79	7.77	8.19	7.98	7.73	8.21	7.97	7.69	8.19	7.94	7.65	8.15	7.90
T_2 : PM	7.31	7.53	7.42	7.37	7.87	7.62	7.46	7.89	7.68	7.48	7.85	7.67	7.44	7.84	7.64	7.37	7.82	7.60
T_3 : SM	7.39	7.50	7.45	7.62	7.79	7.70	7.82	7.91	7.86	7.79	7.88	7.84	7.74	7.85	7.80	7.66	7.81	7.74
T_4 : UC	7.65	7.73	7.69	7.83	8.09	7.96	8.05	8.17	8.11	8.00	8.15	8.08	8.01	8.17	8.09	7.95	8.14	8.05
T_5: CP	7.34	7.58	7.46	7.51	7.74	7.63	7.78	7.82	7.80	7.82	7.83	7.83	7.77	7.85	7.81	7.75	7.81	7.78
T_6 : BC	7.47	7.59	7.53	7.58	7.65	7.62	7.76	7.78	7.77	7.83	7.89	7.86	7.80	7.90	7.85	7.73	7.86	7.79
T_7 : Talc + 10 % VC	7.73	7.78	7.76	8.00	8.38	8.19	8.17	8.46	8.31	8.14	8.52	8.33	8.12	8.52	8.32	7.96	8.48	8.22
T_8 : Talc + 10 % PM	7.33	7.38	7.36	7.52	7.65	7.59	7.68	7.79	7.74	7.70	7.91	7.80	7.65	7.86	7.76	7.59	7.80	7.70
T_9: Talc + 10 % SM	7.44	7.55	7.50	7.68	7.74	7.71	7.88	7.93	7.91	7.94	8.00	7.97	7.88	7.96	7.92	7.80	7.88	7.84
T_{10}: Talc + 10 % UC	7.66	7.69	7.68	7.72	8.18	7.95	7.82	8.33	8.08	7.86	8.27	8.06	7.85	8.26	8.06	7.84	8.19	8.01
T_{11}: Talc + 10 % CP	7.27	7.45	7.36	7.44	7.82	7.63	7.71	7.97	7.84	7.71	7.99	7.85	7.67	7.93	7.80	7.64	7.87	7.75
T_{12}: Talc + 10 % BC	7.60	7.63	7.62	7.69	7.79	7.74	7.60	7.79	7.70	7.64	7.82	7.73	7.57	7.78	7.67	7.54	7.72	7.63
Mean	7.45	7.60		7.62	7.90		7.79	8.00		7.80	8.02		7.77	8.00		7.70	7.96	
	S.Em ±	CD@ 0.05%	CV %	S.Em ±	CD@ 0.05%	CV %	S.Em ±	CD@ 0.05%	CV %	S.Em ±	CD@ 0.05%	CV %	S.Em ±	CD@ 0.05%	CV %	S.Em ±	CD@ 0.05%	CV %
C	0.038	0.108		0.024	0.068		0.021	0.059		0.014	0.039		0.012	0.034		0.009	0.025	
T	0.094	N.S	3.05	0.058	0.164	1.84	0.052	0.147	1.62	0.340	0.966	1.05	0.030	0.085	0.94	0.023	0.065	0.74
C × T	0.132	N.S		0.082	0.233		0.074	0.210		0.048	0.136		0.420	1.194		0.033	0.093	

Note: C_1–Unsterilized, C_2–Sterilized

TABLE II

Survival of Pseudomonas fluorescens in unsterilized and sterilized organic materials

Treatments	Population density (log_{10} CFU/ml) Duration of storage (days)																	
	0 days			10 days			20 days			30 days			60 days			90 days		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
T_1 : VC	7.98	8.05	8.01	8.01	8.07	8.04	7.99	8.11	8.05	8.04	8.09	8.07	7.97	8.02	8.00	7.90	7.95	7.93
T_2 : PM	8.05	8.03	8.04	8.00	8.05	8.03	7.98	8.05	8.01	7.94	8.07	8.00	7.87	8.01	7.94	7.81	7.95	7.88
T_3 : SM	7.95	8.00	7.98	7.99	8.06	8.03	7.97	8.04	8.00	8.00	8.07	8.04	7.97	8.04	8.00	7.90	7.98	7.94
T_4 : UC	7.98	7.99	7.99	8.03	8.07	8.05	7.99	8.04	8.01	8.05	8.11	8.08	8.01	8.07	8.04	7.97	8.06	8.02
T_5 : CP	8.05	8.03	8.04	8.07	8.09	8.08	8.03	8.11	8.07	7.98	8.09	8.04	7.94	8.06	8.00	7.91	8.01	7.96
T_6 : BC	8.02	8.06	8.04	8.00	8.08	8.04	7.97	8.10	8.04	8.01	8.07	8.04	7.98	8.04	8.01	7.93	8.01	7.97
T_7 : Talc + 10 % VC	8.04	8.09	8.06	7.94	8.11	8.03	8.01	8.08	8.04	8.06	8.10	8.08	7.99	8.11	8.05	7.97	8.09	8.03
T_8 : Talc + 10 % PM	7.98	8.04	8.01	7.91	8.01	7.96	8.00	8.06	8.03	8.03	8.05	8.04	7.99	8.02	8.00	7.89	7.98	7.94
T_9 : Talc + 10 % SM	7.99	8.03	8.01	8.02	8.06	8.04	8.03	8.03	8.03	7.97	8.01	7.99	7.92	7.99	7.96	7.89	7.98	7.94
T_{10} : Talc + 10% UC	8.10	8.12	8.11	8.07	8.15	8.11	8.11	8.18	8.14	8.14	8.17	8.16	8.20	8.22	8.21	8.17	8.20	8.19
T_{11} : Talc + 10 % CP	8.04	8.01	8.02	8.01	8.07	8.04	8.03	8.05	8.04	8.00	8.07	8.04	7.99	8.03	8.01	7.95	8.00	7.98
T_{12} : Talc + 10 %BC	8.03	8.00	8.01	8.05	8.05	8.05	8.07	8.10	8.09	8.03	8.04	8.04	7.98	7.99	7.99	7.95	7.95	7.95
Mean	8.02	8.04		8.01	8.07		8.01	8.08		8.02	8.08		7.98	8.05		7.94	8.02	
		S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %
C		0.009	NS		0.004	0.018		0.004	0.017		0.004	0.015		0.003	0.014		0.004	0.016	
T		0.023	N.S	0.71	0.012	0.045	0.37	0.011	0.042	0.34	0.0.10	0.039	0.31	0.009	0.035	0.28	0.010	0.041	0.34
C × T		0.032	N.S		0.017	0.064		0.015	0.060		0.014	0.055		0.013	0.049		0.015	0.058	

Note: C_1–Unsterilized, C_2–Sterilized

TABLE III

Survival of Bacillus megaterium in unsterilized and sterilized organic materials

Treatments	Population density (log_{10}CFU/ml) Duration of storage (days)																	
	0 days			10 days			20 days			30 days			60 days			90 days		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
T_1 : VC	7.65	7.68	7.66	7.70	7.65	7.68	7.68	7.70	7.69	7.76	7.78	7.77	7.75	7.80	7.78	7.70	7.75	7.72
T_2 : PM	7.50	7.55	7.53	7.45	7.52	7.48	7.52	7.60	7.56	7.57	7.65	7.61	7.50	7.62	7.56	7.39	7.55	7.47
T_3 : SM	7.30	7.45	7.38	7.45	7.50	7.48	7.50	7.58	7.54	7.42	7.68	7.55	7.38	7.60	7.49	7.30	7.50	7.40
T_4 : UC	7.71	7.69	7.70	7.60	7.65	7.63	7.63	7.68	7.66	7.75	7.80	7.78	7.65	7.70	7.68	7.58	7.65	7.62
T_5: CP	7.58	7.62	7.60	7.62	7.68	7.65	7.67	7.70	7.69	7.65	7.72	7.69	7.58	7.68	7.63	7.50	7.60	7.55
T_6 : BC	7.45	7.55	7.50	7.55	7.62	7.59	7.58	7.60	7.59	7.62	7.70	7.66	7.63	7.68	7.66	7.55	7.62	7.58
T_7 : Talc + 10 % VC	7.65	7.70	7.67	7.68	7.74	7.71	7.60	7.80	7.70	7.71	7.84	7.78	7.70	7.82	7.76	7.60	7.80	7.70
T_8 : Talc + 10 % PM	7.55	7.61	7.58	7.58	7.58	7.58	7.68	7.63	7.66	7.60	7.68	7.64	7.55	7.62	7.58	7.45	7.60	7.53
T_9 : Talc + 10 % SM	7.45	7.50	7.48	7.57	7.60	7.58	7.52	7.59	7.55	7.63	7.67	7.65	7.58	7.62	7.60	7.54	7.58	7.56
T_{10} : Talc + 10 % UC	7.71	7.68	7.69	7.65	7.72	7.69	7.63	7.79	7.71	7.78	7.86	7.82	7.70	7.89	7.80	7.68	7.78	7.73
T_{11} : Talc + 10 % CP	7.66	7.65	7.66	7.69	7.74	7.72	7.64	7.72	7.68	7.60	7.74	7.67	7.62	7.69	7.66	7.58	7.65	7.62
T_{12} : Talc + 10 % BC	7.70	7.69	7.69	7.72	7.75	7.74	7.68	7.71	7.69	7.71	7.78	7.75	7.65	7.75	7.70	7.60	7.65	7.63
Mean	7.58	7.61		7.61	7.65		7.61	7.67		7.65	7.74		7.61	7.71		7.54	7.64	
	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %
C	0.002	0.010		0.002	0.011		0.001	0.007		0.002	0.007		0.002	0.008		0.001	0.007	
T	0.006	0.024	0.21	0.007	0.027	0.23	0.004	0.017	0.15	0.005	0.019	0.16	0.005	0.020	0.17	0.004	0.017	0.14
C × sT	0.009	0.035		0.010	0.038		0.006	0.025		0.007	0.027		0.007	0.028		0.006	0.024	

Note: C_1–Unsterilized, C_2–Sterilized

TABLE IV

Survival of Azotobacter chroococcum in unsterilized and sterilized organic materials

Treatments	Population density (log_{10} CFU/ml) Duration of storage (days)																	
	0 days			10 days			20 days			30 days			60 days			90 days		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
T_1 : VC	7.61	7.65	7.63	7.58	7.71	7.65	7.66	7.69	7.67	7.72	7.75	7.74	7.65	7.65	7.65	7.60	7.58	7.59
T_2 : PM	7.58	7.55	7.57	7.55	7.60	7.58	7.60	7.64	7.62	7.51	7.67	7.59	7.52	7.60	7.56	7.45	7.52	7.49
T_3 : SM	7.60	7.61	7.61	7.62	7.63	7.63	7.58	7.69	7.63	7.55	7.70	7.63	7.62	7.64	7.63	7.57	7.60	7.59
T_4 : UC	7.62	7.63	7.63	7.58	7.67	7.63	7.65	7.78	7.71	7.70	7.85	7.78	7.61	7.80	7.71	7.59	7.70	7.65
T_5 : CP	7.55	7.58	7.57	7.55	7.65	7.60	7.59	7.60	7.60	7.62	7.70	7.66	7.57	7.66	7.62	7.48	7.54	7.51
T_6 : BC	7.59	7.63	7.61	7.62	7.69	7.65	7.57	7.73	7.65	7.65	7.80	7.73	7.58	7.74	7.66	7.50	7.68	7.59
T_7 : Talc + 10 %VC	7.65	7.66	7.66	7.80	7.77	7.79	7.60	7.83	7.71	7.75	7.90	7.83	7.68	7.85	7.76	7.64	7.78	7.71
T_8 : Talc + 10 %PM	7.58	7.59	7.59	7.54	7.62	7.58	7.58	7.60	7.59	7.61	7.69	7.65	7.65	7.65	7.65	7.48	7.60	7.54
T_9 : Talc + 10 %SM	7.58	7.61	7.59	7.62	7.68	7.65	7.61	7.65	7.63	7.67	7.71	7.69	7.58	7.66	7.62	7.50	7.58	7.54
T_{10} : Talc + 10%UC	7.59	7.62	7.61	7.61	7.70	7.66	7.68	7.77	7.73	7.73	7.89	7.81	7.60	7.84	7.72	7.55	7.81	7.68
T_{11} : Talc + 10 %CP	7.63	7.67	7.65	7.59	7.70	7.65	7.63	7.75	7.69	7.70	7.83	7.76	7.65	7.80	7.72	7.58	7.75	7.66
T_{12} : Talc + 10 %BC	7.60	7.58	7.59	7.81	7.89	7.85	7.89	7.99	7.94	7.83	7.97	7.90	7.75	7.91	7.83	7.60	7.89	7.75
Mean	7.60	7.62		7.62	7.69		7.64	7.73		7.67	7.79		7.62	7.73		7.55	7.67	
	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %	S.Em ±	CD @ 0.05%	CV %
C	0.006	NS		0.004	0.018		0.003	0.012		0.005	0.020		0.002	0.009		0.002	0.009	
T	0.015	NS	0.50	0.011	0.045	0.38	0.008	0.031	0.26	0.013	0.051	0.42	0.006	0.023	0.19	0.006	0.023	0.19
C × T	0.022	NS		0.016	0.064		0.011	0.044		0.019	0.072		0.008	0.032		0.008	0.033	

Note: C_1–Unsterilized, C_2–Sterilized

Pseudomonas fluorescens initially had $\log_{10}$ 8.10 CFU / g in unsterilized and $\log_{10}$ 8.12 CFU / g in sterilized conditions and *Bacillus megaterium* had $\log_{10}$ 7.71 CFU / g in unsterilized and $\log_{10}$ 7.68 CFU / g in sterilized conditions in Talc + 10 per cent Urban compost formulation. *Pseudomonas fluorescens* population remained stable upto 90 days of storage, recorded $\log_{10}$ 8.17 CFU / g in unsterilized and $\log_{10}$ 8.20 CFU / g in sterilized conditions, the same trend observed in *Bacillus megaterium,* recorded $\log_{10}$ 7.68 and $\log_{10}$ 7.78 CFU /g in unsterilized and sterilized conditions which were on par with the vermicompost formulation (Table II and III).

Azotobacter chroococcum recorded $\log_{10}$ 7.60 and $\log_{10}$ 7.58 CFU / g in unsterilized and sterilized formulation of Talc + 10 per cent Biochar in initial storage and population remained constant at 90 days, which recorded $\log_{10}$ 7.60 and $\log_{10}$ 7.89 CFU / g in unsterilized and sterilized conditions, results were on par with the Talc + 10 per cent Vermicompost formulation (Table IV).

Sterilized carrier materials maintained the population of microbial inoculants (*Bradyrhizobium japonicum*, *Bacillus megaterium*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*) for longer period than the unsterilized carrier materials. The results are in conformity with the findings of many research workers who reported substantial proportion of the inoculants produced using unsterilized carrier is unsatisfactory for farmer use because of low populations of microbial inoculants and high numbers of contaminants (Karla *et al.,* 2010; Bashan 1998). Survival of microbial inoculants (*Bradyrhizobium japonicum, Bacillus megaterium, Pseudomonas fluorescens* and *Azotobacter chroococcum*) in organic material based formulations were monitored upto 90 days. The population of microbial inoculants in all the formulations were observed to increase and remained stable upto 90 days of storage period. The optimal performance of the organic material is due to its higher nutrient content and high water-holding capacity. The result of this study is in agreement with the findings of Gandhi and Saravanakumar (2009), reported an increased shelf life of *Azospirillum lipoferum*, *Bacillus megaterium, Pseudomonas fluorescens* in vermicompost based formulation than in lignite formulation and also correlate with the findings of Kalra *et al.,* (2010). Similar results were obtained by Lakshmi (2013), who reported the survival of *Rhizobium, Bacillus megaterium* and *Pseudomonas fluorescens* in different consortial combination in urban compost based formulation upto 180 days. In the present study, the survival of microbial inoculants in sterilized organic material (10 %) in combination with talc as a carrier was found more effective than talc or lignite.

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