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Evaluation of Different Packaging Materials for Microbial Inoculants

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An investigation was carried out to study the influence of packaging materials on survival of microbial inoculants. Survival of *Bradyrhizobium japonicum, Bacillus megaterium* and *Pseudomonas fluorescens* in lignite and talc formulations were monitored upto six month of storage in different packaging materials such as aluminium and polythene bag. Lignite based *B. japonicum* and *B. megaterium* recorded highest viable cells compared to talc based inoculants. *B. japonicum* and *B. megaterium* packed in polythene bag supported 100 % cells from the beginning to the end of storage period. Maximum viable cells of *P. fluorescens* recorded in talc formulation compared to lignite formulation. Survival of *P. fluorescens* was best in aluminium cover compared to polythene bag. Regarding to packaging materials, polythene bag was the best packaging material for *B. japonicum* and *B. megaterium*. Aluminium cover was the best for *P. fluorescens*. However, both packaging materials were not harmful for survival of microorganisms and maintain good viable cells as per the BIS standards up to 180 days of storage.

Keywords: *B. japonicum, B. megaterium, P. fluorescens,* lignite and talc formulations, packaging material.

Biofertilizer are products containing live or latent cells of efficient strains of microorganisms used for application to seed, soil or composting with an objective of increasing number of such microorganisms and accelerate those microbial process which augment the availability of nutrients that can be easily assimilated by plants. Effect of biofertilizer application in different crops have different functional traits such as plant growth and productivity, nutrient profile, plant defense and protection with special emphasis to its function to trigger various growth and defense related genes in signaling network of cellular pathways to cause cellular response and there by crop improvement (Bharadwaj *et al*., 2014).

Different kinds of biofertilizers are recommended for agriculture such as nitrogen fixers, phosphorus solubilizers, phosphorus mobilizers, plant growth promoters and biocontrol agents. Variety of materials used as carriers has been shown to improve the survival and biological effectiveness of inoculants by protecting bacteria from biotic and abiotic stresses (Van Veen *et al.,* 1997).

Packaging of biofertilizer has been defined as microbial inoculants are covered by any materials in order to enhance its protection, handling, delivery and presentation of biofertilizer, from producer to user or farmer. Commonly metals (aluminium, aluminium foil and laminates films) and plastics (polythene, polypropylene, polyolefin and polyesters) are used as packing material for biofertilizer. Packaging plays a vital role in protecting and maintain viable cell as it is transported from the lab to land. The inoculants require protection from climatic conditions such as temperature, humidity, precipitation and solar radiation, which directly or indirectly influence the survival of microbial inoculants during storage.

Packaging materials prevents any

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wastage such as leakage or deterioration which may occur during storage, transportation and distribution. It also facilitate the labeling, which helps to the farmer to identify the biofertilizer and instruct them how to use it, how much to apply, to which crop it is intended *etc.* Good Packaging materials should possess some of the properties such as stable towards gamma irradiation, autoclavable, high gas exchange capacity and not allow high rates of moisture loss for packing of biofertilizer. These attributed materials must be used to ensure that inoculants reaches the farmer field in viable state. Hence, quality of packing materials is an important role in biofertilizer production technology. But virtually no information is available on the effect of packaging materials on microbial inoculants, by keeping all these points in view, the present research work was conducted with the following objectives. 1. To study the suitability of different packaging materials. 2. To study the survival of microbial inoculants.

MATERIALS AND METHODS

Bradyrhizobium japonicum, Bacillus megaterium and *Pseudomonas fluorescens* were grown in Yeast Extract Mannitol Broth (YEMB), Pikovskaya's broth and King's B broth respectively. They were mixed uniformly and separately with sterilized lignite at the rate of 1:3 (170 ml of broth culture in 500 g of lignite) under aseptic condition and packed in aluminium and polythene covers, sealed with the help of electronic sealer. Similarly talc based biofertilizer were prepared at the rate of 1:5 (100 ml of broth culture in 500 g of talc) and packed in aluminium and polythene covers.

Details of Experiment

In this experiment, three microorganisms, two carrier materials and two packaging materials were used. There were four treatments and three replications.

Survivability of microbial inoculants in different treatment

Survival study was done to estimate the microbial population in different treatments at 0, 15, 30, 60, 90, 120, 150 and 180 days of storage by standard plate count technique.

RESULTS AND DISCUSSION

Physico - chemical properties of carrier materials

Physical and chemical properties of carrier materials such as colour, particle size, water holding capacity (WHC), bulk density (BD), pH and electrical conductivity (EC) were analyzed. Colour of lignite was black with 106 µm particle size and it recorded 62.26 per cent of water holding capacity, 0.64 g/cc bulk density, 4.06 pH and 0.50 dS/m EC. Talc was white in colour with particle size of 106 µm. It recorded 72.25 per cent of water holding capacity, 0.82 g/cc bulk density, 9 pH and 0.62 dS/m of EC. Gade *et al.* (2014) reported similar outcomes regarding physical and chemical properties of lignite and talc.

Survival of *Bradyrhizobium japonicum, Bacillus megaterium* and *Pseudomonas fluorescens* in different carrier materials

There were differences in population of *B. japonicum* in lignite and talc based formulations. Survival of *B. japonicum* was better in lignite compared to talc based formulations. At the end of storage period $\log_{10}$ 7.58 cfu/g and $\log_{10}$ 6.97 cfu/g were observed in lignite and talc based formulations of *B. japonicum* respectively (Table 1). The results revealed that maximum population was observed in lignite throughout the storage period. This clearly supports the findings of Kalaivani (1998) that lignite supported higher survival of *P. fluorescens* and *B. japonicum* than peat.

In both formulations, viable cells of *B. megaterium* increased upto 60 days and later declined. Lignite based inoculants recorded maximum population ($\log_{10}$ 7.19 cfu/g) than talc based inoculants ($\log_{10}$ 6.93 cfu/g) at the end of storage period. (Table 1). The results are in agreement with the finding of Sangeetha and Stella (2012), that survival rate of *B. subtilis* increased initially upto 60 days later declined towards the survival period.

Pseudomonas fluorescens recorded its survival from $\log_{10}$ 7.36 to $\log_{10}$ 6.87 cfu/g in lignite formulation. Talc based inoculants recorded its survival from $\log_{10}$ 7.1 to $\log_{10}$ 7.07 cfu/g. The data is statistically on par between the carrier materials during storage period except 60 days of storage (Table 1). A similar finding was reported by Gade *et al*. (2014) talc as a carrier, which supports the

Table 1. Survival of *Bradyrhizobium japonicum, Bacillus megaterium* and *Pseudomonas fluorescens* in different carrier materials

Duration of storage (Days)	*Bradyrhizobium japonicum*			*Bacillus megaterium*			*Pseudomonas fluorescens*		
	Lignite	Talc	CD @ 5 %	Lignite	Talc	CD @ 5 %	Lignite	Talc	CD @ 5 %
	$\log_{10}$ cfu/g			$\log_{10}$ cfu/g			$\log_{10}$ cfu/g		
0	7.40	7.20	NS	7.36	7.26	NS	7.36	7.10	0.19
15	8.81	7.15	0.16	7.28	7.28	NS	7.23	7.15	NS
30	8.42	7.31	0.18	8.01	8.02	NS	7.25	7.28	NS
60	8.82	7.69	0.07	8.60	8.08	0.11	7.87	8.16	0.18
90	8.12	7.85	0.16	8.35	7.75	0.17	7.65	7.74	NS
120	7.96	7.69	0.14	8.23	7.58	0.16	7.50	7.56	NS
150	7.64	7.06	0.15	7.26	7.02	NS	7.04	7.14	NS
180	7.58	6.97	0.35	7.19	7.19	0.25	6.87	7.07	NS

Where: NS – Non Significant Note: Values are mean of three replications

Table 2. Survival of *Bradyrhizobium japonicum, Bacillus megaterium* and *Pseudomonas fluorescens* in different packaging materials

Duration of storage (Days)	*Bradyrhizobium japonicum*			*Bacillus megaterium*			*Pseudomonas fluorescens*		
	Aluminium cover	Polythene bag	CD @ 5 %	Aluminium cover	Polythene bag	CD @ 5 %	Aluminium cover	Polythene bag	CD @ 5 %
	$\log_{10}$ cfu/g			$\log_{10}$ cfu/g			$\log_{10}$ cfu/g		
0	7.30	7.30	NS	7.31	7.31	NS	7.23	7.23	NS
15	7.95	8.00	NS	7.28	7.28	NS	7.10	7.28	NS
30	7.83	7.89	NS	7.28	8.75	0.14	7.15	7.38	0.21
60	8.25	8.26	NS	7.76	8.92	0.11	8.72	7.31	0.18
90	8.32	7.65	0.16	7.51	8.60	0.17	8.25	7.15	0.19
120	8.16	7.49	0.14	7.44	8.37	0.16	7.83	7.23	0.27
150	7.26	7.43	0.15	6.78	7.86	0.38	7.19	6.99	0.14
180	7.21	7.34	NS	6.70	7.42	0.25	7.09	6.86	0.20

Where: NS – Non Significant Note: Values are mean of three replications

maximum number of viable cells of *P. fluorescens* than lignite. Similarly Chandar *et al.* (2013) reported that higher cfu in talc based formulation of *P. fluorescens* as compared to lignite or vermiculite based formulation.

Survival of *Bradyrhizobium japonicum, Bacillus megaterium* and *Pseudomonas fluorescens* in different packaging materials

Maximum survival of *B. japonicum* was observed at 90 days ($\log_{10}$ 8.32 cfu/g) which were placed in aluminium cover as compared to polythene bag ($\log_{10}$ 7.65 cfu/g). However, at the end of six months of storage maximum viable cells of *B. japonicum* recorded in polythene bag ($\log_{10}$ 7.34 cfu/g) than aluminium cover ($\log_{10}$ 7.21 cfu/g) (Table 2). The results were on par between the packaging materials upto 60 days and later, showed a significant difference. It may be due to properties of packaging materials which include gas exchange property, thickness, density of material *etc.* Polythene bag had good gas exchange property and less thickness compared to aluminium cover. Roughley (1968) confirmed that the *Rhizobium* needed a gas exchange through a packing materials. Strijdom and Deschodt (1976) reported a maximum survival of *Azospirillum* sp. (3.5×10^8 cfu/g) in steam sterilized peat, which was packed in low density polythene bag (0.31 – 0.32 mm).

There were significant differences in population of *B. megaterium* with respect to packaging materials after 30 days of storage. *B. megaterium* declined during storage period from

$\log_{10}$ 7.31 to $\log_{10}$ 6.72 cfu/g which packed in aluminium cover. In case of polythene bag, population gradually increased upto 60 days then slowly declined, during storage from $\log_{10}$ 7.31 to $\log_{10}$ 7.42 cfu/g of *B. megaterium* was observed (Table 2). The results are in agreement with the finding of Prihast (2013) who reported that highest population of *Bacillus* sp. and *Paenibacillus* in plastic bag (polythene bag) with 50 to 60 per cent of water than aluminium foil. There were differences in population of *P. fluorescens* with respect to packaging materials. Survival of *P. fluorescens* was better in aluminium cover compared to polythene bag. At the end of storage period $\log_{10}$ 7.09 and $\log_{10}$ 6.86 cfu/g were observed in aluminium and polythene bag respectively (Table 2). According to results obtained, aluminium cover was a suitable packing materials for *P. fluorescens* than polythene bag. The result were on par with work done by Callaghan *et al.* (2006) who reported that greater survival of *P. fluorescens* in aluminium foil than gas transferable bag.

It is evident from this investigation, lignite or talc can be a carrier material for the survival of microbial inoculants. With regard to packaging materials, polythene bag was the best packaging material for *B. japonicum* and *B. megaterium* whereas aluminium cover was the best for *P. fluorescens*. However, both packaging materials were not harmful for survival of microorganisms and maintain good viable cells as per the BIS standards upto 180 days of storage.

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