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EVALUATION OF SHELF LIFE OF LIQUID AND CARRIER BASED INOCULANTS OF *MESORHIZOBIUM* SP. AND *PSEUDOMONAS* SP. UNDER DIFFERENT STORAGE CONDITIONS

Arvind Kumar

Department of Ag. Chemistry and Soil Science, R. K. (P.G.) College, Shamli - 247 776 (U.P.).
E-mail rastogiakumar62@gmail.com

ABSTRACT

Survival of *Mesorhizobium* sp. and *Pseudomonas* sp. at room temperature and under refrigerated conditions in liquid inoculants was compared with their carrier based inoculants. The shelf life of liquid and carrier based inoculants was better under refrigerated conditions than at room temperature conditions. The liquid and carrier inoculants under refrigerated conditions indicated 4.0 and 10.0% more mean viable counts of *Mesorhizobium sp*. and 6.5 and 13.5% of *Pseudomonas* sp. at different intervals. Liquid inoculants by recording 12.5 and 19.5% more mean viable counts of *Mesorhizobium* sp. and 10.6 and 18.4% of *Pseudomonas* sp. count under refrigerated and room conditions, at different intervals gave better shelf life than their carrier based inoculants.

Key words : Carrier based inoculants, Liquid inoculants, *Mesorhizobium* sp., *Pseudomonas* sp., Shelf life.

INTRODUCTION

Bio-fertilisers areidentified to have a supplementary role to chemical fertilisers to increase soil fertility and crop production in sustainable farming. Microbial inoculant of poor quality is one of the major constraints in adoption of bio-fertilisers by the farmers. The quality of inoculants is determined mainly by number of viable cells present in it. Microbial inoculants not only increased the assimilation of nutrients by plants but also improved soil properties such as organic matter content and total nitrogen in soil (W.U. *et al*., 2005). The number of microorganisms in the inoculant also influences their performance in field. The carrier based inoculants can be used more advantageously and they are currently being produced (Dube *et al*., 1980; Kandasamy and Prasad, 1971). These inoculants suffer with drawbacks short shelf life and contamination leading to a poor response in the inoculated crops. The cost of production of carrier based inoculants is high as it is an energy and labour intensive process (Somasegaran and Hoben, 1994). Liquid inoculants have been claimed to provide solutions to these problems associated with carrier based inoculants (Singlton *et al*., 2002). Besides, storage conditions play a vital role in the survival of microorganisms in the inoculants. Liquid inoculants are the new innovations in bio-fertiliser technology. Bio-fertilisers with solid carrier material can be used more advantageously because they can increase the supply of nutrients to plants, resistance to soil borne pathogen and biological degradation of organic pollutants (Warren *et al*., 2009). In the present

study, I compared the shelf life of liquid and carrier based inoculants of *Mesorhizobium* sp. and *Pseudomonas* sp. under room temperature and refrigerated conditions.

MATERIALS AND METHODS

The *Mesorhizobium* strain was obtained from Deptt of Microbiology CCSHAU, Hisar and *Pseudomonas* strain from GBPUA &T, Pantnagar. The purity of the strains was maintained as slant culture onYeast Extract Mannitol Agar and Nutrient Agar, respectively. The liquid inoculants of these microorganisms were separately prepared using modified YEM and nutrients broths using compositions as given in table 1. These media were dispensed in 50ml portions in conical flasks, sterilised at 15psi for 20 minutes and inoculated with a 1ml fresh inoculum of the *Mesorhizobium* sp. and *Pseudomons* sp., separately. The *Mesorhizobium* sp. was grown for 72 hours and *Pseudomonas* sp. for 48 hours at 28±1^{0}C in incubator shaker up to the stationary phase. Carrier based inoculants were prepared by growing *Mesorhizobium* sp. in YEM broth for 72hrs and *Pseudomonas* sp. in nutrient broth for 48hrs and then mixing the broths separately with sterilized charcoal (pH 7.0) in 1:2 ratio. These inoculants were packed in 50 g portion in sterilized polypropylene bags. The prepared liquid and carrier inoculants were stored in refrigerator and room temperature, in triplicate, for 6 months. Samples were taken out aseptically from the stored inoculants at period intervals inoculants and viable counts of *Mesorhizobium sp*. and *Pseudomonas* sp. were recorded by plating the appropriate dilutions on YEMA and Nutrient Agar medium, respectively.

Table 1 : The composition of Yeast Extract Mannitol and Nutrient Broths for liquid inoculant of *Mesorhizobium* sp. and *Pseudomonas* sp.

Ingredients	YEMB (g)	NB (g)
Mannitol	1.0	-
K_2HPO_4	0.5	-
$MgSO_4.7H_2O$	0.2	-
NaCl	0.1	-
Yeast Extract	1.0	-
Beef Extract	-	3.0
Peptone	-	5.0
Glucose	1.0	1.0
Arabinose	0.5	1.0
PVP-40	20.0	20.0
Fe-EDTA	200µg	200µg
Glycerol	4.0ml	1.0ml
Distilled water	1000ml	1000ml

RESULTS AND DISCUSSION

In general, refrigerated conditions allowed better survival of *Mesorhizobium* sp. and *Pseudomonas* sp. in both the liquid and carrier inoculants. However, variations in viable counts at different intervals in the inoculants stored at refrigerated and room conditions were more in carrier based inoculants than in liquid inoculants. The mean *Mesorhizobium* sp. population of different intervals under refrigerated conditions was 10% more in 3.6% in liquid inoculants over room temperature. The same pattern was observed with *Pseudomonas* sp. Averaged over inoculants formulation, inoculant stored under refrigerated conditions recorded maximum and significantly more viable counts 26.5% at 6 weeks in carrier inoculant and 10.5% at 2 weeks in liquid inoculant over room temperature. In refrigerator population of *Mesorhiozobium* sp. in liquid inoculant increased slightly up to 8 weeks. A decline in the *Mesorhizobium* sp. counts was observed in both carrier and liquid inoculants at later stages. Inoculant formulations under refrigerated conditions maintained more counts than at room conditions up to 24 weeks. Such effect of low temperature on the survival of microorganisms is well known because of their reduced metabolic activities. A decreasing trend in the population of*Mesorhizobium* sp. in carrier inoculants at room temperature may be attributed to lose in moisture, high temperature and exhaustion of nutrients owing to continuous growth, which resulted in cell death (Hafeez *et al*., 1991). Irrespective in storage conditions liquid inoculants of *Mesorhizobium* sp. recorded higher population than carrier based inoculants at different intervals. The mean viable population of *Mesorhizobium* sp. in liquid inoculant was 19.1 and 12.4% higher over carrier inoculant at room temperature and refrigerated condition, respectively. The results are in agreement with the report of Bharamprakash *et al*. (2007), who also observed better survival of *Bradirhizobia* in liquid than carrier inoculants.

The variation in *Pseudomonas* sp. viable population due to storage condition was maximum of 23% at 3 weeks in carrier inoculant, and 12.7% at 16 weeks in liquid inoculants. Refrigerated conditions did not show reductions in the viable population of *Pseudomonas* sp. in carrier inoculant up to 3 weeks and in liquid inoculants up to 12 weeks. At termination of the study, carrier and liquid inoculants under refrigerator also recorded 14.9 and 11.2% more counts than at room temperature. A higher survival under refrigerated conditions may be due to the fact that low temperature in refrigerator protects the cell death in inoculum. Liquid inoculants recorded better survival of *Pseudomonas* sp. under both storage

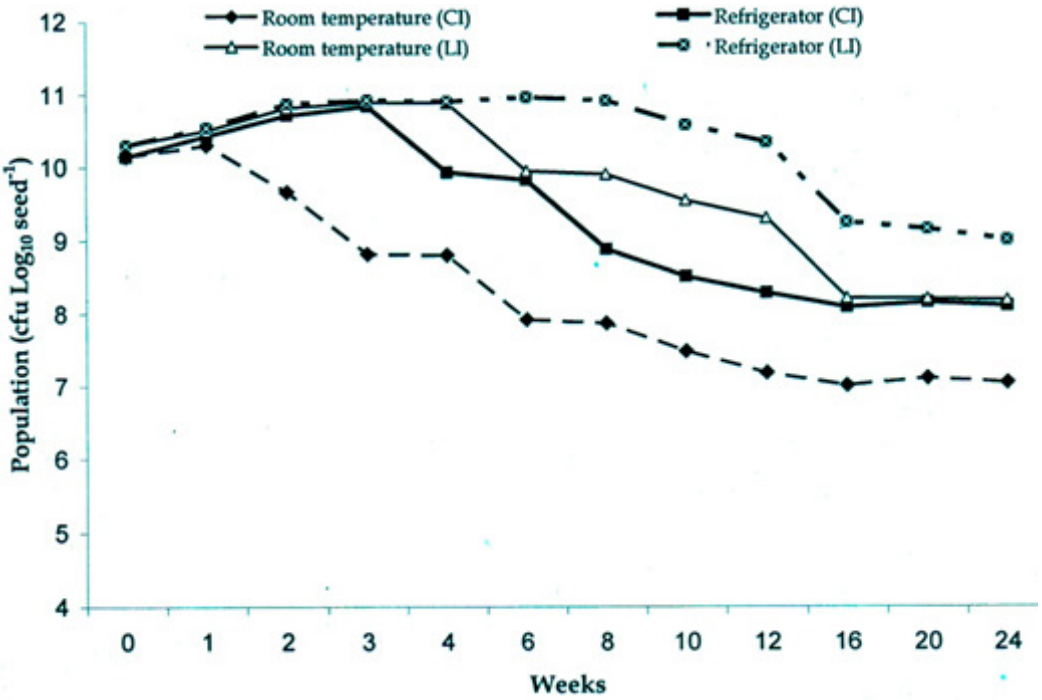

Fig. 1 : Survival of *Mesorhizobium* sp. in carrier (CI) and liquid (LI) inoculants in refrigerated and room conditions.

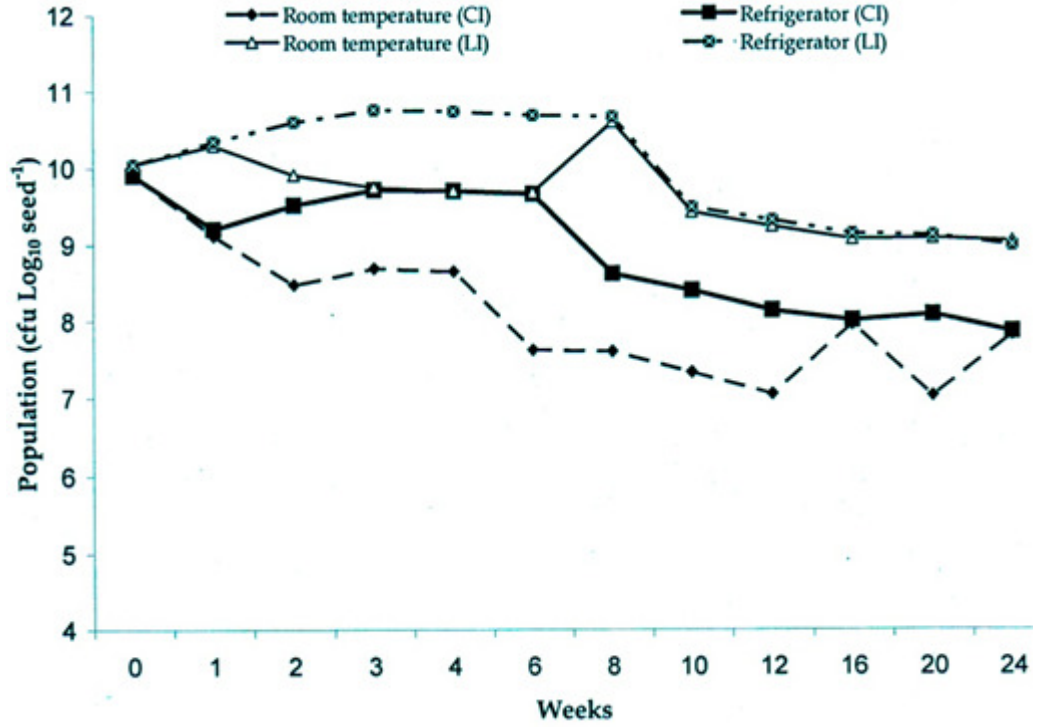

Fig. 2 : Survival of *Pseudomonas* sp. in carrier (CI) and liquid (LI) inoculants in refrigerated and room conditions.

conditions registering significant increase in mean population of 18.3% at room temperature and 10.5% in refrigerated conditions. In contrast, room conditions, where temperature varied 25 to 40^{0}C allowed growth of organisms creating depletion of nutrients (Taylor *et al*., 1968 and Vincent *et al*., 1962). At termination of study liquid inoculants gave 16 and 12.2% more population than carrier inoculants at room temperature and refrigerated conditions, respectively. The better survival in liquid inoculants may be attributed to chemical constituents of liquid inoculants which act as osmoprotectant and alternative source of carbon, and also offered better protection of inoculated organisms against dessication and high temperature resulted in enhanced

shelf life (Panlada *et al*., 2007).

The study suggested that liquid inoculants supported better survival than carrier inoculants of *Mesorhizobium* sp. and *Pseudomonas* sp. and low temperature of refrigerator was better than room conditions for their survival in the inoculants.

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