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The Study of Shelf Life of Potassium Solubilizing Microorganisms for Liquid Biofertilizer

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ABSTRACT

Liquid biofertilizer is increasingly available in the market as one of the alternatives to chemical fertilizer and pesticide. One of the benefits from biofertilizer is a contribution from population of microorganisms available. Traditionally, liquid biofertilizer produced from fermentation of effective microorganisms (EM) was recommended to be used within three months. This experiment showed that shelf life of the potassium solubilizing microorganism used as liquid biofertilizer had high amount of viable microbial population after four months of storage. The formulation study states that it can be also promoted in liquid formulation which has higher shelf life and stress protection The two conditions of storage at 4 ^{0}C, 30 ^{0}C, were tested by using two medium formulation (solid and liquid) upon viable microbial population. However in-depth study is required for the commercialization. The study showed that application of liquid broth formulation increases the shelf life of *Enterobacter hormaechei* which was used as a potassium solubilizer comparing to the solid formulation.

KEYWORDS Biofertilizer, Effective microorganisms, Formulation, *Enterobacter hormaechei,* potassium solubilizer

INTRODUCTION

India is agricultural based country. In order to feed the ever growing populations, India has to increase the per unit area productivity. Further, in order to increase the unit area productivity of agricultural land, the role of different crop nutrients in contributing increased crop yield play a vital role. Among the crop nutrient, nitrogen as well as phosphorus play an important role in increasing the crop productivity. Further, the nitrogenous chemical fertilizers are manufactured industrially using non renewable petroleum products under high temperature and high pressure. Increase in petroleum cost day by day effects the cost of the chemical fertilizers. (Chandra S. Greep *et al*, 2005) On the other hand when increased doses of chemical fertilizers are used to increase crop production it causes environmental pollution and toxic to soil as it kills the beneficial microorganisms and so on. However, plant nutrients like N.P. and K are highly essential for plant growth and metabolism. It is also evidence that plant remove much nutrient from the soil in modern intensive cultivation and needs replenishment. The poor farm management technique and improper use of agrochemicals has also resulted in both soil quality and environmental degradation (Setboonsarng and Gilman 1999).Under such conditions Microbial organisms offer good alternative technology to replenish crop nutrients. As the agriculture industry becomes more aware of the need to adopt more environmental-friendly approach in providing plant nutrition and sustaining soil fertility, in tandem with increased productivity, bio-fertilizers have a substantial role in it. Biofertilizer is commonly referred to as the fertilizer that contains living micro-organisms and it is expected that their activities will influence the soil ecosystem and produce supplementary substance for the plants (Parr *et. al*. 2002).

METHODS AND MATERIALS

The experiment was conducted with *Enterobacter hormaechei* (KSB-8) and *Aspergillus terreus* (KSF-1), which is potassium solubilizing strains isolated from the ceramic industries near by Kad i(North Gujarat, India) region and these microorganisms also studied for their for gowth promoting effect on Okra plant. Their shelf-life are studied under two different storage temperature and two different medium conditions. Formulation of these potassium solubilizing strains were checked in two medium: Liquid (Peptone - 5 g%, Yeast extract- 3 g%, NaCl- 5 g%, pH- 7.2) and Solid on lignite carrier based medium (Lignite-70gm%, CaCO3-8 gm %, Gum Arabic-10 gm%) and two different storage conditions (30 ^{0}C & 4 ^{0}C).

Solid biofertilizer formulation was kept into the polyethylene bag at 100g solid medium per bag and sterilized. The carrier lignite bags were inoculated with KSM (5 x 10^7 CFU /g for Aspergillus terreus and 6 X 10^7 CFU/g for Enterobacter hormaechei) and were stored at 30°C and 4°C.

For liquid biofertilizer formulation, sterilized 100 ml liquid medium was kept in 150 ml plastic bottles and inoculated with KSM (5 x 10^7 CFU /ml for Aspergillus terreus and 6 X 10^7 CFU /ml for Enterobacter hormaechei) and were stored at 30^{0}C and 4^{0}C. Viability of potassium solubilizing microorganisms was checked by using Standard Plate Count (SPC) method after each 15 days interval. Colony forming unit was counted using four replicates for SPC count in each treatment. Potato Dextrose Agar was used for KSF-1 and incubated at 30^{0}C for 72 hrs & Nutrient Agar medium was used for KSB-8 and incubated at 37^{0}C for 48 hrs. The results were interpreted in terms of CFU /ml.

RESULTS AND DISCUSSION

When studying the shelf-life of liquid biofertilizer, viable microorganisms, and formulation medium are considered. The experiment described in this contribution showed that the two storage conditions have no significant effect on the 150 Days storage of liquid biofertilizer formulation.

Formulation of the bacterial and fungal cultures were studied under two different medium (Liquid and solid) and at two different storage conditions (30^0 C & 4^0 C) (Al-Taweil et al., 2010) for 180 days (Table-1 and 2). Bacterial culture is more viable in liquid broth as compared to the solid lignite carrier. While fungal spores are more viable in lignite carrier containing solid medium. There is no any significant effect of storage conditions at two different temperatures on fungal spores but bacterial culture shows good sustainability at 4^{0}C temperature.

Table- 1. Viability of *E. hormaechei* in Liquid Broth and Lignite at two different Storage conditions.

Time Interval (Days)	Liquid Broth (CFU/ ml)		Lignite (CFU/ ml)	
	4^0C	30^0C	4^0C	30^0C
0	$6x10^7$	$6x10^7$	$6x10^7$	$6x10^7$
15	$5.6 x10^7$	$4.48 x10^7$	$4.12 x10^7$	$4.80 x10^7$
30	$4.32 x10^7$	$4.12 x10^7$	$3.84 x10^7$	$4.28x10^7$
45	$3.76 x10^7$	$3.64 x10^7$	$3.56 x10^7$	$3.78 x10^7$
90	$3.56 x10^7$	$3.12 x10^7$	$2.72 x10^7$	$2.88 x10^7$
105	$3.04 x10^7$	$2.68 x10^7$	$2.36 x10^7$	$2.56 x10^7$
120	$2.96 x10^6$	$2.48 x10^6$	$2.04x10^6$	$2.18x10^6$
135	$2.8 x10^6$	$2.32 x10^6$	$1.92x10^6$	$2.08 x10^6$
150	$2.72 x10^6$	$2.20 x10^6$	$1.28x10^6$	$1.44 x10^6$

Table-2. Viability of *A. terreus* in Liquid Broth and Lignite at Two different Storage conditions.

Time Interval (Days)	Liquid Broth (CFU/ ml)		Lignite (CFU/ ml)	
	4^0C	30^0C	4^0C	30^0C
0	$5x10^7$	$5x10^7$	$5x10^7$	$5x10^7$
15	$4.6x10^7$	$4.44 x10^7$	$4.12x10^7$	$4.04 x10^7$
30	$3.64 x10^7$	$3.72 x10^7$	$3.92 x10^7$	$3.84 x10^7$
45	$3.36 x10^7$	$3.24 x10^7$	$3.68 x10^7$	$3.56 x10^7$
90	$3.12 x10^7$	$3.08 x10^7$	$3.52 x10^7$	$3.24 x10^7$
105	$3 x10^7$	$2.84 x10^7$	$3.24 x10^7$	$2.88 x10^7$
120	$2.84 x10^7$	$2.76 x10^7$	$3.08x10^7$	$2.76 x10^7$
135	$2.76x10^6$	$2.64 x10^6$	$2.8x10^6$	$2.6 x10^6$
150	$2.56 x10^6$	$2.44 x10^6$	$2.32x10^6$	$2.48 x10^6$

Potassium mobilizing microorganisms were isolated and authenticated/developed from Banana rhizosphere by Dr. Krishan Chandra and Singh (1999), which had the ability to mobilize the elemental or mixture of potassium (Chandra and Singh 1999; Chandra *et.al.*, 2001; 2002). It can be easily absorbed by plants. By using this Biofertilizer, 50 to 60% of potash chemical fertilizers cost could be reduced. The bacteria are known as *"Frateuria aurentia"* belonging to the family Pseudomonaceae. These bacteria have the extra ability to mobilize K in all types of soils especially, low K content soils, pH range 5-11 and survival temperature is 42^0C. There was no antagonistic/inhibiter effect noticed (Chandra *et al.*, 2000).

The efficacy of talc-based Pf1 formulation has already been demonstrated in different agriculture and horticulture crops against pests, diseases and nematodes under glasshouse and field conditions (Nandakumar *et al.,* 2001; Bharathi *et al.,* 2004; Ramamoorthy *et al.,* 2002; Saravanakumar *et al.,* 2007 and 2009). However, the carrier based bio-inoculants suffer from short shelf-life, high contamination and low field performance (Hegde, 2002). In this context, the development of liquid inoculants assumes greater importance in sustainable crop protection which could increase the shelf-life more than 1 year besides providing tolerance to adverse conditions. Further, the production of quality inoculants with increased shelf-life and user friendly formulation are essential factors for the success of bio-inoculant technology.

The developed formulation in this study avoided contamination and it was able to preserve counts of 10^6 CFU /ml after 150 days. The uses of liquid formulation in the management of several other diseases need to be studied in future for further commercialization process. The Enterobacter hormaechei (KSB-8) bacterial culture have a more viability in liquid formulation while the solid lignite carrier based formulation is not showing higher, so the liquid inoculants can support cell survival better than the solid lignite carrier formulation.

Aspergillus terreus (KSF-1) fungal biofertilizer formulation in both liquid and solid carrier based formulation showed the good viability (2.4 X 10^6 CFU/ml). So the results revealed that the fungal culture can be formulated either in the liquid or carrier based solid medium. There is no any major effect is observed in the viability of the culture in the two storage conditions,

CONCLUSION

The shelf-life study revealed that the survival of bacterial culture is more in liquid broth as compared to the solid lignite carrier, while fungal spores are surviving more on lignite carrier. There is no any significant effect of storage conditions at two different temperatures on fungal spores but bacterial culture shows good sustainability at 4^0C temperature. The study shows that application of lignite formulation sustain the shelf-life of *Aspergillus terreus* (KSF-1) which can be used as a biofertilizer compared to the liquid formulation. While for *Enterobacter hormaechei* (KSB-8) liquid formulation is more preferred as compared to the solid medium formulation.

REFERENCES

1) Al-Taweil, H. I., Osman, M. B., Abdulhamid, A., Mohammad, N. H. and Yussof, W. M. W. (2010). Comparison of Different Delivery System of Trichoderma and Bacillus as Biofertilizer. Advances in Environmental Biology, 4(1): 31-33. | 2) Bharathi, R., Vivekananthan, R., Harish, S., Ramanathan, A. and Samiyappan, R. (2004). Rhizobacteria based bioformulations for the management of fruit rot infection in chillies. Crop Protection, 23: 835–843. | 3) Chandra, K. and Singh, T. (1999). Past and present scenario of RBDC; Edts Chandra. K., Rath. S. and Singh. T. 1999 - Biofertilizer Situation in Orissa. National Institute for sustainable tropical Agriculture and Human Action (NISTHAA), Chintamaniswar, Bhubaneswar. pp: 1-7. | 4) Chandra, K., Ingle, S. R. and Bihari, K. (2002). Biofertilizers and its impact on different crops. National Seminar on "Biotechnology: Microbes to Man". School of Life Science, Utkal University, Bhubaneswar, Orissa. pp: 18-19. | 5) Chandra, K., Greep, S., Ravindranath, P. and Sivathsa, R. S. H. (2005). Liquid biofertilizers. Regional Center for Organic Farming Hebbal, Bangalore. pp: 22-35. | 6) Chandra, K., Ingle, S. R., Bihari, K. and Srivathsa, R. S. H. (2001). Future Inoculum- Biofertilizers in todays Agriculture. National Seminar on Plant Resource Management for Sustainable Development Department of Botany, Utkal University, Bhubaneswar. pp: 63-67. | 7) Chandra, K., Srivathsa, H., Greep, S. and Ingle, S. R. (2000). Liquid inoculum, Biofertilizer ready reckoner vision 2004. Published by – Regional Biofertilizer Dev. Centre, Bangalore. pp: 23-25. | 8) Hegde, S. V. (2002). Liquid biofertilizers in Indian agriculture. Biofertilizer News Letter, 12: 17–22. | 9) Nandakumar, R., Viswanathan, R., Babu, S., Sheela, J., Raguchander, T. and Samiyappan, R. (2001). A new bio-formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. Biocontrol, 46: 493–510. | 10) Parr, J.F. Hornick, S.B. and Papendick, R.I. (2002). Transition from conventional agriculture to natural farming systems: The role of microbial inoculants and biofertilizer. [Internet] Available from: http://www.emtech.org/data/pdf/0103.pdf. [cited March 2006]. | 11) Ramamoorthy, V., Raguchander, T. and Samiyappan, R. (2002). Induction of defense related proteins in tomato roots treated with Pseudomonas fluorescens Pf1 and Fusarium oxysporum f. sp. lycopersici. Plant and Soil, 239: 55–68. | 12) Saravanakumar, D., Lavanya, N., Muthumeena, K., Raguchander, T. and Samiyappan, R. (2009). Fluorescent pseudomonad mixtures mediate disease resistance in rice plants against sheath rot (Sarocladium oryzae) disease. Biocontrol, 54(2): 273–286. | 13) Saravanakumar, D., Muthumeena, B., Lavanya, N., Suresh, S., Rajendran, L., Raguchander, T. and Samiyappan, R. (2007). Pseudomonas induced defense molecules in rice against leaf folder (Cnephalocrocis medinalis) pest. Pest Management Science, 63: 714–721. | 14) Setboonsarng, S. and Gilman, J. (1999). Alternative Agriculture in Thailand and Japan. HORIZON Communications, Yale University, New Haven, Connecticut. |