Science Research Reporter, 4(1): 44-50, (April - 2014)

ISSN: 2249-2321 (Print); ISSN: 2249-7846 (Online)
Received: 21-01-2014, Revised: 27-02-2014, Accepted: 11-03-2014

Full Length Article

Effect of enrichment material on the shelf life and field efficiency of bioformulation of *Rhizobium* sp. and P-solubilizing *Pseudomonas fluorescens*

Sachin Singh[1], Govind Gupta[2], Ekta Khare[3], K. K. Behal[4] and Naveen K. Arora[*5]

[1]Research and Development Division, Prathista Industries Ltd., Hyderabad, India
[2]Department of Microbiology, Barkatullah University, Bhopal, India
[3]Department of Microbiology, CSJM University, Kanpur 208024, India
[4]Department of Life Science, CSJM University, Kanpur 208024, India
[5]Department of Environmental Microbiology, School of Environmental Sciences BBA University, Lucknow – 226 025, Uttar Pradesh, India
*nkarora_net@rediffmail.com

ABSTRACT

In the present investigation seven carriers – talc, saw dust, fuller's earth, rice husk, sugarcane bagasse, charcoal and wheat bran were evaluated for the production of bioformulation. The bacteria used for bioformulation development were root nodulating *Rhizobium* sp. RASH6$^{Chl+Kan+}$ and phosphate solubilizing *Pseudomonas fluorescens* PB6$^{Amp+Str+}$. Both bacterial strains were inoculated in all the carriers separately and in combination with each other (co-inoculants). The bacterial population was determined in each carrier up to six month storage. Sawdust proved to be the best carrier in both water holding capacity (350 %) and also in maintaining the bacterial population for both individual and co-inoculation. Saw dust based formulation was separately amended with CMC, sucrose, molasses and gum. Enrichment of saw dust with molasses brought maximum increment in population both in mono and co-inoculants. Finally the impact of six month-stored enrichment inoculants on plant productivity was determined taking chickpea as a test crop. The co-inoculants proved much better in enhancing the seedling biomass and the nodule number. Molasses enriched saw dust based formulation showed 48.43 %, 52.02 % and 57.41 % enhancement in dry weight with RASH6, PB6 and their co-inoculant respectively after 60 days of sowing. Results showed that enrichment of carrier is expected to permit the retention of cell viability thus increasing the effectiveness of the active material.

Key Words: Bioformulation, molasses, *Pseudomonas*, *Rhizobium*, saw dust

INTRODUCTION

In agriculture one of the limiting factors is providing plant nutrients, particularly nitrogen and phosphorus (Kloepper *et al.,* 1989). Soil micro-organism plays an important role in soil processes that determine plant productivity (Smita *et al.,* 2011). The beneficial effect of rhizobia on legumes in terms of biological N_2 fixation is well known (Smith, 1992). Rhizobia are unique in that they are the only nitrogen fixing bacteria living in a symbiotic relationship with legume (Jadhav, 2013). Biological nitrogen fixation by rhizobia is one of the effective methods to improve the plant growth and productivity (Deshwal *et al.,* 2013). Application of rhizobia is a well-established strategy because of their ability for symbiotic nitrogen fixation with host plants (Deshwal *et al.,* 2003). Phosphorus is second essential nutrient after nitrogen, required for the growth of plant and micro organisms.

Soils are often abundant in insoluble Phosphorus (P), either in organic or inorganic forms, but deficient in soluble phosphates essential for growth of most plants and microorganisms. To enhance phosphorus uptake efficiency, phosphate solubilizing bacteria (PSB) play an important role in supplying phosphate to plants, which is environment friendly and sustainable approach. Fluorescent pseudomonads comprise a major group of root-associating bacteria that stimulate the growth of plants through phosphate solubilizing activity.

For agronomic utility, inoculation of plants with target microorganisms at a higher concentration than those normally found in soil is necessary to take advantages of their beneficial properties for plant yield enhancement (Subba Rao, 1993). The erratic performances of bioinoculants under field conditions have raised concerns about the practical potential offered by microbial releases into soil (Arora *et al.,* 2010). Since formulation protects cells against harsh chemical and environmental conditions (Gentry *et al.,* 2004), and since most bioformulations are meant for field application, it is essential that suitable carrier materials are used to maintain cell viability under adverse environmental conditions (Brar *et al.,* 2006). A good quality formulation promotes survival of bacteria, maintaining aviable population sufficient to exude growth promoting effects on plants (Aeron *et al.,* 2011). The carrier with the ability to carry both the inoculants (rhizobial and *Pseudomonas* spp.) will appear to be revolutionary for the agriculture industry. One such approach to ensure the viability of cells is to enrich the carrier material with suitable enrichment materials. Evidence suggests that the addition of nutrients to seed pellets may be a useful strategy for improving inoculant survival (Moënne-Loccoz *et al.,* 1999). Present study was aimed to examine the potential of different carriers based bioformulations of root nodulating *Rhizobium* RASH6 and phosphate solubilizing *Pseudomonas fluorescens* PB6 as monoinoculant and co-inoculant for the ability of plant growth promotion. Another aim was to check the effect of enrichment materials on inoculums viability and *in vivo* plant growth promotory potential taking chickpea (*Cicer arietinum*) as a test crop.

MATERIALS AND METHODS

Bacterial strains

Nitrogen fixing *Rhizobium* RASH6^{Chl+} indigenously resistant to chloramphenicol and phosphate solubilizing *Pseudomonas fluorescens* PB6^{Amp+} spontaneous-resistant to ampicillin were taken from culture collection of Department of Environmental Microbiology, School of Environmental Sciences BBA University, Lucknow, India. For tracking the populations of bacteria in bioformulations, antibiotic resistance was also introduced in both the strains. *Escherichia coli* WA803 having suicidal plasmid (pGS9) integrated into a transposon Tn5 with a kanamycin-resistant and streptomycin-resistant marker gene was used to confer kanamycin resistance to RASH6^{Chl+} and streptomycin resistance to PB6^{Amp+} respectively according to the method of Kumar *et al.* (2003). *Rhizobium* sp. RASH6$^{Chl+Kan+}$ and *P. fluorescens* PB6$^{Amp+Str+}$ were grown on Yeast Extract Mannitol Agar (YEMA; Hi-Media, Mumbai) and Kings B (KB; Hi-Media, Mumbai) respectively at 28ºC.

Physico-chemical properties of carriers

For the preparation of bioformulation, locally available agricultural and industrial wastes (fuller's earth, charcoal, sawdust, wheat bran, peat, talc and sugarcane bagasse) were selected as carriers. Moisture content was calculated according to Aeron *et al.,* (2012). Water holding capacity of a carrier was determined on a dry weight basis according to Arora *et al.,* (2008) and pH according to Page *et al.,* (1982).

Bioformulation development

Bacterial inoculums were raised by growing *Rhizobium* sp. RASH6 in YEM broth medium and *P. fluorescens* PB6 in KB broth medium for 48 hours at 28ºC. After 48 hours culture was added in each carrier on the basis of their water holding capacity in three sets, (i) Carrier + RASH6, (ii) Carrier + PB6, and (iii) Carrier + RASH6 + PB6.

Shelf life detection

After every 30 days samples were checked for measuring colony forming units (CFU) up to six months, following Somasegran and Hoben (1994). Average cell number was calculated by estimating CFU/g of formulation on YEM agar supplemented with chloramphenicol (50 mg/L) and kanamycin (50 mg/L) for RASH6$^{Chl+Kan+}$ and with ampicillin (50 mg/L) and streptomycin (50 mg/L) for PB6$^{Amp+Str+}$.

Saw dust based bioformulations with enrichment material

Saw dust (SD) was selected for making the bioformulations with different combinations of microbes and enrichment material. Carboxymethyl cellulose (CMC), sucrose, molasses and gum arabic were used as enrichment material. Enrichment martial was mixed in sawdust at 1% concentration (w/w). The culture of RASH6 and PB6 was mixed with saw dust amended with various enrichment materials as mono-inoculant and co-inoculant. The experiment was conducted in triplicate, and one bag of each carrier from each replicate was investigated for inoculums' population density after every 30 days interval up to 180 days.

***In vivo* study**

Chick pea plants were raised from surface-sterilized seeds in plastic pots filled with steam-sterilized local soil (500 g; P = 0.0908 %, pH = 8.4). Saw dust bioformulation (25%) was mixed with sterilized water to form slurry of the formulation and surface sterilized chickpea seeds were soaked in slurry of bioformulations enriched with certain enrichment material for 10 min, then dried under sterilized conditions. The experiment was carried out with fifteen different treatments of saw dust bioformulations: (i) Seeds + RASH6 (ii) Seeds + PB6 (iii) Seeds + RASH6 + PB6 (iv) Seeds + CMC + RASH6 (v) Seeds + CMC + PB6 (vi) Seeds + CMC + RASH6 + PB6. For other supplements (sucrose, molasses and gum) sets were repeated as above. Seeds without any treatment were taken as control. All experiments were done in five replicates. At harvest (60 days), root length, shoot length, nodule number per plant, fresh weight and dry weight were determined. Chlorophyll content of chickpea leaves was estimated according to Arnon, (1949).

Results and discussion

Formulations that transfer the growth promoting activities of rhizobacteria(s) from laboratory to field would have a major impact in agriculture. Amongst all tested carriers used for development of bioformulations, saw dust showed maximum inherent moisture content (15.2 %) and water holding capacity (350 %) respectively followed by talc (8.5 % and 275 %), charcoal (7.02 % and 255 %), sugarcane bagasse (6.76 % and 230 %), rice husk (5.65 % and 210 %), fuller's earth soil (2.1 % and 175 %) and wheat bran (3.2 % and 160 %). The pH of all the carriers was near neutral (6.9 to 7.7). After six month of storage the inherent moisture content of the saw dust was maintained to 12.5 % which was found maximum over all other tested carriers. Brockwell and Bottomley (1996) observed that materials having high water holding capacity, high inherent moisture content and good aeration have been considered as good carriers for bio-inoculants. Similar result for saw dust was observed by Arora *et al.,* (2008). Arora *et al.,* (2001) have also suggested the use of sawdust as carrier, especially when easily available.

Saw dust based bioformulation showed best results for population density during storage with both *Rhizobium* RASH6 and *P. fluorescens* PB6, followed by talc. Population density of the isolate RASH6$^{Chl+Kan+}$ and PB6$^{Amp+Str+}$ in the saw dust based mono-inoculant (bioformulation) was observed to be 10.4 log CFU/g and 10.8 log CFU/g after one month of storage at room temperature. There was gradual decrease in the population due to cell shock and the population of RASH6$^{Chl+Kan+}$ and PB6$^{Amp+Str+}$ declined up to 8.4 and 8.1 log CFU/g respectively after six months of incubation which is within the permissible limit (Brahmaprakash and Sahu, 2012). During co-inoculation saw dust again proved to be the best carrier for both RASH6 and PB6. In coinoculant on sawdust, population density of RASH6$^{Chl+Kan+}$ was maintained at 7.9 log CFU/g after six months of storage while that of phosphate solubilizing *P. fluorescens* PB6$^{Amp+Str+}$ was 7.7 log CFU/g. Sawdust based bioformulations with RASH6 and PB6 supported more than 7.0 log CFU/g of population after six months, which is in accordance with the Bureau of Standards in India (Brahmaprakash and Sahu, 2012). Maintenance of optimum viability of propagules in formulations suggests the robust nature of the bacterium under room temperature storage conditions (Shanmugam *et al.,* 2011). Minimum population was supported by wheat bran and it was 5.2 and 4.9 log CFU/g for RASH6$^{Chl+Kan+}$ and PB6$^{Amp+Str+}$ respectively, in coinoculant after six months of storage. There are reports that carriers possessing high water-holding capacity and near-neutral pH can support large populations (Arora *et al.,* 2008, Roughley and Vincent, 1967).

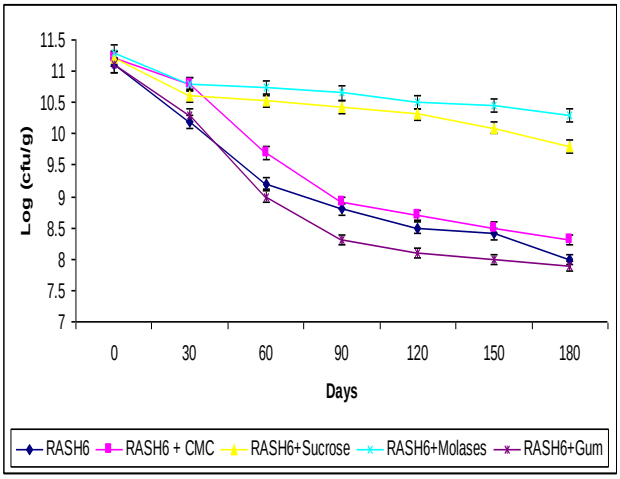
The results of saw dust based bioformulation enriched with molasses showed maximum population density of RASH6$^{Chl+Kan+}$ and PB6$^{Amp+Str+}$ in both mono-inoculant and co-inoculant followed by sucrose and CMC (Fig. 1).

Table 1. Detection of plant growth promotory ability of sawdust based bioformulations of RASH6$^{Chl+Kan+}$ and PB6$^{Amp+Str+}$ under field conditions.

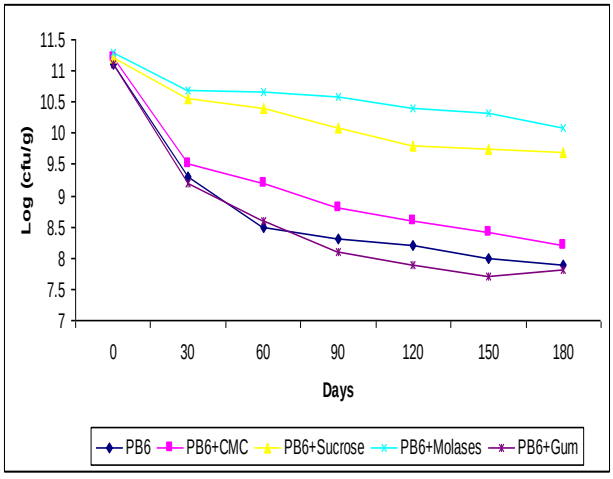
Treatments	Root Length (cm)	Shoot Length (cm)	No of Pods/ plant	Nodules weight/ plant (g)	Fresh Weight (g)	Dry Weight (g)	Chl a+b (mg/g)
Control (Seed)	10.12±0.04^{a}	21.27±0.03^{a}	16.32±0.04^{a}	-	11.46±0.03^{a}	06.05±0.04^{a}	0.011±0.04^{a}
RASH6	11.98±0.03^{b}	30.45±0.04^{b}	25.54±0.03^{c}	3.1±0.03^{a}	24.82±0.03^{b}	13.09±0.03^{c}	0.014±0.03^{b}
PB6	11.56±0.04^{b}	32.12±0.03^{d}	22.32±0.03^{b}	-	25.65±0.02^{c}	12.34±0.02^{b}	0.015±0.03^{c}
RASH6 + PB6	13.34±0.02^{d}	38.56±0.03^{a}	31.12±0.03^{f}	5.2±0.02^{b}	28.32±0.03^{fe}	15.45±0.03^{e}	0.014±0.03^{b}
RASH6 + CMC	12.21±0.03^{c}	32.98±0.04^{d}	27.22±0.04^{d}	3.4±0.03^{a}	26.54±0.03^{d}	15.76±0.03^{e}	0.016±0.03^{d}
PB6 + CMC	12.43±0.03^{c}	34.32±0.02^{e}	25.34±0.02^{c}	-	27.54±0.02^{e}	14.87±0.02^{d}	0.014±0.04^{b}
RASH6 + PB6 + CMC	13.21±0.03^{cd}	36.23±0.03^{f}	34.23±0.03^{g}	4.5±0.02^{ab}	30.12±0.03^{h}	17.45±0.04^{g}	0.017±0.03^{e}
RASH6 + Sucrose	12.97±0.03^{c}	39.12±0.03^{h}	34.23±0.02^{h}	5.1±0.03	33.54±0.03^{i}	18.67±0.02^{h}	0.017±0.03^{e}
PB6 + Sucrose	13.11±0.02^{cd}	40.12±0.03^{i}	29.56±0.03^{e}	-	34.23±0.03^{j}	18.54±0.04^{h}	0.016±0.02^{d}
RASH6 + PB6 + Sucrose	13.98±0.03^{d}	44.23±0.04^{l}	37.23±0.04^{j}	5.7±0.02^{b}	38.12±0.04^{m}	19.76±0.03^{i}	0.019±0.03^{g}
RASH6 + Molasses	13.21±0.02^{cd}	43.12±0.04^{k}	38.83±0.04^{k}	5.9±0.03^{bc}	37.87±0.04^{l}	19.43±0.02^{i}	0.018±0.03^{f}
PB6 + Molasses	13.98±0.03^{d}	45.12±0.03^{m}	36.43±0.03^{l}	-	35.49±0.03^{k}	18.76±0.03^{l}	0.017±0.04^{e}
RASH6 + PB6 + Molasses	14.87±0.04^{e}	47.58±0.02^{n}	39.85±0.03^{j}	6.1±0.02^{c}	39.89±0.02^{n}	24.32±0.03^{j}	0.020±0.03^{h}
RASH6 + Gum	12.43±0.02^{c}	38.32±0.03^{g}	32.12±0.04^{g}	5.2±0.03^{b}	28.56±0.03^{fe}	16.87±0.02^{f}	0.015±0.03^{c}
PB6 + Gum	12.21±0.03^{c}	37.21±0.04^{f}	34.67±0.03^{h}	-	27.78±0.04^{ed}	16.43±0.03^{f}	0.014±0.02^{b}
RASH6 + PB6 + Gum	13.56±0.03^{d}	41.12±0.04^{i}	37.23±0.02^{j}	5.5±0.02^{b}	30.13±0.04^{h}	17.49±0.03^{g}	0.015±0.03^{c}

Results are the mean of 05 replicates ±SD. Means in the column followed by same letters indicate no significant difference (P=0.05) by Duncan's multiple range test.

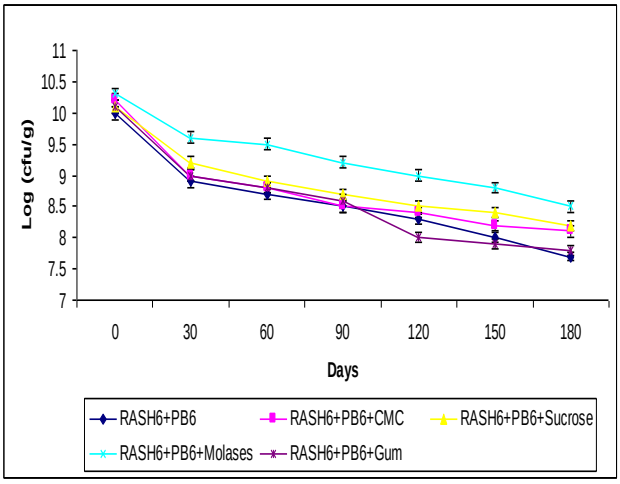
There was no increase in population density of RASH6 and PB6 in gum arabic enriched bioformulations over non-enriched sawdust based bioformulation. The formulation enrichment with molasses maintain the population density to 10.3 and 10.1 log CFU/g in the monoinoculant of RASH6$^{Chl+Kan+}$ and PB6$^{Amp+Str+}$, respectively after six months of storage. Bioformulation of RASH6$^{Chl+Kan+}$ + PB6$^{Amp+Str+}$ enriched with molasses maintained their population density to 8.5 and 8.3 log CFU/g respectively, after six months of storage. Results clearly showed that enrichment of carrier with molasses enhances the cell viability of the bioformulation over non-enriched sawdust based bioformulations. These are in agreement with previous research which showed that high-molecular-weight (C6 to C12) compounds such as glucose, sucrose and trehalose enhanced survival of bacteria in dried biopolymers (Aeron *et al.,* 2012, Omer, 2010, Ilyina *et al.,* 2000). Brar *et al.,* (2006) also found that the combination of carrier with enrichment material is expected to permit the retention of cell viability thus increasing the effectiveness of the formulation. Caesar and Burr (1991), found that amendment of sucrose (0.72M) in King's B medium increased population and shelf life of *P. fluorescens* (P7NF, TL3) in talc-based formulation up to 12 months.



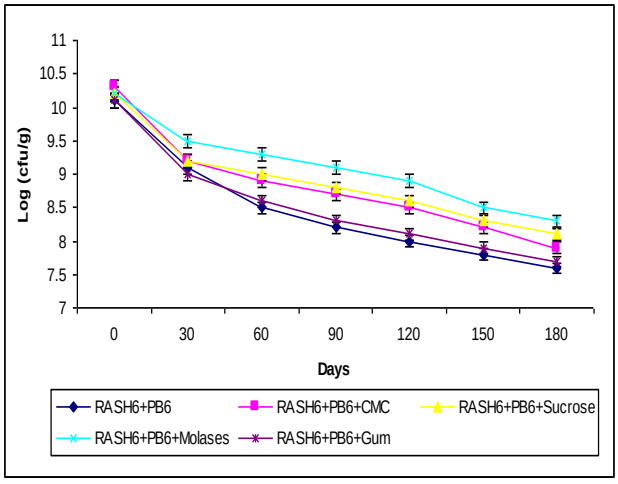
A) Population density of *Rhizobium* sp. RASH6$^{Chl+Kan+}$ in monoinoculant



B) Population density of *P. fluorescens* PB6$^{Amp+Str+}$ in monoinoculant



C) Population density of *Rhizobium* sp. RASH6$^{Chl+Kan+}$ when co-inoculated with *P. fluorescens* PB6$^{Amp+Str+}$



D) Population density of *P. fluorescens* PB6$^{Amp+Str+}$ when co-inoculated with *Rhizobium* sp. RASH6$^{Chl+Kan+}$

Fig 1. Population density of *Rhizobium* sp. RASH6 and *P. fluorescens* PB6 in different sawdust based bioformulations enriched with different materials.

Ting *et al.,* (2009), indicated that bentonite-based bioformulation enriched with sucrose can produce both good viability and efficacy results, even after exposure to sunlight. It has been proposed that a uniform coating of approximately 10^7 CFU of bacteria per seed is necessary for successful bacterization (Suslow, 1982).

Saw dust based bioformulation enrichment with molasses showed maximum enhancement of plant growth parameters of chickpea followed by sucrose and CMC over control (Table 1). Molasses enriched saw dust bioformulation of RASH6$^{Chl+Kan+}$, PB6$^{Amp+Str+}$ and their co-inoculant showed 48.43 %, 52.02 % and 57.41 % enhancement in dry weight respectively, over non-enriched sawdust bioformlations after 60 days of sowing. Earlier studies also reported significant increase in plant fresh and dry weight on inoculation with *Pseudomonas* and *Rhizobium* spp. (Pandey *et al.,* 2005). Earlier reports are available which suggest the usefulness of co-inoculants over monoinoculants, and especially of microorganisms that are synergistic to each other. Use of co-inoculants has the advantage of enhancing the plant productivity by diverse mechanisms (Dashti *et al.,* 1998). Nakkeeran *et al.,* (2005) mentioned that the performance of bioformulations can be increased by the incorporation of water soluble adjuvants, oils, stickers and emulsions.

The present study recommends the use of molasses as enrichment material in bioformulations. These data suggest that *Rhizobium sp.* and *Pseudomonas* sp. isolates can be used in further investigations as a potential agent of new biofertilizer for improved chick pea production.

Acknowledgement

The authors Singh and Arora are grateful to Vice Chancellor of CSJM, University, Kanpur and Vice Chancellor of BBA University, Lucknow, India for providing facilities and support.

LITERATURE CITED

Aeron A, Khare E, Arora NK and Maheshwari DK, 2012. Practical use of CMC-amended rhizobial inoculant for *Mucuna pruriens* cultivation to enhance the growth and protection against *Macrophomina phaseolina*. *J. Gen. Appl. Microbiol.,* **58**:121–127.

Aeron A, Dubey RC, Maheshwari DK, Pandey P, Bajpai VK and Kang SC, 2011. Multifarious activity of bioformulated *Pseudomonas fluorescens* PS1 and biocontrol of *Sclerotinia sclerotiorum* in Indian rapeseed (*Brassica campestris* L.). *Eur. J. Plant Pathol.,* **131**:81–93.

Arnon DI, 1949. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*, *Plant Physiol*., **24**:1-15.

Arora NK, Kang SC and Maheshwari DK, 2001. Isolation of siderophore inducing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of ground nut. *Curr. Sci*., **81**:673-677.

Arora NK, Khare E, Naraian R and Maheshwari DK, 2008. Sawdust as a superior carrier for production of multipurpose bioinoculant using plant growth promoting rhizobial and pseudomonad strains and their impact on productivity of *Trifolium repense*, *Curr. Sci.,* **95**:90-94.

Arora NK, Maheshwari DK and Khare E, 2010. PGPR: Constraints in bioformulation, commercialization and future strategies. Bacteria and plant Health, D.K. Maheshwari, eds., *Springer Publication*, Netherland, 97-116.

Brahmaprakash GP and Sahu PK, 2012. Biofertilizers for Sustainability. *J. Indian Inst. Sci*., **92**(1):37-62.

Brar SK, Verma M, Tyagi RD and Valero JR, 2006. Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides," *Process Biochem*., **41**:323-342.

Brockwell J and Bottomley PJ, 1996. Recent advances in inoculant technology and prospects for the future. *Soil Biol. Biochem.*, **27**:683–697.

Caesar AJ and Burr TJ, 1991. Effect of conditioning, betaine, and sucrose on survival of rhizobacteria in powder formulations. *Appl. Environ. Microbiol.,* **57**:168-172.

Dashti N, Zhang F, Hynes R and Smith DL, 1998. Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean (*Glycine max* L. Merr.) under short season conditions. *Plant and Soil,* **200**:205–213.

Deshwal VK, Dubey RC and Maheshwari DK, 2003. Isolation of plant growth promoting *Bradyrhizobium* (*Arachis*) sp. with biocontrol potential against *Macrophomina phaseolina* causing charcoal rot of peanut. *Curr. Sci.,* **84**:443-448.

Deshwal VK, Singh SB, Kumar P and Chubey A, 2013. Rhizobia Unique Plant Growth Promoting Rhizobacteria: A Review. *Inter. J. of Life Sci.,* **2**(2): 74-86.

Gentry TJ, Rensing C and Pepper IL, 2004. New approaches for bioaugmentation as a remediation technology. *Critical Rev. Environ. Sci. Tech*., **34**(5):447-494.

Ilyina AD, Villarreal SJA, Rivera RE, Garza GY and Rodriguez MJ, 2000. Using of biadac (Absorbent granules produced from paper industry residues) as carrier to microorganisms for soil inoculation. Vestnik Moskovskogo univaestteta. Khimiya, **41**(6):135-138.

Jadhav RN, 2013. Isolation of rhizobia from soybean cultivated in Latur area & study of its phosphate solubilization activity**.** *Biosci. Discov*.**, 4(1)**:100-103.

Kloepper JW, Lifshitz R and Zablotowicz RM, 1989. Free-living bacterial inocula for enhancing crop productivity. *Trends Biotechnol*. **7**(2):39–43.

Kumar A, Vij N and Randhawa GS, 2003. Isolation and symbiotic characterization of transposon *Tn-5* induced arginine auxotrophs of *Sinorhizobium meliloti. Ind. J. Exp. Biol*., **41**:1198–1204.

Moënne-Loccoz Y, Naughton M, Higgins P, Powell J and O' Gara F, 1999. Effect of inoculum preparation and formulation on survival and biocontrol efficacy of *Pseudomonas fluorescens* F113. *J. Appl. Microbiol*., **86**:108–116.

Nakkeeran S, Dilantha Fernando WG and Siddiqui WA, 2005. Plant growth promoting rhizobacteria formulations and its scope in commercialization for

the management of pests and diseases," PGPR, biocontrol and biofertilization, Z.A. Siddiqui, eds., *Springer*, Dordrecht, The Netherlands, 257-296.

Omer AM, 2010. Bioformulations of *Bacillus* spores for using as biofertilizers. Life Science J **7**(4):124-131.

Page AL, Miller RRH and Keeny DR, 1982. Methods of soil analysis. Chemical and microbiological properties, part 2, American Society of Agronomy, Inc., Soil Science Society of America, Inc. Madison, Wisconian, U.S.A., pp. 1159.

Pandey P, Kang SC and Maheshwari DK, 2005. Isolation of endophytic plant growth promoting *Burkholderia* sp. MSSP from root nodules of *Mimosa pudica*. *Curr. Sci.,* **89**:170–180.

Roughley RJ and Vincent JM, 1967. Growth and survival of Rhizobium spp. in peat culture. *J. Appl. Bacteriol.,* **30**:362–376.

Shanmugam V, Kanoujia N, Singh M, Singh S and Prasad R, 2011. Biocontrol of vascular wilt and corm rot of gladiolus caused by *Fusarium oxysporum f.* sp. *gladioli* using plant growth promoting rhizobacterial mixture. *Crop Protec.,* **30**:807–813.

Smita S, Sinhal SK, Sharma VK and Shahi VK, 2011. Phenotypic and physiological characterization of rhizobia strains isolated from different areas of Bihar India. *Biosci. Disc.,* **02** (2):281 -287.

Smith RS, 1992. Legume inoculant formulation and application. *Can. J. Microbiol.,* **38**: 485–492.

Somasegran P and Hoben HJ, 1994. Handbook of Rhizobia: Methods in Legume *Rhizobium* Technology. *Springer-Verlag, New York*.

Subba Rao NS, 1993. Biofertilizers in agriculture and forestry. Oxford and IBH publishing Co. Pvt. Ltd., New Delhi, 242.

Suslow TV, 1982. Role of root-colonizing bacteria in plant growth. *Phytopathogenic Prokaryotes*, M. S. Mount, and G. Lacy, eds., **1**:187-223.

Ting ASY, Fang MT and Tee CS, 2009. Assessment on the effect of formulative materials on the viability and efficacy of *Serratia marcescens* a biocontrol agent against *Fusarium oxysporum* F. sp. *cubense* race 4. *American J. Agri. Biol. Sci.,* **4**(4):283-288.

How to Cite this Article:

Sachin Singh, Govind Gupta, Ekta Khare, KK Beha and Naveen K Arora, 2014. Effect of enrichment material on the shelf life and field efficiency of bioformulation of *Rhizobium* sp. and P-solubilizing *Pseudomonas fluorescens*. *Sci. Res. Rept.,* **4**(1):44-50.