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Carrier Based Bioformulations of PGPR- Characteristics, Shelf life and Application in Improving Health Status of Crop Plants- A Mini Review

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ABSTRACT

The thin layer of soil (about 1 to 2mm thick) surrounding crop roots and the volume of soil occupied by roots are known as the rhizosphere. Plant growth results from interaction of roots and shoots with the environment. The environment for roots is the soil or planting medium which provide structural support as well as water and nutrients to the plant. Roots also support the growth and functions of a complex of microorganisms that can have a profound effect on the growth and survival of plants. These microorganisms constitute rhizosphere microflora and can be categorized as deleterious, beneficial, or neutral with respect to root/plant health. Beneficial interactions between roots and microbes are common in rhizosphere and can be enhanced. Increased plant growth and crop yield can be obtained upon inoculating seeds or roots with certain specific root-colonizing bacteria- plant growth promoting rhizobacteria (PGPR). Isolation of microorganisms, screening for desirable characters, selection of efficient strains and production of inocula are important steps for making use of this microbe-based technology. One of the common means of application of bacterial inoculants to soil is in the form of bioformulations. However, for easy handling of such bacteria, it is necessary to pack such bacteria in inert materials which can also be packaged and stored. The review presented below has been compiled mainly on important aspect on development of bioformulations of beneficial bacteria.

Keywords: PGPR, Carrier based bioformulations, Shelf life, Potential use, Health status of crops.

INTRODUCTION

One of the common means of application of bacterial inoculants to soil is in the form of bioformulations. However, for easy handling of such bacteria, it is necessary to pack such bacteria in inert materials which can also be packaged and stored. Initially, it is essential to determine whether the bacteria can survive in the bioformulations for a reasonable period of time and whether they can induce similar effects to those observed by live bacterial cells. For commercialization, viability of bioinoculant in a prescribed formulation for a certain period with preservation of strain characteristics is also desirable (Fages 1992; Smith 1992). Krishnamurthy and Gnanamanickam (1998) developed talc based formulation of *Pseudomonas fluorescens* for the management of rice blast caused by *Pyricularia grisea*, in which methyl cellulose and talc was mixed at 1: 4 ratio and blended with equal volume of bacterial suspension at a concentration of 10^{10}cfu/ml. Treatment of cucumber seeds with strain mixtures comprising of *Bacillus pumilus* - INR7, *B. subtilis* – GB03 and *Curtobacterium flaccumfaciens* – ME1 with a mean bacterial density of $5x10^{9}$cfu/seed reduced intensity of angular leaf spot and anthracnose equivalent to the synthetic elicitor actigard and better than seed treatment with individual strains (Raupach and Kloepper 1998). For field applications, the inoculum is required in an appropriate formulation. Viability of inoculum in an

appropriate formulation for a certain length of time is important for commercialization of the technology (Bashan 1998). PGPR can also survive in alginate beads for longer periods of time (Bashan and Gonzalez 1999). Press mud is a by product of sugar industries. It was composted using vermin-composting technique and later used as a carrier for *Azospirillum* spp. This carrier maximizes the survival of *Azospirillum* spp than lignite, which is predominantly used as a carrier material in India (Muthukumarasamy et al. 1999). Formulation development must consider factors such as shelf life, compatibility with current application practices, cost, and ease of application. Health and safety testing may be required to address such issues as non-target effects on other organisms including toxigenicity, allergenicity and pathogenicity, persistence in the environment, and potential for horizontal gene transfer.

CHARACTERISTICS AND CATEGORIES OF BIOFORMULATIONS

According to Jeyarajan and Nakkeeran (2000), an ideal formulation should have some characteristics:- 1. Should have increased shelf life. 2. Should not be phytotoxic to the crop plants.3. Should dissolve well in water and should release the bacteria. 4. Should tolerate adverse environmental conditions. 5. Should be cost effective and should give reliable control of plant diseases. 6. Should be compatible with other agrochemicals. 7. Carriers must be cheap and readily available for formulation development.

There are mainly two categories of bioinoculants as presented below (Table 1).

Table 1: Two Categories of Bioformulations

Liquid Based	Carrier based
Easy for application as a foliar spray	Time consuming
Dosage per acre 250 ml	Dosage per acre 5 kg
Contamination is Nil	Contamination chances are high
Very good shelf life 12 months from the manufacture	Poor shelf life 3- 6 months
Cell count 1 x 10^9cfu/ml	Cell count 1 x 10^7cfu/ml
Adequate moisture	Moisture loss may occur
Cells protected with additives from biotic stress	Cells are not protected

For the development of successful formulations of PGPR, rhizobacteria should also possess following characteristics: 1. High rhizosphere competence. 2. High competitive saprophytic ability. 3. Enhanced plant growth. 4. Ease for mass multiplication. 5. Broad spectrum of action. 6. Excellent and reliable control. 7. Safe to environment. 8. Compatible with other rhizobacteria. 9. Should tolerate desiccation, heat, oxidizing agents and UV radiations (Jeyarajan and Nakkeeran 2000).

SHELF LIFE OF DIFFERENT CARRIER BASED BIOFORMULATIONS OF PGPR

Seed treatment of lettuce with either vermiculite or kaolin based carrier of *B. subtilis* (BACT-0) significantly reduced root rot caused by *P. aphanideramtum* and it also increased the fresh weight of lettuce under greenhouse conditions. Treatment of tomato seeds with powder formulation of PGPR (*B. subtilis, B. pumilus*) reduced symptom severity of ToMoV and increased the fruit yield (Murphy et al. 2000). Seed treatment with vermiculite based *P. putida* also reduced fusarium root rot of cucumber and increased the yield and growth of cucumber (Amer and Utkhede 2000). Viveganandan and Jauhri (2000) also reported the superiority of alginate-based formulations over charcoal-based ones in maintaining the population of two phosphate solubilizing bacteria during storage at different temperatures and moisture content. Due to the limitations of direct inoculation and the use of various solid-phase bacterial inoculants, several polymer- based

formulations, such as alginate beads, wet and dry alginate microbeads and gum-arabic preparations of bacterial species like *Azospirillum brasilense* Cd, *Pseudomonas fluorescens*, and *Rhizobium sp*. have been evaluated (Forestier et al. 2001; Bashan et al. 2002). Alginate beads have also been reported to preserve the beneficial properties of PGPRs under storage (Russo et al. 2001). Soaking of rice seeds in water containing 10g of talc based formulation of P. fluorescens consisting mixture of PF1 and PF2 (10^8cfu/g) for 24h controlled rice sheath blight under field condition (Nandakumar et al. 2001). Experimental formulations of *Bacillus* spp that have effectively reduced plant diseases have included ca- alginate, alginate manucol (Schmidt et al. 2001); peat and chitin (Manjula and Podile 2001; Sid Ahmed et al. 2003).

Yuen et al. (2001) also found that incorporation of chitin in the medium increased bacterial population when compared to the non-chitin amended medium and improved the efficiency of PGPR strains in reducing the severity of rust disease in bean plants. Three plant growth promoting rhizobacterial (PGPR) strains of Pseudomonas spp, PF1, FP7 and PB2, were also tested alone and in combinations for suppression of rice sheath blight disease and promotion of plant growth under glasshouse and field conditions. The mixture of PGPR strains significantly reduced the sheath blight incidence when applied as either bacterial suspension through seed, root, foliar and soil application in glasshouse conditions, or as talc-based formulation under field conditions, compared to the respective individual strains (Nandakumar et al. 2001). Chitin amendment of soil may have effects in the rhizosphere, such as the stimulation of growth of chitinolytic microorganisms (Ahmed et al. 2003), their increased biocontrol activity and elicitation of plant defense proteins (Bharathi et al. 2004). All these effects may culminate in enhancing plant protection. Five plant growth promoting rhizobacterial formulations, each consisting of two bacilli strains with chitosan as a carrier were tested for their capacity to promote growth and induce resistance against downy mildew in pearl millet under both greenhouse and field conditions. Three modes of applications were tested: seed treatment, soil amendment, and seed treatment+soil amendment. In general, irrespective of application method, most of the formulations, in comparison with the control, increased plant growth and vigor as measured by seed germination, seedling vigour, plant height, fresh and dry weight, leaf area, tillering capacity, number of earheads, length and girth of earhead, 1000 seed weight and yield. The time of flowering was also advanced by 4-5 days over the control. Likewise all the formulations significantly reduced downy mildew incidence relative to the nontreated control. However, the rate of growth enhancement and disease suppression varied considerably with the formulations. Formulations LS256 and LS257 besides being the best growth promoters were also the most efficient resistance inducers. None of the formulations matched the level of the fungicide metalaxyl in offering protection against downy mildew. Among the application methods tested, soil amendment was found to be the most suitable and desirable way of delivering the formulations. Combination of seed treatment and soil amendment produced the same effect that was produced by soil amendment alone. This study by Raj et al. (2003) demonstrated a potential role for plant growth promoting rhizobacterial formulations in downy mildew management. Application of PGPR strains GB03 (*Bacillus subtilis*) and IN937a (*Bacillus amyloliquefaciens*) with the carrier chitosan to the tomato led to protection against cucumber mosaic virus (Murphy et al. 2003). Though there are several reports of PGPR strains amended with chitin against plant pathogens, there is only little information available about the induction of defence enzymes against virus diseases.

Further, the development of commercial formulations require newer molecules in order to enhance the survival and efficacy of the plant growth-promoting rhizobacterial (PGPR) strains. Sharathchandra et al. (2004) also reported that a bioformulation of *Bacillus* was able to induce plant growth promotion and induce resistance in pearl millet. Endospores of *B. megaterium* were formulated in granule formulations with sodium alginate, lactose and poly vinyl pyrrolidone (PVP K-30) by the wet granulation technique. The granule formulation exhibited good physical characteristics, such as high-water solubility and optimal viscosity that would be suitable for spray application. The bacteria remained viable in the dry granule formulation at 10^9 c.f.u./g after 24 months storage at room temperature. Under laboratory conditions, aqueous solutions of the formulation showed high activity against mycelial growth of R. solani (99.64 ± 0.14% mycelial inhibition). High viability of the bacterial antagonist on leaf sheath and leaf blade at day 7 after spraying with the formulation was observed (approximately 10^6 c.f.u./g of plant). Application of an equivalent number of un-formulated endospores resulted in much loss of the bacterial endospores even 1 day after application. A commercially developed aqueous Chitosan formulation Elexa was also used in different concentrations viz,1:5,1:10,1:15,1:19 and 1:25 as seed soaking treatment to pearl millet for 3,6 and 9 h duration to test for its effect of germination and vigor index. Among the treatments used 1: 19 for 6 h soaking recorded maximum germination and seedling vigor. Seed treatment, foliar spray and combination of seed treatment and foliar spray were tested for control of downy mildew diseases caused by *Scleropora garminicola* in pearl millet under greenhouse and field conditions. Metalaxyl at the rate of 2.1% a.i. in the form of Apron 35SD seed treatment was used as cheek. Under greenhouse conditions seed treatment offered 48% protection. Foliar spray was carried out to two, seven and 14-dy-old seedling and there was marked reduction in downy mildew severity to 42.5% and recorded 38% protection, whereas foliar spray to 7day-old seedling gave 67% protection and reduced severity to 25% and combination of seed treatment by 23%. The nature of disease control mechanisms has been investigated and the results indicated that it is due to induction of systemic resistance. The indication of resistance was observed as early as 24 h time gap between the inducer treatment and pathogen inoculation and the maximum resistance developed at 24-48 h time gap and maintained thereafter. Elexa treated to pearl millet seeds offered growth promoting effect under greenhouse conditions and recorded increase in plant, ear head length and seed weight. Hence, authors inferred that Elexa is a good downy mildew management commercial formulation and also exhibits growth-promoting effect in pearl millet.

Shelf life of formulation is an important factor which also differs according to the use of different bacterial inoculants as well as carrier materials. Different rhizobacterial formulations and their shelf life are given below (Tables 2 a & b).

Table 2a: Shelf life of different formulations in talc and lignite carrier materials

Carriers	Bacteria	Shelf life	References
Talc	Rhizobacteria	2 months	Kloepper and Schroth (1981)
Talc	*P. fluorescens* (P7NF, TL3)	12 months (8.4 Log cfu/g)	Caesar and Burr (1991)
Talc	*P. fluorescens*(Pf1)	8 months ($1.3x10^7$cfu/g)	Vidhyasekaran and Muthamilan (1995)
Talc	*B. subtilis*	45 days (1.0×10^6 cfu/g)	Amer andUtkhede (2000)
Talc	*P. putida*	45 days (1.0×10^3 cfu/g)	Amer and Utkhede (2000)
Talc	*P. putida* strain30 and 180	6 months (>1×10^8cfu/g)	Bora et al. (2004)
Lignite	*P. fluorescens* (Pf1)	4 months (2.8×10^6cfu/g)	Vidhyasekaran and Muthamilan (1995)

FIELD APPLICATION OF DIFFERENT BIOFORMULATIONS OF PGPR IN IMPROVING HEALTH STATUS OF CROP PLANTS AND SUPPRESSION OF DISEASES

The plant growth-promoting rhizobacteria Pseudomonas fluorescens (FP7) amended with chitin sprayed at fortnightly intervals gave the maximum induction of flowering, a yield attribute in the preharvest stage, consequently reduced latent symptoms were recorded at the postharvest stage. An enormous induction of the defence-mediating lytic enzymes chitinase and β-1,3-glucanase was recorded in colorimetric assay and the expression of discrete bands in native PAGE analysis after FP7 + chitin treatment. The enhanced expression of defence-mediating enzymes may collectively contribute to suppress the anthracnose pathogen, leading to improved yield attributes. In China, for instance, a liquid formulation has been extensively tested to control sheath blight disease in farm rice field conditions (Mew et al. 2004). Although this type of formulation can be produced in large quantity using a simple fermentation and formulation processes, it may be difficult to store and may have a relatively short shelf life. Talc-based bioformulations containing cells of *Pseudomonas fluorescens*, *Bacillus subtilis* and *Saccharomyces cerevisiae* were also evaluated for their potential to attack the mango (Mangifera indica L.) anthracnose pathogen *Colletotrichum gloeosporioides* Penz. under endemic conditions by Vivekananthan et al. (2004). Talc and peat based formulations of *P. chlororaphis* and *B. subtilis* were prepared and used for the management of turmeric rhizome rot (Nakkeeran et al. 2004). *Pseudomonas putida* strain 30 and 180 also survived up to 6 months in talc based formulations. The population load at the end of 6th month was 10^8 cfu/g of the product (Bora et al. 2004).

Table 2b: Shelf life of different formulations in peat and other carrier materials

Carriers	Bacteria	Shelf life	References
Peat	*P. fluorescens* (Pf1)	8 months (7.0×10^6cfu/g)	Vidhyasekaran and Muthamilan (1995)
Peat supplemented with chitin	*B. subtilis*	6 months (>1 x 10$^{?}$cfu/g)	Manjula and Podile(2001)
Peat	*P. chlororaphis* (PA23) and *B. subtilis* (CBE4)	6 months (>1 x 10^8cfu/g)	Nakkeeran *et al*.(2004)
Vermiculite	*P. fluorescens* (Pf1)	8 months (1.0×10^6cfu/g)	Vidhyasekaran and Muthamilan (1995)
Vermiculite	*B. subtilis*	45days (>1.0×10^6 cfu/g)	Amer and Utkhede(2000)
Vermiculite	*P. putida*	45days (>1.0×10^3 cfu/g)	Amer and Utkhede(2000)
Farm yard manure	*P. fluorescens* (Pf1)	8 months (1.0×10^6cfu/g)	Vidhyasekaran and Muthamilan (1995)
Kaolinite	*P. fluorescens* (Pf1)	4 months (2.8×10^6cfu/g)	Vidhyasekaran and Muthamilan (1995)

Carrier-based preparations of two plant growth-promoting rhizobacteria (PGPR) viz. *Bacillus subtilis* and *Pseudomonas corrugata*, developed in five formulations were also evaluated for their growth promotion, rhizosphere colonization, and viability under storage. The effect of these formulations as fresh preparations, and after 6 months of storage at 4ºC and room temperature, was also determined. The bacterial inoculants in all the formulations were found to enhance the growth parameters of the test plant species; best results were obtained in case of alginate-based formulations. Maximum numbers of inoculated bacteria were recovered from the rhizosphere of alginate-based formulation-treated plants after 6 weeks of growth. Viability of bacterial inoculants was maximal in alginate beads, and alginate

beads supplemented with skim milk formulations, after 180 days of storage at 4ºC (Trivedi et al. 2005). The bacterial populations that were recorded, initially lower in the case of alginate-based formulations, increased with time. In contrast to this, the maximum number of bacteria in the case of charcoal- and broth-based formulations was recovered after 7 days of inoculation declined after 14 and 21 days in case of both bacteria. Maximum colonization of rhizosphere (5.819 and 5.431 log10 c.f.u. g^{-1} of soil) was recorded in alginate-coated seeds by *B. subtilis* and in the alginate bead formulation by *P. corrugata*, respectively. Minimum colonization was recorded in case of the broth based formulation (3.125 and 3.410 log10 c.f.u. $g)^{-1}$ of soil) by *B. subtilis* and *P. corrugata*, respectively. The formulations of strain of *Bacillus subtilis* AUBS-1 inhibitory to the growth of damping-off pathogen, *Pythium aphanidermatum*, for seed treatment was provided by Jayraj et al. (2005). The formulation included the talc-based powder, lignite based powder, lignite and fiyash-based powder, wettable-powder, bentonite-paste, polyethylene glycol (PEG) paste and water dispersible tablet. Formulations were stored at room temperature for 2 years and frequently sampled to test their shelf life. Populations of bacteria in the formulations were stable for up to 2 years storage at room temperature 28^{o}C. Viability of propagules was significantly reduced in talc, wettable powder, and PEG paste and tablet formulations beyond 1 year of storage. Seed treatment of tomato with these formulations resulted in effective control of damping-off caused by *P. aphanidermatum* and also enhanced plant biomass under glasshouse and field conditions. Active rhizosphere colonization by the bacterium was observed on tomato plants grown from the seeds treated with above formulations. Fluorescent pseudomonads based bioformulation was evaluated for their ability to control *Macrophomina* root rot disease in mungbean (*Vigna mungo*). *P. fluorescens* isolate Pf1 showed the maximum inhibition in mycelial growth of *Macrophomina phaseolina* under in vitro conditions. Bioformulation of Pf1 with chitin was effective in reducing the root rot incidence in green gram both under glasshouse and field conditions. The rhizosphere colonization of *P. fluorescens* was observed appreciable with the green gram plants. However, Pf1 amended with chitin colonized effectively. Furthermore, the induction of defence-related enzymes and chemicals in plants by Pf1 amended with or without chitin and neem were tested. Increased accumulation of defence enzymes viz., phenyl alanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), chitinase, β-1,3-glucanse and phenolics were observed in Pf1 bioformulation amended with chitin, pre-treated plants challenge inoculated with *M. phaseolina* under glasshouse conditions. The present study revealed that in addition to direct antagonism and plant-growth promotion, PGPR strains amended with chitin bioformulation induced defence-related enzymes and pathogenesis related (PR) proteins which collectively enhance the resistance in green gram against the infection of *M. phaseolina* (Saravanakumar et al. 2005). Different formulations of Bacillus licheniformis were evaluated on their own and in combination with prochloraz and stroburilin for their ability to reduce mango post-harvest fruit diseases [anthracnose and stem-end rot (SR)] when applied as a dip treatment in a mango pack house. Untreated fruit and fruit treated with either prochloraz or stroburilin alone served as controls. In these trials treatments integrating chemical pesticides with *B. licheniformis* controlled anthracnose and SR as effectively as the chemical control. The antagonist was more effective especially in the control of post-harvest diseases when fruit were kept in cold storage to simulate export conditions. In two of the three trials, results obtained when fruit was treated with the antagonist in combination with the commercial chemical were comparable to

that obtained with the commercial chemical control. In a study by Govender and Korsten (2006), it was found that the antagonist when used in mango pack house treatments could provide an effective alternative to fungicides. Furthermore, the powder formulation of the antagonist can be successfully incorporated into the existing pack line. Previous reports are also available where *Bacillus* bioformulations could survive upto one year or more in several bioformulations. Plant growth-promoting rhizobacteria (PGPR) bioformulations (*Pseudomonas* and *Bacillus*) were also tested for their efficacy against blister blight (Exobasidium vexans) disease in tea (*Camellia sinensis*) under field conditions for two seasons. Among the bioformulations tested, foliar application of *Pseudomonas fluorescens* Pf1 at 7-d intervals consistently reduced the disease incidence of blister blight for two seasons, almost comparable with that of chemical fungicide. In addition to disease control, it also increased tea yield significantly compared to the untreated control. Induction of defense enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, chitinase, β-1,3-glucanase and phenolics were studied. The enzyme accumulation was greater in *P. fluorescens* Pf1-treated plants compared to control. The study revealed the probable influence of plant growth promotion and induced systemic resistance (ISR) in enhancing the disease resistance in tea plants against blister disease by PGPR bioformulations (Saravanakumar et al. 2005).

Pseudomonas fluorescens strains CHA0 and Pf1 were investigated for their biocontrol efficacy against Banana bunchy top virus (BBTV) in banana (Musa spp.) alone and in combination with chitin under glasshouse and field conditions. Bioformulation of *P. fluorescens* strain CHA0 with chitin was effective in reducing the banana bunchy top disease (BBTD) incidence in banana under glasshouse and field conditions. In addition to disease control, the bioformulation increased the economic yield significantly compared to the untreated control. Increased accumulation of oxidative enzymes, peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), pathogenesis-related (PR) proteins, chitinase, β-1,3-glucanase and phenolics were observed in CHA0 bioformulation amended with chitin-treated plants challenged with BBTV under glasshouse conditions. Indirect ELISA indicated the reduction in viral antigen concentration in P. fluorescens strain CHA0 with chitin-treated banana plants corresponding to reduced disease ratings. Induction of defence enzymes by *P. fluorescens* with chitin amendment reduced the BBTD incidence and increased bunch yield in banana (Kavino et al. 2008). According to Kim et al. (2008), *Phytophthora* blight of pepper caused by *Phytophthora capsici* has devastating consequences when combined with other pathogens, including *Rhizoctonia solani, Fusarium oxysporum*, and *Fusarium solani*. In order to develop a field-effective biocontrol strategy against *Phytophthora* blight of pepper, three chitinolytic bacteria, *Serratia plymuthica* strain C-1, strongly antagonistic to *P. capsici*, *Chromobacterium sp*. strain C-61, strongly antagonistic to *R. solani,* and *Lysobacter enzymogenes* strain C-3, antagonistic to *R.solani* and *Fusarium spp.*, were selected. In pot studies, application of cultures combining the three bacterial strains effectively suppressed *Phytophthora* blight more than application of any single bacterial strain. Bioformulations developed from growth of the strains in a simple medium containing chitin under large batch conditions resulted in effective control in field applications. Efficacy of the bioformulated product depended on both the dose and timing of application. In a small pilot field study, an aqueous solution of the formulation (3%w/v) applied by spraying at days 1, 5 and 10 after pathogen inoculation of the rice plants was more effective in suppressing rice sheath blight disease than one application of a fungicide (Iprodione) at day

1. Additionally, rice plants sprayed with the aqueous solution of the granule formulation had higher panicle and whole kernel weights than those of fungicide-treated and control (untreated) plants (Chumthong et al. 2008). *Pseudomonas fluorescens* Pf1 along with chitin amendment was effective for survival and colonization of bacteria under field conditions. The efficacy of the talc-based bioformulation of *Beauveria* (B2) strain was tested as seed treatment + seedling dip + soil application + foliar spray against rice leaffolder under in vitro and greenhouse conditions. The percentage damage was significantly less (5.5) in B2 as compared to untreated healthy control (25.8). In addition, the same treatment increased the activities of defenserelated enzymes, namely peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, chitinase, and phenolics in rice (Sivasundaram et al. 2008). Two plant growth-promoting bacteria, *Bacillus subtilis* and *Pseudomonas corrugata*, immobilized in a sodium alginate based formulation were evaluated for their survival, viability and plant growth-promoting ability after 3 years of storage at 4 °C. Populations of both of the bacterial isolates recovered from the immobilized sodium alginate beads were in the order of 10^8 cfu g^{-1}. The plant-based bioassay indicated that the plant growth promotion ability of both of the bacterial isolates was equal to those of fresh broth-based formulations. After 42 days, cfu g-1dry weight of root was 5.60 and 5.10 for alginate beads as compared to 3.24 and 3.31 for fresh broth formulations of *B. subtilis* and *P. corrugata*, respectively. The bacterial isolates retained the root colonization, and antifungal and enzyme activities in the alginate-based formulation during storage (Trivedi and Pandey 2008).

In a study by Chakraborty et al. (2009) talc based formulation of *Ochrobactrum anthropi* was prepared and its survival determined every month up to a period of 12 months. *O. anthropi* could survive in the formulation up to a period of 9 months with a concentration of 7.0 $\log_{10}$ CFU g^{-1}, after which there was a decline. Talc formulation was as effective as aqueous suspensions in both plant growth promotion and disease suppression. Chakraborty et al. (2010) also reported that *Serratia marcescens* (TRS 1), either as aqueous suspensions or in bioformulations of saw dust, rice husk and tea waste, promoted growth in tea saplings as evidenced by increase in height, emergence of new leaves and branches, as well as increase in leaf biomass. *Bacillus megaterium* (TRS 4) and *Serratia marcescens* (TRS 1) could survive in bioformulations of saw dust, rice husk and tea waste in the range of 6.1-8.0×$\log_{10}$ cfu/ml for more than 9 months *in vitro*.Saw dust, rice husk and tea waste bioformulations of these bacteria were found to enhance growth of tea varieties-TV-18, TV-23, TV-25, TV-26 and T-17 (Chakraborty et al. 2012). A greenhouse experiment by Atieno et al. (2012) was set to assess the formulation effect of one strain i.e. *Bradyrhizobium japonicum*, 532c (granules, liquid and broth) and also to determine the efficiency of coinoculation of *Bacillus* with two commercial strains of *B. japonicum* (532c and RCR 3407) on 2 soybean (*Glycine max* L.) varieties. PCR–RFLP analysis was used to determine the nodule occupancy in each treatment. Most of the inoculants showed increased nodulation and biomass yields (by approximately 2–5 and 4–10 g $plant^{-1}$ respectively) as compared to the uninoculated controls. TGx1740-2F showed no significant differences in nodule fresh weights for the formulation effect while the co-inoculants increased the nodule fresh weights by up to 4 g $plant^{-1}$. The liquid and granule-based inoculants induced higher biomass yields (4– 8 g $plant^{-1}$) suggesting a possible impact of formulation on the effectiveness of the inoculants. The coinoculants also gave higher yields but showing no significant differences to the rhizobial inoculants. Nodule occupancy was 100 % for the rhizobial inoculants as well as the co-inoculants emphasizing the

infectivity and high competitiveness of 532c and RCR 3407 strains despite the high population of indigenous rhizobia. In a study by Chakraborty et al. (2013), Bioformulations of *Bacillus amyloliquefaciens*, *Serratia marcescens* and *B. pumilus* in talc powder, saw dust and rice husk were prepared and their viability tested. The bacteria showed good survivability even up to 9 months of storage.

CONCLUSION

Application of plant growth promoting rhizobacteria (PGPR) into the soil, taking into account suitable carriers, shelf life and sustainability of the rhizobacteria in field have been focused in this review. It is difficult to predict the actual happening in the soil environment but probably the PGPR secrete metabolites into the soil which in turn elicit responses in the host. It is quite evident that initial information regarding their suitability, ability to survive for sufficiently long periods in the bioformulations as well as sustainability of the applied bacteria in soil has to be generated. Once this is done, commercial mix of PGPR in suitable formulations for various aims such as in improvement of crop yield or suppression of pests and disease may be used as an alternative where use of biological products to replace or supplement chemical use is the need of the hour.

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