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Development and evaluation of compost based PGPR consortium in gelatin capsules

Sangamesh Kayakad, Brahmaprakash GP and Ameer Pasha B

Abstract
An investigation was conducted to determine the effect of inoculation of microbial consortium by using capsule-based formulation and its comparison with vermicompost and talc powder. Survival study was conducted in laboratory conditions from the results it is evident that survival of the *Azotobacter chroococcum* and *Bacillus megaterium* both in single and dual inoculants of vermicompost and capsule-based formulations were declined gradually at the end of 180 days of storage. *Azotobacter chroococcum* in vermicompost based formulation recorded $\log_{10}$ 7.85 CFU/g at the beginning of storage. Later, gradually declined with an intermittent increase in the population. At the end of 180 days of storage *A. chroococcum* population declined from $\log_{10}$ 7.85 CFU/g to $\log_{10}$ 6.22 CFU/g. *Bacillus megaterium* population was maximum up to 90 days of storage. Later, the population was declined from $\log_{10}$ 7.93 CFU/g to $\log_{10}$ 5.97 CFU/g at the end of 180 days of storage. Survival of dual inoculants containing *Azotobacter chroococcum* + *Bacillus megaterium* in capsule based formulation was studied up to 180 days of storage. The population of *Azotobacter chroococcum* + *Bacillus megaterium* showed $\log_{10}$ 7.90, $\log_{10}$ 7.76 cells/g at the beginning, reduced to $\log_{10}$ 5.90, $\log_{10}$ 6.46 cells/g at the end of 180 days of storage. It is clearly evident from this investigation that gelatin capsules are the best alternate formulations to carrier-based inoculants and also from results found that consortial application of microbial inoculants was found more advantageous than in individual inoculation in terms of enhancing crop growth parameters.

Keywords: Consortium, gelatin, dual inoculants, capsule

Introduction
Biofertilizers are low cost, environment-friendly and economically viable technology which improve plant growth and development. They have several advantages, high-cost benefit ratio, enhance plant growth and yield by increasing soil fertility and nutrient availability, reduce the environmental pollution caused by chemical fertilizers and protect plants against many soilborne pathogens and also acts as an integral part of organic system by helping the plant to grow under stress condition (Sahu and Brahmaprakash, 2012) [6].

Biofertilizers play a vital role in improving soil fertility. They improve soil structure and texture, thereby enhancing plant growth, yield, and quality parameters when inoculated with biofertilizer consortial tablets containing nitrogen fixers (*Azotobacter chroococcum*), phosphate solubilizing bacteria (*Bacillus megaterium*) and other beneficial strains of bacteria in tomato crop (Nair and Brahmaprakash, 2017) [3].

Plant growth promoting rhizobacteria (PGPR) are the group of bacteria that enhance growth directly by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of plant growth regulators, production of siderophores and phytohormones. Some of bacteria support plant growth indirectly by boosting growth restricting conditions either via inducing host resistance towards plant pathogens or by production of antagonistic substances. Hence, biofertilizer has a potential role in agriculture.

Selection of carrier material is very important while preparing biofertilizers. Although, there are no clear cut criteria for the selection of carrier materials but some general characteristics should be present in the material which is used as a carrier for biofertilizer such as it should be cost-effective, contain non-toxic compounds and high organic content, easy to process, more than 50% water holding capacity, high buffering capacity, sticky in nature and available in bulk quantity. A variety of materials can be used as a carrier but there is need to find out the most suitable carrier which fulfils all the above stated properties. (Bazilah *et al*., 2011) [1].

The criteria for new materials as carriers for PGPR is the capability to support high population densities of the inoculant when incorporated into the soil.

The carrier material should not affect the activity of the introduced bacteria, for example by adsorbing signal compounds, antibiotics, and plant growth hormones that are excreted by the cells. Many PGPR activities which have been correlated to increase the total root length, branching, and root hair formation (Patten and Glick, 2002; Spaepen *et al*., 2008) [4, 8].

Material and Methods

Culture Collection and Maintenance

Plant Growth Promoting Microorganisms (PGPM) used for this study *Azotobacter chroococcum* (free-living dinitrogen fixer) and *Bacillus megaterium* (phosphate solubilizer) pure cultures were procured from Department of Agricultural Microbiology, GKVK, Bangalore-65. Loop full of inoculum is transferred aseptically into 250 ml conical flask containing 100 ml of Waksman No. 77 broth and Pikovskaya`s broth respectively and incubated on a rotary shaker up to 3 days. Slant cultures stored in the refrigerator which further served as mother culture for future studies.

Compatibility study

The standard pure cultures maintained in the Department of Agricultural Microbiology, UAS, GKVK, Bangalore were taken and used for compatibility study. The compatibility test of *Azotobacter chroococcum* and *Bacillus megaterium* were done as dual inoculant under in vitro condition.

Development of Capsule-Based Formulation

Treatment Detail

T1-Talc based formulation for *Bacillus megaterium*
T2-Talc based formulation for *Azotobacter chroococcum*
T3-Talc based formulation for combination of *Bacillus megaterium* and *Azotobacter chroococcum*
T4-Vermicompost based formulation for *Bacillus megaterium* in gelatin capsules
T5-Vermicompost based formulation for *Azotobacter chroococcum* in gelatin capsules
T6-Vermicompost based formulation for combination of Bacillus megaterium and *Azotobacter chroococcum* in gelatin capsules

Multiplication of Microorganisms

The beneficial microorganisms like *Azotobacter chroococcum* and *Bacillus megaterium* mass multiplication was done in Waksman No. 77 broth and Pikovskaya`s broth respectively. Further, both the beneficial microorganisms in the broth medium were kept for incubation under shaking condition for 5-6 days at 28±2 °C

Vermicompost and talc powder were used as a carrier material for the development of biofertilizers from *Azotobacter chroococcum* and *Bacillus megaterium*. Air dried vermicompost passed through a 2 mm mesh sieve then the vermicompost was mixed with CaSO4 at the rate of 20 g/100 g vermicompost to bring down pH from 7.58 to 7.00.

To study survival of microbial inoculants in capsules

Survivability study was done to evaluate the microbial population in different treatments. Survival study was carried up to 180 days of storage. Samples were drawn at 0, 15, 30, 60, 90, 120, 150 and 180 days intervals of storage at ambient temperature.

Survivability study was done by using standard plate count method used for enumeration of viable cells. One gram of sample was taken from each prepared consortium, serially diluted up to 10-5, 10-6 and 10-7 dilutions were plated on Petri plates.

About 20 ml of the autoclaved and cooled medium was poured into Petri plates which contains one ml of inoculum of respective dilutions. Then the plates were rotated clockwise and anticlockwise for uniform mixing of suspension into medium and allowed for solidification and incubated at room temperature at 28±2 °C. The colonies developed were counted at 3 days after incubation and the microbial population was calculated by using a formula as follows.

$$\text{Number of microorganisms per gram of sample (CFU/g)} = \frac{\text{Average number of colonies}}{\text{Weight of sample}} \times \text{dilution factor}$$

To study the efficacy of microbial consortia in capsules under greenhouse experiment

Effectiveness of developed inoculants was tested on finger millet crop (*Eleusine coracana* L. Gaertn.) in pot culture under greenhouse conditions. The experiment was carried out in the Department of Agricultural Microbiology UAS, GKVK, Bangalore. The replications were made on a random basis. The above treatments were tried with a Factorial CRD analysis by considering, with NPK and without NPK, RDF of finger millet crop. There were 48 experimental units from 2 levels of nutrients, 6 treatments, and 4 replications.

Specifications of Gelatin Capsules

Sl. No.	Properties of gelatin	capsules Content
1	Size	"0"
2	Colour	Orange/White
3	Moisture	8-13%
4	Relative density	1.3-1.4
5	Carbon	50.5%
6	Hydrogen	6.8%
7	Oxygen	25.2%
8	Nitrogen	17%

Source: Amazon.in (www.patcopharma.com)

Results and Discussion

Experimental results of the research work taken up on novel consortial formulations of agriculturally beneficial microorganisms are exhibited in this chapter. Laboratory experiments were conducted to determine the effect of capsule-based formulation on the survival of *Azotobacter chroococcum* and *Bacillus megaterium* in both single and dual inoculant formulations. Green house investigations were conducted to determine the effectiveness of these capsule based formulations on plant growth parameters of finger millet (*Eleusine coracana* L. Gaertn.) crop.

Compatibility Study

The compatibility between *Azotobacter chroococcum* and *Bacillus megaterium* were tested by plating these two microorganisms on nutrient agar medium. There was no antagonistic interaction between these organisms

The growth of *Azotobacter chroococcum* and *Bacillus megaterium* inoculants used in the study were compatible and found no suppression, indicated their synergistic activity. Similar results were reported before developing carrier-based formulation by (Shilpa and Brahmaprakash, 2016) [7].

Microbial analysis of vermicompost

Vermicompost collected from Zonal Agricultural Research Station (ZARS) GKVK, Bengaluru was examined for total microbial load by plating vermicompost on respective medium using standard dilution plate count technique.

The results showed that total bacterial population of 5.78 $\log_{10}$ CFU/g of vermicompost, followed by 3.32 $\log_{10}$ CFU/g of fungal population and 4.15 $\log_{10}$ CFU/ g of actinomycetes population was recorded (Table 1).

Physico-chemical properties of vermicompost

Physical and chemical properties of the vermicompost such as water holding capacity, bulk density, pH, EC, organic carbon, nitrogen, phosphorus, and potassium were estimated. The results are described below.

The vermicompost had water holding capacity of 123.5 %, pH 7.34, EC 2.13 dS/m, organic carbon content 29.12 %, nitrogen content 1.32 %, phosphorus content 0.537 % and potassium content 0.89 % respectively (Table 2).

Survival study

Survival of single inoculant

Survival of *Azotobacter chroococcum* and *Bacillus megaterium* was observed up to 180 days of storage in both single and dual inoculant combinations. Observations were recorded at 0, 15, 30, 60, 90, 120, 150 and 180 days of storage.

Survival of single microbial inoculants in the vermicompost-based formulation

Azotobacter chroococcum in vermicompost based formulation recorded $\log_{10}$ 7.85 CFU/g at the beginning of storage. Later, gradually declined with an intermittent increase in the population. At the end of 180 days of storage *A. chroococcum* population declined from $\log_{10}$ 7.85 CFU/g to $\log_{10}$ 6.22 CFU/g (Table 3). Per cent survival of 79.24 was recorded at the end of 180 days of storage (Table 4).

In vermicompost based formulation *Bacillus megaterium* population was maximum up to 90 days of storage. Later, the population was declined from $\log_{10}$ 7.93 CFU/g to $\log_{10}$ 5.97 CFU/g at the end of 180 days of storage (Table 3). per cent survival of 75.28 was recorded at the end of 180 days of storage (Table 4).

Survival of single microbial inoculants in the capsule-based formulation

The population of *Azotobacter chroococcum* in capsule-based formulation maintained at its maximum $\log_{10}$ 8.21 cells/g in the first 30 days of storage. After 30 days the population declined from $\log_{10}$ 8.21 cells/g to $\log_{10}$ 6.33 cells/g at the end of 180 days of storage (Table 5). Per cent survival of 77.57 was recorded at the end of 180 days of storage (Table 6).

Bacillus megaterium in capsule based formulation recorded $\log_{10}$ 7.92 cells/g at the beginning of storage. Later, gradually declined with an intermittent increase in the population. At the end of 180 days of storage *B. megaterium* population declined from $\log_{10}$ 7.92 cells/g to $\log_{10}$ 6.21 cells/g (Table 5). Per cent reduction of *B. megaterium* at the end of 180 days of storage found to be 78.40 (Table 6).

After analyzing the results obtained from survival study in all intervals of storage, the survival of single inoculants of both *A. chroococcum* and *B. megaterium* population found to be stable in capsule based formulation compared to that of vermicompost based formulation. This may be because of the presence of moisture content in the formulation and also the gelatin capsules acts as a protector for organisms to survive under storage condition. The same outcomes were obtained by the investigations of Nair and Brahmaprakash (2017) [3].

Survival of Dual Inoculants

Survival of dual microbial inoculants in the vermicompost-based formulation

Vermicompost based formulation containing *Azotobacter chroococcum* and *Bacillus megaterium* (dual) inoculants maintained a population of $\log_{10}$ 7.88, $\log_{10}$ 7.62 CFU/g in initial days. At the end of 180 days of storage population of cells will gradually reduce to $\log_{10}$ 5.77, $\log_{10}$ 6.97 CFU/g respectively (Table 3). 73.22 and 91.47 per cent cells on log values were recorded in *Azotobacter chroococcum* and *Bacillus megaterium* respectively at the end of 180 days of storage (table 4).

Survival of dual microbial inoculants in capsule-based formulation

Survival of dual inoculants containing *Azotobacter chroococcum* + *Bacillus megaterium* in capsule based formulation was studied up to 180 days of storage. The population of *Azotobacter chroococcum* + *Bacillus megaterium* showed $\log_{10}$ 7.90, $\log_{10}$ 7.76 cells/g at the beginning, reduced to $\log_{10}$ 5.90, $\log_{10}$ 6.46 cells/g at the end of 180 days of storage (Table 5). In dual inoculants survival of 74.83 and 83.24 per cent cells were noticed at the end of 180 days of storage for *A. chroococcum* and *B. megaterium* respectively (table 6).

In case of dual inoculants survival of *Azotobacter chroococcum* was found to be maximum in capsule based formulation as compared to vermicompost based formulation. Similarly, the survival of *Bacillus megaterium* was found to be maximum in vermicompost based formulation as compared to capsule based formulation in dual inoculant formulation. The results were confirmed the findings of Rajasekar *et al.* (2012), Lavanya (2014) [5, 2].

Table 1: Microbial population of vermicompost

Sl. No.	Organisms	Population (log10 CFU/g)
1	Bacteria	5.78
2	Fungi	3.32
3	Actinomycetes	4.15

Table 2: Physico-chemical properties of vermicompost.

Sl. No.	Properties	Content
1	Water holding capacity	123.5%
2	Bulk density	0.75 g/cc
3	pH	7.34
4	Electrical conductivity	2.13 dS/m
5	Organic carbon	29.12%
6	Total Nitrogen	1.32
7	Total Phosphorus	0.537
8	Total Potassium	0.89

Table 3: Survival of beneficial microbial population in vermicompost based bio-fertilizer formulation under ambient temperature

Inoculants	Microorganisms	Per cent population density							
		Duration of storage (days)							
		0	15	30	60	90	120	150	180
Inoculant 1	*Azotobacter chroococcum*	100^{a}	94.27^{cd}	101.15^{a}	91.46^{ef}	90.57^{cde}	87.26^{d}	87.64^{bc}	79.24^{de}
Inoculant 2	*Bacillus megaterium*	100^{a}	97.10^{ab}	92.43^{bc}	98.87^{a}	97.60^{a}	91.17^{bc}	85.25^{c}	75.28^{ef}
Inoculant 3	*Azotobacter chroococcum*	100^{a}	98.98^{a}	99.49^{a}	96.45^{bc}	96.45^{ab}	93.40^{bc}	82.23^{d}	73.22^{f}
	Bacillus megaterium	100^{a}	103.54^{a}	102.62^{a}	98.43^{c}	98.95^{ab}	96.98^{dab}	95.80^{a}	91.47^{ab}

Note: Means with the same superscript within similar days of storage do not differ significantly at P=0.05 with DMRT

Table 4: Per cent survival (based on log_{10}CFU) of beneficial microbial population in vermicompost based bio-fertilizer formulation under ambient temperature

Inoculants	Microorganisms	Population density (log_{10} CFU)							
		Duration of storage (days)							
		0	15	30	60	90	120	150	180
Inoculant 4	*Azotobacter chroococcum*	8.16^{a}	7.97^{a}	8.21^{a}	7.99^{a}	7.81^{a}	7.42^{a}	6.85^{b}	6.33^{ab}
Inoculant 5	*Bacillus megaterium*	7.92^{b}	7.96^{ab}	7.70^{bc}	7.68^{b}	7.97^{a}	7.59^{a}	7.06^{a}	6.21^{b}
Inoculant 6	*Azotobacter chroococcum*	7.90^{b}	7.80^{a}	7.84^{b}	7.77^{b}	7.41^{b}	7.14^{b}	6.54^{c}	5.90^{c}
	Bacillus megaterium	7.76^{b}	7.70^{b}	7.62^{c}	7.58^{b}	7.51^{b}	7.47^{a}	6.97^{ab}	6.46^{a}

Note: Means with the same superscript within similar days of storage do not differ significantly at P=0.05 with DMRT

Table 5: Survival of beneficial microbial population in capsule-based biofertilizer formulation under ambient temperature

Inoculants	Microorganisms	Per cent population density							
		Duration of storage (days)							
		0	15	30	60	90	120	150	180
Inoculant 4	*Azotobacter chroococcum*	100^{a}	97.67^{a}	100.61^{a}	97.91^{a}	95.71^{a}	90.93^{a}	83.94^{b}	77.57^{ab}
Inoculant 5	*Bacillus megaterium*	100^{b}	100.50^{ab}	94.36^{bc}	96.96^{b}	100.63^{a}	95.83^{a}	89.14^{a}	78.40^{b}
Inoculant 6	*Azotobacter chroococcum*	100^{b}	98.73^{a}	99.24^{b}	98.35^{b}	93.79^{b}	90.37^{b}	82.71^{c}	74.83^{c}
	Bacillus megaterium	100b	99.22^{b}	98.19^{c}	97.68^{b}	96.77^{b}	96.26^{a}	89.81^{ab}	83.24^{a}

Note: Means with the same superscript within similar days of storage do not differ significantly at P=0.05 with DMRT

Table 6: Percent survival (based on log_{10} CFU) of beneficial microbial population in capsule-based biofertilizer formulation under ambient temperature

Inoculants	Microorganisms	Population density (log_{10} CFU)							
		Duration of storage (days)							
		0	15	30	60	90	120	150	180
Inoculant 1	*Azotobacter chroococcum*	7.85^{ab}	7.40^{cd}	7.94^{a}	7.18^{ef}	7.11^{cde}	6.85^{d}	6.88^{bc}	6.22^{de}
Inoculant 2	*Bacillus megaterium*	7.93^{a}	7.70^{ab}	7.33^{bc}	7.84^{a}	7.74^{a}	7.23^{bc}	6.76^{c}	5.97^{ef}
Inoculant 3	*Azotobacter chroococcum*	7.88^{ab}	7.80^{a}	7.84^{a}	7.60^{bc}	7.60^{ab}	7.36^{bc}	6.48^{d}	5.77^{f}
	Bacillus megaterium	7.62^{b}	7.89^{a}	7.82^{a}	7.50^{c}	7.54^{ab}	7.39^{ab}	7.30^{a}	6.97^{ab}

Note: Means with the same superscript within similar days of storage do not differ significantly at P=0.05 with DMRT

Reference

1. Bazilah ABI, Sariah M, Abidin MAZ, Yasmeen S. Effect of carrier and temperature on the viability of *Burkholderia* sp. (UPMB3) and Pseudomonas sp. (UPMP3) during storage. Int. J Agric. Biol. 2011;13:198-202.
2. Lavanya G. Formulation and effectiveness of fluid bed dried consortia of agriculturally beneficial microorganisms. Ph.D Thesis, Univ. Agric. Sci. Bengaluru, India; c2014.
3. Nair SS, Brahmaprakash GP. Effect of effervescent biofertilizer consortial tablets on growth of tomato (*Lycopersicon esculentum* Mill.). Int. J Curr. Microbiol. App. Sci. 2017;6(9):615-623.
4. Patten CL, Glick BR. Role of Pseudomonas putida indole acetic acid in development of the host plant root system. Appl. Environ. Microbiol., 2002;68:3795-3801.
5. Rajasekar K, Daniel T, Karmegam N. Microbial enrichment of vermicompost. ISRN Soil Sci. 2012;54(2):946079.
6. Sahu PK, Brahmaprakash GP. Bio-fertilizers for sustainability., J. Ind. Inst. Sci. 2012;92:37-62.
7. Shilpa ME, Brahmaprakash GP. Evaluation of selected organic materials as carriers for agriculturally important microorganisms. Mysore J Agric. Sci. 2016;2:266-271.
8. Spaepen S, Dobbelaere S, Croonrnborghs A, Vanderleyden J. Effects of *Azospirillum brasilense* indole-3-acetic acid production on inoculated wheat plants. Plant. Soil. 2008;312:15-23.