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ORIGINAL RESEARCH ARTICLE

Survival of Plant Growth Promoting Bacterial Inoculants in Different Carrier Materials

D. Sangeetha* and D. Stella

Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram – 608 002, India

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ABSTRACT

In this present study, the survival of PGPR isolates was investigated by using different carrier materials. The carrier based PGPR consortium with four selected strains *viz*., *Azospirillum lipoferum* VAZS-18, *Azotobacter chroococcum* VAZB-6, *Bacillus megaterium* VBA-2, *Pseudomonas fluorescens* VPS-19 was prepared and the shelf life for each inoculants was studied upto six months of storage. The surviving population in the lignite based consortium was 1.64×10^8 cfu g^{-1} for *Azospirillum lipoferum* VAZS-18, 1.46×10^8 cfu g^{-1} for *Azotobacter chrococcum*, VAZB-6, 1.22×10^8 cfu g^{-1} for *Bacillus megaterium* VBA-2 and 2.01×10^8 cfu g^{-1} for *Pseudomonas fluorescens* VPS-19 after six month of storage. The surviving population in vermiculite based consortium was 4.32×10^8 cfu g^{-1} for *Azospirillum lipoferum* VAZS-18, 1.98×10^8 cfu g^{-1} for *Azotobacter chroococcum* VAZB-6, 1.14×10^8 cfu g^{-1} for *Bacillus megaterium* VBA-2 and 3.32×10^8 cfu g^{-1} for *Pseudomonas fluorescens* VPS-19 after six months of storage. In the pressmud based consortium, the surviving population was 3.25×10^8 cfu g^{-1} for *Azospirillum lipoferum* VAZS-18, 3.00×10^8 cfu g^{-1} for *Azotobacter chroococcum* VAZB-6, 2.14×10^8 cfu g^{-1} for *Bacillus megaterium* VBA-2 and 3.42×10^8 cfu g^{-1} for *Pseudomonas fluorescens* VPS-19 after six months of storage. In the alginate bead based consortium the surviving population was 64.61×10^8 cfu g^{-1} for *Azospirillum lipoferum* VAZS-18, 56.81×10^8 cfu g^{-1} for *Azotobacter chroococcum* VAZB-6, 47.83×10^8 cfu g^{-1} for *Bacillus megaterium* VBA-2 and 63.89×10^8 cfu g^{-1} for *Pseudomonas fluorescens* VPS-19 after six months of storage.

Key words: PGPR isolates, Lignite, Vermiculite, Pressmud and Alginate bead.

1. INTRODUCTION

Vetiveria zizanioides (L.) Nash (Poaceae), popularly known as khus grass, has been known in India since ancient times. It is the major source of the well-known oil of vetiver, which is used in medicine and in perfumery [1]. In India, the roots have been used for making screens, mats, hand fans, and baskets. Different morphological parts of the grass are used for various ailments, such as boils, burns, epilepsy, fever, scorpion sting, snake bite, and sores in the mouth. The root extract is used for headache and toothache, the leaf paste is used for lumbago, sprain, and rheumatism, the stem decoction for urinary tract infection, the leaf juice as an anthelmintic, the vapors for malarial fever, and the root ash is given for acidity relief [2,3].

Rhizospheric bacterial communities have efficient systems for uptake and catabolism of organic compounds present in root exudates [4]. Several bacteria have the ability to attach to the root surfaces (rhizoplane) making them to derive maximum benefit from root exudates. Few of them are more specialized, as they possess the ability to penetrate inside the root tissues (endophytes) and have direct access to organic compounds present in the apoplast. By occupying this privileged endophytic location, bacteria do not have to face competition from their counterparts as encountered in the rhizosphere or in soil.

Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. The rhizosphere concept was first introduced by Hiltner to describe the narrow zone of soil surrounding the roots

where microbe populations are stimulated by root activities. The original concept has now been extended to include the soil surrounding a root in which physical, chemical and biological properties have been changed by root growth and activity [5]. A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant

***Corresponding Author:** D. Sangeetha**, Email:** maran_5@rediffmail.com

among them. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates creating a very selective environment where diversity is low [6]. Since bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization [7].

Bacteria associated with plants can be either harmful or beneficial. PGPR may promote growth directly, by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solublize and sequester iron, or production of plant growth regulators, phytohormones [8]. Some bacteria support plant growth indirectly by improving growth restricting conditions either via production of antagonistic substances or by inducing host resistance towards plant pathogens. Since associative interactions of plant and microorganisms must have come into existence as a result of convolution; the use of either former or latter groups as bioinoculants forms one of the vital components for a long-term sustainable agriculture system [9]. In this present study, the survival of PGPR isolates was investigated by using different carrier materials.

2. MATERIALS AND METHODS

2.1. Preparation of carrier based inoculant

The selected isolates were multiplied in large quantities in appropriate culture broths by incubating at 28 ± 2°C in an incubator shaker till they attained log phase with a cell load of $l \times l0^9$ cfu ml^{-1} and were used for inoculant preparation. Lignite collected from Neyveli Lignite Corporation (NLC), Neyveli and Vermiculite collected from Tamil Nadu Minerals Ltd. Chennai and Pressmud collected from EID parry, Nellikuppam, Cuddalore were used as carriers. The individual carrier materials were powdered and the pH was brought to neutral by adding $CaCO_3$ if necessary and sterilized at 15 psi for 1 hour and allowed to cool over night and then mixed with the log phase culture ($l \times l0^9$ cfu ml^{-1}) of the selected plant growth promoting bacterial isolates *viz., Azospirillum lipoferum, Azotobacter chroococcum, Bacillus megaterium* and *Pseudomonas fluorescens* individually in separate quantities of sterile carrier in shallow trays. The moisture content was adjusted to 30-35 per cent. Curing in shallow trays for 24 hours in aseptic rooms and packed in high density opaque polythene bag (300 gauge) at the rate of 200 g bag^{-1} and sealed. Individual inoculant was prepared by mixing equal volumes of each culture broth with sterile carrier and combined inoculant was also prepared by mixing equal volumes of broth with the carrier materials. The populations of individual plant growth promoting rhizobacteria in the inoculant carriers were assessed at monthly intervals upto six months.

2.2. Preparation of alginate beaded inoculant

The *Azospirillum lipoferum, Azotobacter chroococcum, Bacillus megaterium* and *Pseudomonas fluorescens* were grown in respective medium to get a population of $l \times l0^9$ cfu ml^{-1}. Sodium alginate beaded inoculant was prepared as per the methods described by Hegde and Brahmaprakash (1992)[10]. Two gram of sodium alginate was added to 100 ml of culture broth of PGPR and mixed for 30 minutes in a magnetic stirrer. The mixture was added drop wise through a 10 ml syringe into 100 ml sterile 0.1N $CaCl_2$ to obtain uniform alginate beads. One gram of material contained 16 to 17 beads, each bead approximately weighing 60 mg. The beads were washed twice in sterile distilled water and incubated in respective broth containing PGPR isolates for seven days in a psychrotherm (model environ shaker) incubator at 28 ± 2°C to allow PGPR to multiply inside the beads. The beads were again washed in sterile distilled water and air dried in laminar air flow chamber under aseptic condition. The alginate beads were then stored in polythene bags at room temperature upto 6 months.

2.3. Determination of surviving population in the carrier based inoculant by serial dilution and plating technique

A quantity of 10 g of carrier based inoculant was suspended in 100 ml sterile water in 250 ml Erlenmeyer flask. After thorough agitation over a shaker for 10 min, one ml of the supernatant was aseptically transferred to 9 ml sterile water blank in a test tube. Dilution process was continued till 10^{-8} dilution was reached. From this dilution, 1.0 ml aliquots were withdrawn and transferred to sterile petriplates. Appropriate medium for different groups of PGPR was prepared and sterilized in an autoclave at 15 psi for 20 min. The medium was cooled and poured in petriplates and rotate the plats in clockwise and anticlockwise directions for even spreading and allowed for solidification. The plates were incubated in inverted position at room temperature. The developed bacterial colonies on the plates were counted and the population was determined and expressed as cfu g^{-1} of carrier material on oven dry basis.

2.4. Determination of surviving population in the alginate beaded inoculant

To study the survival of bacterial population in the alginate beaded inoculant containing plant growth promoting rhizobacteria (PGPR) as individual inoculant and as consortium of inoculants. The beads were immersed in potassium phosphate buffer of 0.2 M (pH 6.8 ± 0.1), at the rate of one bead per ml of buffer in a test tube for 1 to 2 hours in a rotary shaker at 100 g at 30°C which released the bacteria entrapped in (or) covering the microbeads. The released bacteria were counted using the conventional plate count method on Nutrient agar [11].

2.5. Influence of storage temperature on the survival of the inoculants as consortium in different carrier materials

The carrier based microbial inoculants prepared with different carrier material was kept in different temperature levels *viz.,* 20, 25, 30, 35 and 40°C after one month incubation period. The surviving populations of PGPB at different temperatures were determined and population was enumerated by Dilution plate technique after one month of incubation period.

3. RESULTS AND DISCUSSION

3.1. Survival of plant growth promoting rhizobacteria as consortium in lignite carrier

The survival of plant growth promoting rhizobacterial strains *viz*., *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Bacillus megaterium* and *Pseudomonas fluorescens* in consortium as well as single inoculants prepared in lignite carrier materials was assessed upto six months storage (**Table 1**). The initial population PGPR strains in consortium 73.21 × 10^8 cfu g^{-1} for *Azospirillum*, 59.33 × 10^8 cfu g^{-1} for *Azotobacter*, 52.20 × 10^8 cfu g^{-1} for *Bacillus* and 71.32 ×10^8 cfu g^{-1} for *Pseudomonas*. While the corresponding population in the carrier material prepared with single inoculant strains were 74.43 × 10^8 cfu g^{-1} for *Azospirillum*, 60.33 × 10^8 cfu g^{-1} for *Azotobacter*, 53.00 × 10^8 cfu g^{-1} for *Bacillus* and 72.13 ×10^8 cfu g^{-1} for *Pseudomonas*.

The surviving population after one month of storage was 75.87 × 10^8 cfu g^{-1} for *Azospirillum* 63.84 × 10^8 cfu g^{-1} for *Azotobacter*, 56.23 × 10^8 cfu g^{-1} for *Bacillus* and 74.00 × 10^8 cfu g^{-1} for *Pseudomonas* as single inoculant. The surviving population in consortium packet was 74.18 × 10^8 cfu g^{-1} for *Azospirillum*, 63.00 × 10^8 cfu g^{-1} for *Azotobacter*, 56.23 × 10^8 cfu g^{-1} for *Bacillus* and 74.00 × 10^8 cfu g^{-1} for *Pseudomonas* after one month storage in lignite carrier material. The surviving population of PGPR strain in consortium and single inoculant preparations after one month storage was on par. The inoculant populations increased in both consortium and in single inoculants packet during the first month of storage and there after reduced on 2nd month sampling onwards and on further storage the cell populations of different inoculants gradually decreased upto six months of storage. The shelf life of PGPR strains was satisfactory upto six months in both single inoculant and consortium of inoculant packets.

Table 1: Survival of plant growth promoting rhizobacteria (PGPR) as consortium in lignite carrier

Storage Period in month	Number of cfu x $10^8 g^{-1}$ of lignite							
	Azospirillum in single inoculant	*Azospirillum* inconsortium	*Azotobacter* in single inoculant	*Azotobacter* in consortium	*Bacillus* in single inoculant	*Bacillus* in consortium	*Pseudomonas* in single inoculant	*Pseudomonas* in consortium
0	74.43	73.21	60.33	59.33	53.00	52.20	72.13	71.32
1	75.87	74.18	63.84	63.00	56.23	55.21	74.00	73.00
2	64.98	63.88	50.20	49.21	46.33	46.00	61.23	61.44
3	23.64	23.00	16.54	15.54	12.36	11.36	21.55	20.35
4	17.31	17.00	11.02	9.03	7.56	6.58	15.00	14.21
5	5.30	5.21	3.32	2.42	4.46	3.23	11.00	10.01
6	2.64	1.64	2.46	1.46	2.00	1.22	2.33	2.01

Table 2: Survival of plant growth promoting rhizobacteria (PGPR) as consortium in Vermiculite carrier

Storage period in months	Number of Cfu × 10^8 g^{-1} Vermiculite							
	Azospirillum in single inoculant	*Azospirillum* in consortium	*Azotobacter* in single inoculant	*Azotobacter* in consortium	*Bacillus* in single inoculant	*Bacillus* in consortium	*Pseudomonas* in single inoculant	*Pseudomonas* in consortium
0	73.66	71.61	64.23	63.21	56.61	55.00	72.11	71.12
1	77.00	76.21	66.15	65.14	58.21	57.02	75.23	74.14
2	66.66	65.42	54.66	53.66	48.12	47.13	61.23	60.23
3	27.66	26.17	21.00	20.12	16.36	15.35	27.62	26.52
4	19.33	18.33	13.02	12.01	11.54	9.63	19.65	17.23
5	10.66	9.65	4.62	3.42	3.22	2.33	9.32	8.11
6	5.33	4.32	3.00	1.98	2.16	1.14	5.43	3.32

Table 3: Survival of plant growth promoting rhizobacteria (PGPR) as consortium in pressmud carrier

Storage period in months	Number of Cfu × 10^8 g^{-1} pressmud							
	Azospirillum in single inoculant	*Azospirillum* in consortium	*Azotobacter* in single inoculant	*Azotobacter* in consortium	*Bacillus* in single inoculant	*Bacillus* in consortium	*Pseudomonas* in single inoculant	*Pseudomonas* in consortium
0	73.64	72.64	63.34	62.32	55.83	54.73	74.63	73.14
1	77.21	76.11	66.82	65.81	58.23	57.26	76.63	75.62
2	69.34	68.32	53.65	52.63	49.63	48.34	66.82	65.81
3	27.89	26.42	21.32	20.23	14.32	15.23	24.21	23.12
4	19.84	18.53	12.02	11.01	9.00	8.31	18.46	17.43
5	11.43	8.42	5.00	4.21	4.23	3.98	9.32	8.31
6	5.42	3.25	3.42	3.00	3.13	2.14	3.93	3.42

Table 4: Survival of plant growth promoting rhizobacteria (PGPR) as consortium in Alginate beads

Storage period in months	Number of Cfu × 10^8 g^{-1} Alginate beads							
	Azospirillum in single inoculant	*Azospirillum* in consortium	*Azotobacter* in single inoculant	*Azotobacter* in consortium	*Bacillus* in single inoculant	*Bacillus* in consortium	*Pseudomonas* in single inoculant	*Pseudomonas* in consortium
0	75.32	74.00	62.64	60.64	56.31	55.31	74.34	73.41
1	78.61	77.62	63.23	62.21	57.54	56.32	75.83	74.38
2	76.04	75.12	62.03	61.11	55.24	54.13	73.23	72.23
3	71.01	70.13	60.40	60.00	53.04	51.24	71.42	70.42
4	69.64	68.53	59.34	58.32	52.38	50.31	68.36	67.38
5	67.54	66.12	58.01	57.23	53.00	52.92	67.23	66.21
6	65.61	64.61	57.61	56.81	49.82	47.83	64.92	63.89

Table 5: Influence of storage temperature on the survival of plant growth promoting rhizobacteria (PGPR) inoculant as consortium in Lignite carrier

Storage temperature	Inoculant populations (Cfu × 10^8 g^{-1} Lignite)							
	Azospirillum in single inoculant	*Azospirillum* in consortium	*Azotobacter* in single inoculant	*Azotobacter* in consortium	*Bacillus* in single inoculant	*Bacillus* in consortium	*Pseudomonas* in single inoculant	*Pseudomonas* in consortium
25°C	75.03	73.14	63.14	62.21	53.12	52.13	73.14	71.66
30°C	76.21	75.18	64.66	63.06	55.66	54.01	74.83	72.65
35°C	65.14	63.14	57.48	53.47	48.21	47.12	63.64	61.32
40°C	46.17	45.24	40.23	39.17	28.64	27.64	40.24	38.14

Table 6: Influence of storage temperature on the survival of plant growth promoting rhizobacteria (PGPR) inoculant in Vermiculite carrier

Storage temperature	Inoculant populations (Cfu × 10^8 g^{-1} Vermiculite carrier)							
	Azospirillum in single inoculant	*Azospirillum* in consortium	*Azotobacter* in single inoculant	*Azotobacter* in consortium	*Bacillus* in single inoculant	*Bacillus* in consortium	*Pseudomonas* in single inoculant	*Pseudomonas* in consortium
25°C	73.15	72.11	63.14	62.11	55.42	54.00	74.33	72.33
30°C	75.13	73.00	62.37	61.00	56.31	55.31	76.00	73.12
35°C	70.01	68.13	55.48	54.46	49.47	47.00	65.21	64.21
40°C	50.46	40.32	42.78	40.73	38.12	36.12	50.01	48.00

Table 7: Influence of storage temperature on the survival of plant growth promoting rhizobacteria (PGPR) inoculant as Consortium in pressmud carrier

Storage temperature	Inoculant populations (Cfu × 10^8 g^{-1} pressmud)							
	Azospirillum in single inoculant	*Azospirillum* in consortium	*Azotobacter* in single inoculant	*Azotobacter* in consortium	*Bacillus* in single inoculant	*Bacillus* in consortium	*Pseudomonas* in single inoculant	*Pseudomonas* in consortium
25°C	73.06	71.04	61.24	59.21	54.41	52.21	72.36	70.22
30°C	72.64	70.01	63.82	61.00	53.82	53.00	73.58	71.52
35°C	62.21	60.21	53.69	51.64	47.81	47.22	63.28	61.23
40°C	43.13	41.00	38.39	36.32	28.25	26.31	34.13	32.42

Table 8: Influence of storage temperature on the survival of plant growth promoting rhizobacteria (PGPR) inoculant as Consortium in Alginate beads

Storage temperature	Inoculant population (Cfu × 10^8 g^{-1} Alginate beads)							
	Azospirillum in single inoculant	*Azospirillum* in consortium	*Azotobacter* in single inoculant	*Azotobacter* in consortium	*Bacillus* in single inoculant	*Bacillus* in consortium	*Pseudomonas* in single inoculant	*Pseudomonas* in consortium
25°C	75.13	74.65	64.13	63.18	57.28	55.11	75.41	73.45
30°C	76.21	75.00	65.56	64.33	58.11	56.22	76.67	74.11
35°C	72.28	71.54	60.25	56.42	50.21	51.23	71.12	70.23
40°C	56.31	52.12	44.00	42.21	40.11	38.02	55.36	54.21

3.2. Survival of plant growth promoting rhizobacteria as consortium in vermiculite carrier

Vermiculite supported the required population levels of PGPR strains upto six months (**Table 2**). The shelf life of PGPR strains was satisfactory upto six months in both single and consortium packets. The initial population in vermiculite carrier prepared as single inoculant was 73.66 × 10^8 cfu g^{-1}, 64.23 × 10^8 cfu g^{-1}, 56.61 × 10^8 cfu g^{-1} and 72.11 × 10^8 cfu g^{-1} for *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas* respectively.

While the corresponding populations of *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas* in vermiculite carrier prepared as consortium were 71.61×10^8 cfu g^{-1}, 63.21×10^8 cfu g^{-1}, 55.00×10^8 cfu g^{-1} and 71.12×10^8 cfg^{-1} respectively.

The surviving population was 77.00×10^8 cfu g^{-1} for *Azospirillum*, 66.15×10^8 cfu g^{-1} for *Azotobacter*, 58.21×10^8 cfu g^{-1} for *Bacillus* and 75.23×10^8 cfu g^{-1} for *Pseudomonas* in single inoculant preparation after one month of storage. The surviving populations were 76.21×10^8 cfu g^{-1} for *Azospirillum*, 65.14×10^8 cfu g^{-1} for *Azotobacter*, 57.02×10^8 cfu g^{-1} for *Bacillus* and 74.14×10^8 cfu g^{-1} for *Pseudomonas* is consortium inoculant preparation after one month of storage. The PGPR populations recorded in consortium and single inoculant preparations after one month of storage was on par. The inoculant populations increased in both consortium and single inoculant preparations during the first month of storage and there after reduced with increase in storage period upto six months. However the required inoculant cell load of each group was maintained upto six months in both consortium and in single inoculant preparations.

3.3. Survival of plant growth promoting rhizobacteria as consortium in pressmud carrier

The initial populations of *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas* were 72.64×10^8 cfu g^{-1}, 62.32×10^8 cfu g^{-1}, 54.73×10^8 cfu g^{-1} and 73.14×10^8 cfu g^{-1} respectively in the inoculant consortium. While the corresponding population in pressmud carrier material in single inoculant preparations was 73.64×10^8 cfu g^{-1} for *Azospirillum* 63.34×10^8 cfu g^{-1} for *Azotobacter*, 55.83×10^8 cfu g^{-1} for *Bacillus* and 74.63×10^8 cfu g^{-1} for *Pseudomonas* respectively (**Table 3**). The surviving populations were 77.21×10^8 cfu g^{-1} for *Azospirillum*, 66.82×10^8 cfu g^{-1} for *Azotobacter*, 58.23×10^8 cfu g^{-1} for *Bacillus* and 76.63×10^8 cfu g^{-1} for *Pseudomonas* in single inoculant preparation after one month of storage. The surviving populations were 76.11×10^8 cfu g^{-1} for *Azospirillum*, 65.81×10^8 cfu g^{-1} for *Azotobacter* 57.26×10^8 cfu g^{-1} for *Bacillus* and 75.62×10^8 cfu g^{-1} for *Pseudomonas* in consortium inoculant.

The inoculant population increased in both consortium and as single inoculant packets during the first month of storage and thereafter reduced with increased in the period of storage upto six months. However, the required inoculant cell load was maintained upto six months after preparation. The difference in population load recorded between single inoculant preparation and consortium preparation was on par in pressmud carrier.

3.4. Survival of plant growth promoting rhizobacteria as consortium in alginate bead

The initial population of *Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas* in alginate based on consortium were 74.00×10^8 cfu g^{-1} 60.64×10^8 cfu g^{-1}, 55.31×10^8 cfu g^{-1} and 73.41×10^8 cfu g^{-1} respectively, while the corresponding population in alginate bead in single inoculant alginate beads were 75.32×10^8 cfu g^{-1} for *Azospirillum*, 62.64×10^8 cfu g^{-1} for *Azotobacter*, 56.31×10^8 cfu g^{-1} for *Bacillus*, 74.34×10^8 cfu g^{-1} for *Pseudomonas* respectively. The surviving populations were 78.61×10^8 cfu g^{-1} for *Azospirillum*, 63.23×10^8 cfu g^{-1} for *Azotobacter*, 57.54×10^8 cfu g^{-1} for *Bacillus*, 75.83×10^8 cfu g^{-1} for *Pseudomonas* in single inoculant preparation after one month storage. The surviving populations were 77.62×10^8 cfu g^{-1} for *Azospirillum*, 62.21×10^8 cfu g^{-1} for *Azotobacter*, 56.32×10^8 cfu g^{-1} for *Bacillus* and 74.38×10^8 cfu g^{-1} for *Pseudomonas* in consortium after one month storage.

Alginate beads supported the required population levels of PGPR isolate upto six months (**Table 4**). The inoculant survivability in different carrier materials considerably varied based on the physiochemical characteristics. In the present study, the survivability was the best in alginate beads followed by lignite, vermiculite and pressmud. The inoculant population increased in both consortium and as single inoculant packets during the first month of storage and thereafter reduced with increase in the period of storage upto six months. However the required inoculant cell load was maintained upto six months after preparation in alginate bead.

3.5. Influence of storage temperature on the survival of PGPR strains in different carrier materials prepared as single inoculant and as consortium

The inoculants of PGPR were prepared using different carrier materials like lignite, vermiculite, pressmud and in alginate as bead. They were stored at four different temperatures of 25°C, 30°C, 35°C and 40°C and the surviving population of PGPR strains per gram of carrier material was estimated after one month of packaging.

3.5.1. Influence of storage temperature on the survival of PGPR inoculant as consortium in lignite carrier

The inoculant populations of PGPR strains in lignite at 25°C storage after one month of packaging in single inoculant and in consortium were respectively 75.03 × 10^8 cfu g^{-1} and 73.14 × 10^8 cfu g^{-1} for *Azospirillum*, 63.14 × 10^8 cfu g^{-1} and 62.21 × 10^8 cfu g^{-1} for *Azotobacter*, 53.12 × 10^8 cfu g^{-1} and 52.13 × 10^8 cfu g^{-1} for *Bacillus* and 73.14 × 10^8 cfu g^{-1} and 71.66 × 10^8 cfu g^{-1} for *Pseudomonas*. The inoculant populations of PGPR strains in lignite at 30°C storage after one month of packaging in single inoculant and in consortium were respectively 76.21 × 10^8 and 75.18 × 10^8 cfu g^{-1} for *Azospirillum*, 64.66 × 10^8 and 63.06 × 10^8 cfu g^{-1} for *Azotobacter*, 55.66 × 10^8 and 54.01 × 10^8 cfu g^{-1} for *Bacillus* and 74.83 × 10^8 and 72.65 × 10^8 cfu g^{-1} for *Pseudomonas*. The inoculant population survived between storage temperatures of 25 and 30°C were on par (**Table 5**).

At the storage temperature of 35°C, the surviving population in lignite was less than that of 30°C. In single inoculant and consortium preparations the PGPR estimated were respectively 65.14 × 10^8 and 63.14 × cfu g^{-1} for *Azospirillum*, 57.48 × 10^8 and 53.47 × 10^8 cfu g^{-1} for *Azotobacter*, 48.21 × 10^8 and 47.12 × 10^8 cfu g^{-1} for *Bacillus* and 63.64 × 10^8 and 61.32 × 10^8 cfu g^{-1} *Pseudomonas*. The surviving population of PGPR strains in lignite prepared with both single inoculation as well as consortium significantly reduced at 40°C. the corresponding population after one month were respectively 46.17 × 10^8 and 45.24 × 10^8 cfu g^{-1} for *Azospirillum*, 40.23 × 10^8 cfu g^{-1} and 39.17 × 10^8 cfu g^{-1} for *Azotobacter*, 28.64 × 10^8 and 27.64 × 10^8 cfu g^{-1} for *Bacillus* and 40.24 × 10^8 and 38.14 × 10^8 cfu g^{-1} for *Pseudomonas*.

3.5.2. Influence of storage temperature on the survival of PGPR inoculant in vermiculite carrier

The inoculant populations of PGPR strains in vermiculite at 25°C storage after one month of packaging in single inoculant and in consortium were respectively 73.15 × 10^8 and 72.11 × 10^8 cfu g^{-1} for *Azospirillum*, 63.14 × 10^8 and 62.11 × 10^8 cfu g^{-1} for *Azotobacter*, 55.42 × 10^8 and 54.00 × 10^8 cfu g^{-1} for *Bacillus* sand 74.33 × 10^8 and 72.33 × 10^8 cfu g^{-1} for *Pseudomonas* (**Table 6**). The inoculant populations of PGPR strains in vermiculite at 30°C storage after one month of incubation period in single inoculant and in consortium were 75.13 × 10^8 and 73.00 × 10^8 cfu g^{-1} for *Azospirillum*, 62.37 × 10^8 and 61.00 × 10^8 cfu g^{-1} for *Azotobacter*, 56.31 × 10^8 and 55.31 × 10^8 cfu g^{-1} for *Bacillus* and 76.00 × 10^8 and 73.12 × 10^8 cfu g^{-1} for *Pseudomonas*.

At the storage temperature of 35°C, the surviving population in vermiculite was less than that recorded at 30°C. In single inoculant and consortium preparations of PGPR estimated were respectively 70.01 × 10^8 and 68.13 × 10^8 cfu g^{-1} for *Azospirillum*, 55.48 × 10^8 and 54.46 × 10^8 cfu g^{-1} for *Azotobacter*, 49.47 × 10^8 and 47.00 × 10^8 cfu g^{-1} for *Bacillus* and 65.21 × 10^8 and 64.21 × 10^8 cfu g^{-1} for *Pseudomonas*. The surviving population of PGPR strains in vermiculite prepared with single inoculant as well as consortium significantly reduced at 40°C. The populations were 50.46 × 10^8 and 40.32 × 10^8 cfu g^{-1} for *Azospirillum*, 42.78 × 10^8 and 40.73 × 10^8 cfu g^{-1} for *Azospirillum*, 42.78 × 10^8 and 40.73 × 10^8 cfu g^{-1} for *Azotobacter*, 38.12 × 10^8 and 36.12 × 10^8 cfu g^{-1} for *Bacillus* and 50.01 × 10^8 and 48.00 × 10^8 cfu g^{-1} for *Pseudomonas*.

3.5.3. Influence of storage temperature on the survival of PGPR strains in the pressmud carrier.

The inoculant populations of PGPR strains in pressmud at 25°C storage after one month of packaging in single inoculant and in consortium were respectively 73.06 × 10^8 and 71.04 × 10^8 cfu g^{-1} for *Azospirillum*, 61.24 × 10^8 and 59.21 × 10^8 cfu g^{-1} for *Azotobacter*, 54.41 × 10^8 and 52.21 × 10^8 cfu g^{-1} f for *Bacillus* and 72.36 × 10^8 and 70.22 × 10^8 cfu g^{-1} f for *Pseudomonas* (**Table 7**). The inoculant populations of PGPR strains in pressmud at 30°C storage after one month of packaging in single inoculant and in consortium were respectively 72.64 × 10^8 and 70.01 × 10^8 cfu g^{-1} for *Azospirillum*, 63.82 × 10^8 and 61.00 × 10^8 cfu g^{-1} for *Azotobacter*, 53.82 × 10^8 and 53.00 × 10^8 cfu g^{-1} for *Bacillus* and 73.58 × 10^8 and 71.52 × 10^8 cfu g^{-1} for *Pseudomonas*. The inoculant population survived between the storage temperatures of 25°C and 30°C were on par.

At the storage temperature at 35°C, the surviving population in pressmud was less than that recorded at 30°C. In single inoculant and consortium preparations, the PGPR estimated were 62.21 × 10^8 and 60.21 × 10^8 cfu g^{-1} for *Azospirillum*, 53.69 × 10^8 and 51.68 × 10^8 cfu g^{-1} for *Azotobacter*, 47.81 × 10^8 and 47.22 × 10^8 cfu g^{-1} for *Bacillus* and 63.28 × 10^8 and 61.23 × 10^8 cfu g^{-1} for *Pseudomonas*. The surviving population of PGPR strains in pressmud prepared with single inoculant as well as consortium significantly reduced at 40°C. The populations were 43.13 × 10^8 and 41.00 × 10^8 cfu g^{-1} for

Azospirillum, 38.39 × 10^8 and 36.32 × 10^8 cfu g^{-1} for *Azotobacter*, 28.25 × 10^8 and 26.31 × 10^8 cfu g^{-1} for *Bacillus* and 34.13 × 10^8 and 32.42 × 10^8 cfu g^{-1} for *Pseudomonas*.

3.5.4. Influence of storage temperature on the survival of PGPR strains in alginate bead

The inoculant populations of PGPR strains in alginate bead at 25°C storage after on month of packaging in single inoculant and in consortium preparations were 75.13 × 10^8 and 74.65 × 10^8 cfu g^{-1} for *Azospirillum*, 64.13 × 10^8 and 63.18 × 10^8 cfu g^{-1} for *Azotobacter*. 57.28 × 10^8 and 55.11 × 10^8 cfu g^{-1} for *Bacillus* and 75.41 × 10^8 and 73.45 × 10^8 cfu g^{-1} for *Pseudomonas* (**Table 8**). The inoculant populations of PGPR strains in alginate beads at 30°C storage after one month was 76.21 × 10^8 cfu g^{-1} for *Azospirillum*, 65.56 × 10^8 cfu g^{-1} for *Azotobacter*, 58.11 × 10^8 cfu g^{-1} for *Bacillus* and 76.67 × 10^8 cfu g^{-1} for *Pseudomonas* in single inoculant preparation while the survival of different inoculants in the consortium preparations at 30°C were 75.00 × 10^8 cfu g^{-1} for *Azospirillum*, 56.42 × 10^8 cfu g^{-1} for *Azotobacter*, 56.22 × 10^8 cfu g^{-1} for *Bacillus* and 74.11 × 10^8 cfu g^{-1} for *Pseudomonas*. The inoculant population recorded between 25 to 30°C were on par.

At the storage temperature of 35°C, the surviving population in alginate bead was less than that recorded at 30°C. IA single inoculant and consortium preparations the PGPR strains were estimated respectively 72.28 × 10^8 and 71.54 × 10^8 cfu g^{-1} for *Azospirillum*, 60.25 × 10^8 cfu g^{-1} 56.42 × 10^8 cfu g^{-1} for *Azotobacter*, 50.21 × 10^8 and 51.23 × 10^8 cfu g^{-1} for *Bacillus* and 71.12 × 10^8 and 70.23 × 10^8 cfu g^{-1} for *Pseudomonas*. The surviving population of PGPR in alginate beads prepared with single inoculant as consortium not considerably reduced at 40°C. The populations were respectively 56.31 × 10^8 and 52.12 × 10^8 cfu g^{-1} for *Azospirillum*, 44.00 × 10^8 and 42.21 × 10^8 cfu g^{-1} for *Azotobacter*, 40.11 × 10^8 and 38.02 × 10^8 cfu g^{-1} for *Bacillus* and 55.36 × 10^8 and 54.21 × 10^8 cfu g^{-1} for *Pseudomonas*.

4. DISCUSSION

PGPR are indigenous to soil and the plant rhizosphere and play a major role in the biocontrol of plant pathogens. They can suppress a broad spectrum of bacterial, fungal and nematode diseases. PGPR can also provide protection against viral diseases. The use of PGPR has become a common practice in many regions of the world. Although significant control of plant pathogens has been demonstrated by PGPR in laboratory and greenhouse studies, results in the field have been inconsistent. Recent progress in our understanding of their diversity, colonizing ability, and mechanism of action, formulation and application should facilitate their development as reliable biocontrol agents against plant pathogens. Some of these rhizobacteria may also be used in integrated pest management programmes. Greater application of PGPR is possible in agriculture for biocontrol of plant pathogens and biofertilization [12].

The carrier based inoculants of bacterial biofertilizer consortium have got several advantages such as increased shelf life, protection from adverse conditions, better survival on seed, etc. The carrier based individual inoculant effect on several crop plants has been studied [13]. The physico-chemical characters of carrier materials have got profound influence on the survival of inoculants. The ideal characteristics of an inoculant carrier include more surface area, rich in organic matter, high water holding capacity, neutral pH, easy availability and in expensiveness [14].

Carrier based biofertilizers consortium using the best strains from each of the genus *viz.*, *A. lipoferum* VAZS-18, *A. chroococcum* VZB-6, *B. megaterium* VBA-2, and *P. fluorescens* VPS-19 was prepared. The biofertilizer consortium and the respective single inoculants were prepared using lignite, vermiculite and pressmud as carrier and beaded inoculum using sodium alginate and tested their suitability for medicinal crops. To healthy inoculation practice, besides production of efficient strain of inoculants, it is equally important to maintain the viability of microbial cells at a satisfactory level for a longer period of storage. Higher survival of inoculants strains in consortium as well as single inoculant preparations was noted with alginate beads. The level of contamination as well as reduction in cell density were comparatively less in alginate and vermiculite probably due to protection offered to inoculants against competitiveness and to withstand stress conditions. The population level in alginate beaded inoculum was not significantly reduced upto 180 days.

The surviving population per gram of alginate bead prepared with PGPR consortium was 64.61 × 10^8 of *Azospirillum*, 56.81 × 10^8 of *Azotobacter*, 47.83 × 10^8 of *Bacillus* and 63.89 × 10^8 of *Pseudomonas* in consortium inoculant and it was found to be the best carrier material and shelf life of 6 months was achieved followed by vermiculite, the population of which was 4.32 × 10^8 for *Azospirillum*, 1.98 × 10^8 for *Azotobacter*,

1.14×10^8 for *Bacillus* and 3.32×10^8 for *Pseudomonas* in consortium. The vermiculite is the next best ideal carrier for biofertilizer consortium and can be successfully employed for large scale preparation of commercial inoculants. The lignite as well as pressmud also maintained the required populations significantly well in single inoculant preparations. However, the comparative survivals in consortium in these two carriers were less than that observed with vermiculite carrier. However, significant variation in the inoculant population level was noted in alginate bead followed by vermiculite, lignite and pressmud.

Lakshmi Priya (1997)[15] observed no antagonistic effect between *A. lipoferum* and *B. megaterium* that were found to be compatible with each other and hence suggested their use in combined inoculation for crop production. Similarly, *Azospirillum, Bacillus, Pseudomonas* were found to be compatible both under *in vitro* and *in vivo* conditions [16]. In the present study, *Azospirillum, Azotobacter, Bacillus* and *Pseudomonas* and hence used as consortium of inoculant for crop production.

The factors that affect the longevity of the cells of bio-inoculants include temperature, moisture, carrier material etc. The optimum moisture level of 35 to 50 per cent and a temperature of 30°C is required for maximum survival of the cells in the carrier based inoculants for longer period of storage and was found that the upto 40°C there was no serious mortality [17]. The influence of storage temperatures on the survival of rhizobia depends on the purity of the culture and the moisture loss during storage [18]. The effect of storage temperature on the survival of PGPR strains in consortium as well as in single inoculant preparations was studied at four temperature levels of 25°C, 30°C, 35°C and 40°C and the surviving population of each inoculant after one month of incubation period in different carrier materials was determined. The temperature effect on the surviving population in the carrier depends on the initial load and the moisture loss of the carrier materials. Storage temperature effect although significant between carriers materials, a general trend was the increase in temperature reduced inoculant population except in alginate beads. From this finding, it is recommended that alginate beaded inoculum could be effectively used under stress condition. The increase in temperature influenced the moisture level and brought down the population level.

The results of the present study indicated that the storage temperature of 25°C as well as 30°C was optimum for the survival of inoculants in consortium and single inoculants preparation of different carrier materials. The surviving inoculant population at 25°C and 30°C was on par. The population level of 75.00×10^8 for *Azospirillum*, 64.33×10^8 for *Azotobacter*, 56.22×10^8 for *Bacillus* and 74.11×10^8 for *Pseudomonas* was maintained in consortium preparation at 30°C storage temperature in alginate bead. The storage temperature of 40°C recorded the least surviving population of PGPR strains in both consortium and single inoculant preparations of the different carriers. The reduction at 35° C and 40°C was proportionally increasing with concomitant reduction in shelf life of both single inoculant and consortium of inoculant preparations.

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