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Screening of Carrier Materials for Improved Shelflife of Microbial Consortia

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ABSTRACT

Screening of different carrier materials for the improved shelf life of microbial consortia was carried under invitro conditions. Two different microbial consortia (MC_1 and MC_2) were screened against different out carrier materials like peat, lignite, vermiculite and charcoal powder. Peat, lignite and vermiculite supported highest viability of KRB in microbial consortium 1 and PSB in microbial consortium 2. Charcoal powder showed highest viability of PSB in MC_1 and KRB in MC_2. *Azospirillium*, PSB, KRB, ZnSB and PGPR isolate of MC_1 survived better in peat (T_1) compared to lignite (T_2) followed by vermiculite (T_3) and charcoal powder (T_4). While, *Azotobacter*, PSB, KRB, ZnSB and PGPR isolate of MC_2 survived better in lignite (T_6) compared to peat (T_5) followed by vermiculite (T_7) and charcoal powder (T_8). While *Azospirillium* survived well in lignite (T_6) compared to peat (T_5) followed by charcoal powder (T_8) and vermiculite (T_7). Peat and lignite performed better compared to other carrier materials.

Key words*: Microbial consortia, Carrier materials, Peat, Lignite, Vermiculite, Charcoal powder*

Introduction

Plants need several macro and micronutrients for their growth and development. Now-a-days extensive use of chemical fertilizers is the practice to supply these nutrients. In order to reduce the usage of chemical fertilizers, people are exploiting various microbes to supplement the nutrient requirement of the crops. There are several microbial inoculants formulations which supply those nutrients. But individual application of each of the bio inoculants would be expensive and is not practical. Hence, development of consortia with consistency under field conditions and long shelf life could pave the way for successful commercialization of the microbial inoculant technology. Earlier research work has been carried out on the effect of N_2 fixers, P solubilizers as single and/or consortia on several crops. Simultaneous inoculation with different plant growth promoting rhizobacteria (PGPR) often resulted in increased growth and yield as compared to single inoculation through improved nutrient uptake (Amal raj *et al*. 2015).

Mixed inoculants (combinations of microorganisms) that interact synergistically yield better and show quick results. Microbial consortium for growth promotion has been suggested and the development of plant growth promoting consortium, could be a feasible strategy for increased activity and better viability of plant growth promoting rhizobacteria (PGPR). When these strains are made into consortium, each of the constituent strains of the consortium not only competes with others for rhizospheric establishment but also complement functionally for plant growth promotion (Pandey and Maheshwari, 2007).

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The shelf life of bacteria varies depending on the bacterial genera, carriers and their particle size. Carriers with smaller particle size had increased surface area, which increase resistance to desiccation of bacteria by increased coverage of bacterial cells (Dandurand *et al.,* 1994).

Material and Methods

Shelf life study was conducted with different carrier materials inorder to know the suitable carrier material for the development of carrier based microbial consortia.

Carrier Materials Used in the Study

Different carrier materials like peat, lignite, vermiculite, charcoal powder were selected and screened against developed efficient microbial consortia for improved shelf life. Peat was collected from Nilgiri hills, Coimbatore, Tamil Nadu. Lignite was collected from Bio fertilizers unit, ARS, Amaravthi, ANGRAU, Guntur. Vermiculite was collected from Althosiff Minerals Company, Guduru, Nellore district. Charcoal powder was collected from Quality Traders, Guntur.

Physico-Chemical Properties of Carrier materials

For the preparation of bioformulation, the collected carrier materials such as peat, lignite, vermiculite and charcoal powder were tested for their moisture content and pH (Table: 1).

Table 1. Physico-chemical properties of carrier materials

Parameters	Peat	Lignite	Vermiculite	Charcoal Powder
pH	5.4	6.1	5.5	7.5
Moisture (%)	45	49	41	42
Bulk Density (%)	1.02	1.06	0.63	0.50

Preparation of carrier based bio-formulations

Microbial consortium-1 (MC_1) (*Azospirillium* + PSB + KRB + PGPR Isolate) and Microbial consortium - 2 (MC_2) (*Azotobacter* + *Azospirillium* +PSB + KRB+ PGPR Isolate) were selected as microbial consortia in the study .The selected isolates were multiplied in large quantities in appropriate culture broth by incubating at 28 ± 2 °C in an incubator shaker till they attained log phase with a cell load of 1×10^9 CFU ml^{-1} and were used for microbial consortia preparation. The individual carrier materials were powdered and the pH was brought to neutral by adding $CaCO_3$ after that sterilized at 15 psi for three successive days and allowed to cool over night and then mixed with the log phase culture (1×10^9cfu ml^{-1}) of the selected plant growth promoting microbial consortia in separate quantities in shallow trays. The optimum moisture content was adjusted to (30-40%) prior to preparation, followed by curing in shallow trays for 24 hours in aseptic conditions and then packed in high density opaque polythene bag (12g) at the rate of 500g bag^{-1} and sealed. Individual microbial consortia was then prepared by mixing with peat, lignite, vermiculite and charcoal powder in 1:3 volumes of each culture broth of microbial consortia with sterile carrier materials. The populations of individual Plant Growth Promoting Rhizobacteria in the inoculant carriers were assessed at monthly intervals upto 6 months.

Treatments

- T_1 : Peat + Microbial consortium 1
- T_2 : Lignite + Microbial consortium 1
- T_3 : Vermiculite + Microbial consortium 1
- T_4 : Charcoal powder + Microbial consortium 1
- T_5 : Peat + Microbial consortium 2
- T_6 : Lignite + Microbial consortium 2
- T_7 : Vermiculite + Microbial consortium 2
- T_8 : Charcoal powder + Microbial consortium 2

Results and Discussion

Peat, lignite, vermiculite and charcoal powder were used as carrier materials to assess the viability of microbial consortia over a period of six months in the incubator at 25^{0}C. The microbial consortia used in the study were Microbial consortium-1 (MC_1) (*Azospirillium* + PSB + KRB + PGPR Isolate) and Microbial consortium -2 (MC_2) (*Azotobacter* + *Azospirillium* +PSB + KRB+ PGPR Isolate).

Viability of *Azotobacter* in different carrier materials

In MC_2, 8.56 Log CFU ml^{-1} of *Azotobacter* cells were present while it was mixed with the sterilized carrier materials. After mixing on zero day the population of *Azotobacter* was 8.54 Log CFU g^{-1} carrier.

There was a significant increase in the population of *Azotobacter* upto the end of 2nd month (60th day) and it was 8.98 Log CFU g^{-1} carrier in T_5 (Peat + MC_2), 8.99 Log CFU g^{-1} carrier in T_6 (Lignite + MC_2), 8.79 Log CFU g^{-1} carrier in T_7 (Vermiculite + MC_2)

and 8.83 Log CFU g^{-1} carrier in T_8 (Charcoal powder + MC_2). At the end of storage on 180th day the *Azotobacter* population in T_5 (Peat + MC_2) was 6.64 Log CFU g^{-1} carrier, in T_6 (Lignite + MC_2) was 6.81 Log CFU g^{-1} carrier, in T_7 (Vermiculite + MC_2) was 6.50 Log CFU g^{-1} carrier and in T_8 (Charcoal powder + MC_2) was 6.05 Log CFU g^{-1} carrier (Figure1).

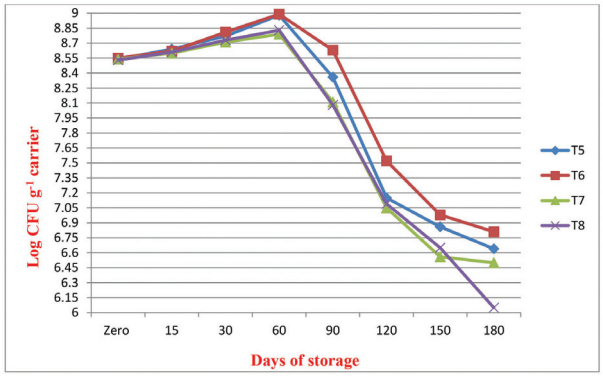

Fig. 1. Shelf life of *Azotobacter* in carrier materials at different intervals of storage

Azotobacter of microbial consortia-2 was significantly better viable in lignite (T_6) followed by peat (T_5), vermiculite (T_7) and charcoal powder (T_8) (Figure: 1). Feng *et al*. (2002) reported the viability of bacteria in peat carrier material which maintained a high number of 10^8 cells g^{-1} carrier even after 85 days. This study was on par with the present results where the PGPR population of microbial consortia showed more than 10^8 cells g^{-1} even after 90 days. The increase in the viable counts in carrier materials like peat, lignite, vermiculite and charcoal powder may be attributed to high carbon content, other sufficient nutrients, moisture and temperature during storage period. The results were in line with those of Bashan (1986).

Viability of *Azospirillum* in different carrier materials

After mixing on zero day *Azospirillium* count was 8.51 Log CFU g^{-1} carrier. There was a significant increase in viability upto the end of 2nd month (60th day) and it was 9.36 Log CFU g^{-1} carrier in T_1 (Peat + MC_1) 9.34 Log CFU g^{-1} carrier in T_5 (Peat + MC_2), in T_2 (Lignite + MC_1) it was 9.05 Log CFU g^{-1} carrier, in T_6 (Lignite + MC_2) it was 9.34 Log CFU g^{-1} carrier, in T_3 (Vermiculite + MC_1) it was 9.03 Log CFU g^{-1} carrier, T_7 (Vermiculite + MC_2) it was 9.04 Log CFU g^{-1} carrier, in T_4 (Charcoal powder + MC_1) and it was 9.08 Log CFU g^{-1} carrier and in T_8 (Charcoal powder + MC_2) it was 9.04 Log CFU g^{-1} carrier.

At the end of storage period in peat (180 days) *Azospirillium* of MC_1 (6.62 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_2 (6.51 Log CFU g^{-1} carrier), in lignite (180 days) *Azospirillium* of MC_2 (6.71 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_1 (6.31 Log CFU g^{-1} carrier), in vermiculite *Azospirillium* of MC_1 (6.04 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_2 (6.00 Log CFU g^{-1} carrier) and in charcoal powder (180 days) *Azospirillium* of MC_2 (6.15 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_1 6.00 Log CFU g^{-1} carrier) (Figure 2).

Carrier material provides a medium on which microorganisms multiply to a reasonably high number with long shelf life. Carriers increase the survival rate of bacteria by protecting it from dessication and death of cells (Heijnen *et al*., 1993). This investigation was similar to the present results where the PGPR organisms of both M.C multiplied to high number compared to the zero day and showed good shelf life. Previously various workers also observed good survival percentage of some bacterial strains using vermiculite as a carrier similar to our studies; however, continued existence of PGP bacterial consortia in carrier seems to be a novelty as we found out in our results (Sarma *et al*. 2011). *Azospirillium* in microbial consortia-1 was significantly better viable in peat (T_1) followed by lignite (T_2), vermiculite (T_3)

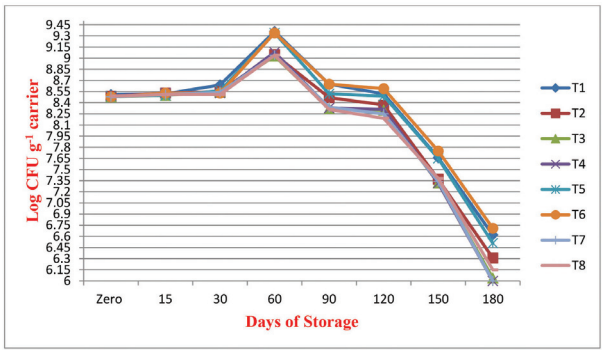

Fig. 2. Shelf life of *Azospirillium* in carrier materials at different intervals of storage

T_1: Peat + MC_1 T_5: Peat + MC_2
T_2: Lignite + MC_1 T_6: Lignite + MC_2
T_3: Vermiculite + MC_1 T_7: Vermiculite + MC_2
T_4: Charcoal powder+ MC_1 T_8: Charcoal powder + MC_2
MC_1 : Microbial consortium 1: *Azospirillium* + PSB + KRB + ZnSB + PGPR isolate
MC_2 : Microbial consortium 2: *Azotobacter* + *Azospirillium* + PSB + KRB + ZnSB + PGPR isolate

and charcoal powder (T_4). *Azospirillium* in microbial consortia-2 was significantly better viable in lignite (T_6) followed by peat (T_5), charcoal powder (T_8) and vermiculite (T_7) (Figure 2).

Viability of phosphate solubilizing bacteria (PSB) in different carrier materials

After mixing on zero day the population of PSB was 8.51 Log CFU g^{-1} carrier. There was a significant increase in the population of PSB upto the end of 2nd month (60th day) in T_1 (Peat + MC_1) it was 9.39 Log CFU g^{-1} carrier, in T_5 (Peat + MC_2) 9.25 Log CFU g^{-1} carrier, in T_2 (Lignite + MC_1), 9.26 Log CFU g^{-1} carrier, in T_6 (Lignite + MC_2) 9.32 Log CFU g^{-1} carrier, in T_3 (Vermiculite + MC_1) 9.18 Log CFU g^{-1} carrier, in T_7 (Vermiculite + MC_2) 9.08 Log CFU g^{-1} carrier, in T_4 (Charcoal powder + MC_1) 9.08 Log CFU g^{-1} carrier and in T_8 (Charcoal powder + MC_2) and it was 9.04 Log CFU g^{-1} carrier.

At the end of storage period in peat (180 days) PSB of MC_1 (6.78 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_2 (6.74 Log CFU g^{-1} carrier), in lignite PSB of MC_2 (6.89 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_1 (6.33 Log CFU g^{-1} carrier), in vermiculite (180 days) PSB of MC_2 (6.52 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_1 (6.17 Log CFU g^{-1} carrier) and in charcoal powder (180 days) PSB of MC_1 (6.15 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_2 (6.05 Log CFU g^{-1} carrier) (Figure 3).

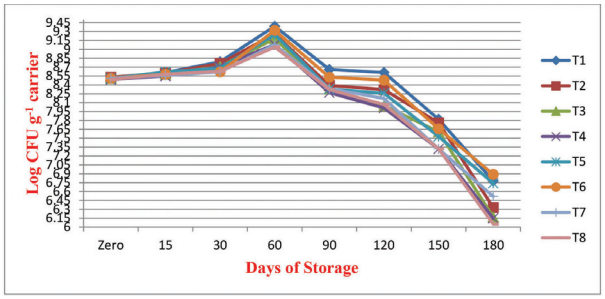

Fig. 3. Shelf life of Phosphorus Solubilizing Bacteria in carrier materials at different intervals of storage

PSB in microbial consortia-1 was significantly better viable in peat (T_1) followed by lignite (T_2), vermiculite (T_3) and charcoal powder (T_4). PSB in microbial consortia-2 was significantly better viable in lignite (T_6) followed by peat (T_5), vermiculite (T_7) and charcoal powder (T_8) (Figure 3). Mahdi *et al*. (2010) stated the suitability of carrier materials for the development of powder based microbial inoculants were in the order of peat > lignite > charcoal> rice husk. The present results of the study were on par with this investigation where *Azospirillum*, PSB, KRB, ZnSB and PGPR isolates population of MC_1 was comparitively better viable in peat in comparision with lignite, vermiculite and charcoal powder.

Viability of potassium releasing bacteria (KRB) in different carrier materials

After mixing on zero day the population of KRB was 8.31 Log CFU g^{-1} carrier. There was a significant increase in the population of PSB upto the end of 2nd month (60th day) in T_1 (Peat + MC_1) 9.40 Log CFU g^{-1} carrier, in T_5 (Peat + MC_2) 9.15 Log CFU g^{-1} carrier, in T_2 (Lignite + MC_1) 9.04 Log CFU g^{-1} carrier, in T_6 (Lignite + MC_2) 9.32 Log CFU g^{-1} carrier, in T_3 (Vermiculite + MC_1) Log 9.07 Log CFU g^{-1} carrier, in T_7 (Vermiculite + MC_2) 9.32 Log CFU g^{-1} carrier, in T_4 (Charcoal powder + MC_1) 9.10 Log CFU g^{-1} carrier and in T_8 (Charcoal powder + MC_2) 9.07 Log CFU g^{-1} carrier.

At the end of storage period in peat (180 days) KRB of MC_1 (6.82 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_2 (6.61 Log CFU g^{-1} carrier), in lignite (180 days) KRB of MC_2 (6.85 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_1 (6.70 Log CFU g^{-1} carrier), in vermiculite (180 days) KRB of M.C -2 (6.49 Log CFU g^{-1} carrier) showed comparitively better viability than that of M.C-1 (6.32 Log CFU g^{-1} carrier) and in charcoal powder (180 days) KRB of MC_2 (6.34 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_1 (6.01 Log CFU g^{-1} carrier) (Figure 4).

Jain *et al*. (2010) stated that carrier materials may enhance the survival of inocula by providing microorganisms with a protective environment, which allow them to survive in unfavourable conditions during the preservation and soil colonization process. In particular, once the microbe was introduced into soil, it must be able to survive in the subsurface zone to effectively solubilize the nutrients independently of the ecological conditions. This study was on par with the present results where different carrier materials have improved microbial inoculants growth and survival.

KRB in microbial consortia-1 was significantly better viable in peat (T_1) followed by lignite (T_2), vermiculite (T_3) and charcoal powder (T_4). While

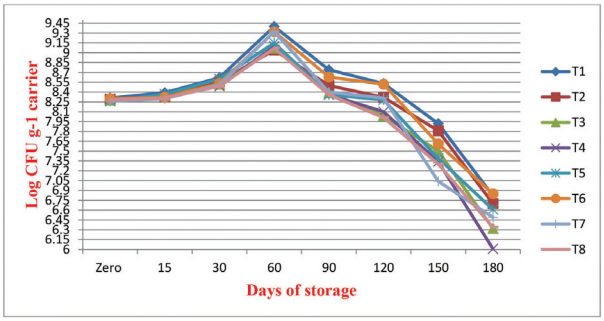

Fig. 4. Shelf life of Potassium Releasing Bacteria in carrier materials at different intervals of storage

T_1: Peat + MC_1 T_5: Peat + MC_2
T_2: Lignite + MC_1 T_6: Lignite + MC_2
T_3:Vermiculite + MC_1 T_7: Vermiculite + MC_2
T_4:Charcoal powder+ MC_1 T_8: Charcoal powder + MC_2
MC_1: Microbial consortium 1: *Azospirillium* + PSB + KRB + ZnSB + PGPR isolate
MC_2: Microbial consortium 2: *Azotobacter* + *Azospirillium* + PSB + KRB + ZnSB + PGPR isolate

KRB in microbial consortia-2 was significantly better viable in lignite (T_6) followed by peat (T_5), vermiculite (T_7) and charcoal powder (T_8) (Figure 4).

Viability of Zinc solubilizing bacteria (ZnSB) in different carrier materials

After mixing on zero day the population of ZnSB was 8.37 Log CFU g^{-1} carrier. There was a significant increase in viability upto the end of 2nd month (60th day) in T_1 (Peat + MC_1) 8.96 Log CFU g^{-1} carrier, in T_5 (Peat + MC_2) 8.93 Log CFU g^{-1} carrier, in T_2 (Lignite + MC_1) 8.92 Log CFU g^{-1} carrier, in T_6 (Lignite + MC_2) 8.94 Log CFU g^{-1} carrier, in T_3 (Vermiculite + MC_1) 8.75 Log CFU g^{-1} carrier, in T_7 (Vermiculite + MC_2) 8.70 Log CFU g^{-1} carrier, in T_4 (Charcoal powder + MC_1) 8.70 Log CFU g^{-1} carrier and in T_8 (Charcoal powder + MC_2) 8.72 Log CFU g^{-1} carrier.

At the end of storage period in peat (180 days) ZnSB of MC_1 (5.85 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_2 (5.64 Log CFU g^{-1} carrier), in lignite (180 days) ZnSB of MC_2 (5.75 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_1 (5.50 Log CFU g^{-1} carrier), in vermiculite (180 days) ZnSB of MC_1 (5.34 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_2 (5.30 Log CFU g^{-1} carrier) and in charcoal powder (180 days) ZnSB of MC_1 (5.04 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_2 (5.03 Log CFU g^{-1} carrier) (Figure:5).

Roughley (1968) reported that effect of storage on growth and survival of PGPR organisms was influenced by both the purity of the culture and the amount of moisture lost during storage. If cultures were prepared in sterilized carriers, incubation at 26°C immediately after inoculation promotes initial rapid growth of organisms and had little or no effect on long term survival if moisture content is maintained. This study was on par with the present study where the incubation temperature was 25 to 26°C immediately after inoculation in sterilized carriers.

ZnSB population of microbial consortia-1 was significantly better viable in peat (T_1) followed by lignite (T_2), vermiculite (T_3) and charcoal powder (T_4). While, ZnSB population of microbial consortia-2 was significantly better viable in lignite (T_6) followed by peat (T_5), vermiculite (T_7) and charcoal powder (T_8) (Figure 5).

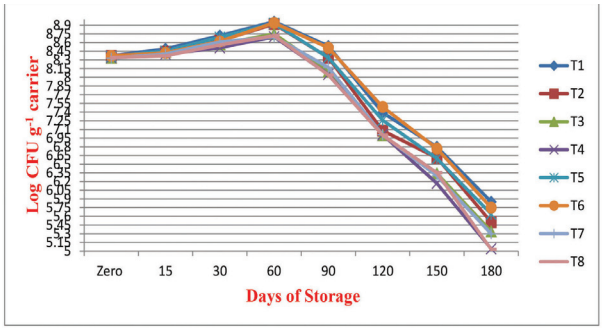

Fig. 5. Shelf life of Zinc Solubilizing Bacteria in carrier materials at different intervals of storage

Viability of PGPR isolate in different carrier materials

After mixing on zero day the population of PGPR isolate was 8.37 Log CFU g^{-1} carrier. There was a significant increase in the population of PGPR isolate upto the end of 2nd month (60th day) in T_1 (Peat + MC_1) 9.32 Log CFU g^{-1} carrier, in T_5 (Peat + MC_2) 9.31 Log CFU g^{-1} carrier, in T_2 (Lignite + MC_1) 9.30 Log CFU g^{-1} carrier, in T_6 (Lignite + MC_2) 9.32 Log CFU g^{-1} carrier, in T_3 (Vermiculite + MC_1) 9.16 Log CFU g^{-1} carrier, in T_7 (Vermiculite + MC_2) 9.08 Log CFU g^{-1} carrier, in T_4 (Charcoal powder + MC_1) 9.20 Log CFU g^{-1} carrier and in T_8 (Charcoal powder + MC_2) 9.17 Log CFU g^{-1} carrier.

At the end of storage period in peat (180 days) PGPR isolate of MC_1 (5.57 Log CFU g^{-1}) showed comparitively better viability than that of MC_2 (5.30 Log CFU g^{-1}), in lignite (180 days) PGPR isolate of MC_2 (5.60 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_1 (5.01

Log CFU g^{-1} carrier), in vermiculite (180 days) PGPR isolate of MC_2 (5.00 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_1 (4.99 Log CFU g^{-1} carrier), in charcoal powder (180 days) PGPR isolate of MC_2 (4.93 Log CFU g^{-1} carrier) showed comparitively better viability than that of MC_1 (4.85 Log CFU g^{-1} carrier) (Figure 6). PGPR isolate of microbial consortia-1 was significantly better viable in peat (T_1) followed by lignite (T_2), vermiculite (T_3) and charcoal powder (T_4). While, PGPR isolate of microbial consortia-2 was significantly better viable in lignite (T_6) followed by peat (T_5), vermiculite (T_7) and charcoal powder (T_8) (Figure 6). The decrease in viable counts of *fluorescent Pseudomonads* from 37.5 X 10^7 CFU g^{-1} carrier to 1.5 x 10^7 CFU g^{-1} carrier after 8 months has also been noted by Vidyasekaran and Muthaamilan (1995).

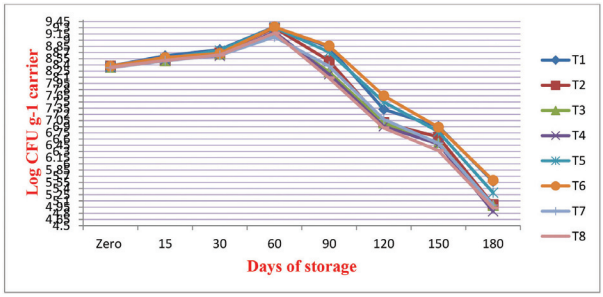

Fig. 6. Shelf life of PGPR Isolate in carrier materials at different intervals of storage

T_1: Peat + MC_1 T_5: Peat + MC_2
T_2: Lignite + MC_1 T_6: Lignite + MC_2
T_3:Vermiculite + MC_1 T_7: Vermiculite + MC_2
T_4:Charcoal powder+ MC_1 T_8: Charcoal powder + MC_2
MC_1 : Microbial consortium 1: *Azospirillium* + PSB + KRB + ZnSB + PGPR isolate
MC_2 : Microbial consortium 2: *Azotobacter* + *Azospirillium* + PSB + KRB + ZnSB + PGPR isolate

Conclusion

Microbial consortia-1 population of *Azospirillium*, PSB, KRB, ZnSB and PGPR isolates survived better in peat (T_1) compared to lignite (T_2) followed by vermiculite (T_3) and charcoal powder (T_4). While, Microbial consortia-2 population of *Azotobacter*, PSB, KRB, ZnSB and PGPR isolate survived better in lignite (T_6) compared to peat (T_5) followed by vermiculite (T_7) and charcoal powder (T_8). While *Azospirillium* survived well in lignite (T_6) compared to peat (T_5) followed by charcoal powder (T_8) and vermiculite (T_7).

Peat, lignite and vermiculite showed highest viability of KRB population in MC_1 and PSB population in MC_2. Charcoal powder showed highest viability of PSB population in MC_1 and KRB population in MC_2

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