Selection of Carrier Materials for Production of Biofertilizer with Diazotrophic Bacteria

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ABSTRACT

The experiment was conducted at soil microbiology laboratory, Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University for sixty days during 1 October to 30 November 2012. Two promising diazotrophic bacterial strains isolated from sugarcane rhizosphere viz., *Bacillus cereus* (BUSo 13), *Acinetobacter calcoaceticus* (BUSo 9) and one strain of *Rhizobium* sp. (BULs 6) isolated from grasspea root nodule were used to conduct the experiment. *Rhizobium* strain (BULs 6) was considered as standard strain. Four liquid polymeric blend viz., CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ ZnO (1% w/w), CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ MgO (1% w/w), CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ ZnO (1% w/w), CMC-1.54 gL^{-1} +Starch-1.02 gL^{-1}+ MgO (1% w/w) and three solid carrier materials viz., peat Inoculant, pressmud inoculant and 50% peat + 50% pressmud were used as carrier materials to conduct the experiment. The experiment was laid out in a Completely Randomized Design (CRD) with four replications having 21 treatment combinations. For liquid carrier material 50 mL test tube and for solid carrier materials 100 g size poly ethylene bags were used to store carrier materials after inoculation of respective diazotrophs and the survival rate of diazotrophs was evaluated during two months storage period. Results revealed that the population of diazoytophs differed significantly due to interaction of carrier materials (C) days (D). The highest population of *Bacillus* sp., *Acinetobacter* sp. and *Rhizobium* sp. were obtained from liquid carrier material C_4 (CMC-1.54 gL^{-1}+ Starch-1.02 gL^{-1}+ MgO (1% w/w) in 30 days storage period. It was found that population of diazotrophs differed significantly due to different carrier materials. The highest survival rate of nitrogen fixing bacteria viz., *Bacillus* sp., *Acinetobacter* sp. *and Rhizobium* sp. were found in carrier material C_4 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w)) which was followed by solid carrier material C_7 (50% Peat soil + 50% pressmud) throughout the storage period of sixty days. Liquid carrier material CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w) and solid carrier materials of 50% Peat soil + 50% pressmud may be recommended for making biofertilizers with different diazotrophs.

Key word: Selection, carrier materials, biofertilizer, diazotrophic bacteria

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INTRODUCTION

Selection of carrier materials for biofertilizer production is important because, suitable carrier materials provide long time survival of microorganisms. The rhizobia-legume association is exploited in agriculture and forestry by rhizobial inoculants production. The rhizobial inoculant is a cheap and non-polluting source of bacteria that can provide high rates of nitrogen to the legume, and the use of improved inoculants is a strategy to increase the BNF rates in agro ecosystems (Deaker *et al.,* 2004; Xavier *et al.,* 2010; Araújo *et al*., 2011). Peat is the most common material used as carrier to cowpea inoculants in Brazil (Fernandes Júnior *et al.,* 2009). However, peat is not the most suitable material to produce rhizobial inoculants and studies have been carried out focusing alternative carriers. Among the alternative carriers evaluated, polymeric materials stand out because of its low costs, microbial compatibility and easy field application (Fernandes Júnior *et al.,* 2009; Silva *et al.,* 2009). The carboxymethyl cellulose (CMC) / starch blend is a polymer composition that has shown the ability to sustain a high rhizobial concentration up to six months of storage, and the inoculant produced with these carriers shows the same performance to peat inoculants in greenhouse conditions (Fernandes Júnior *et al*., 2009). These blends are also feasible to produce inoculants containing associative diazotrophic bacteria to important grasses such as sugarcane (Silva *et al.,* 2009). The use of peat inoculants is also restricted due to variations in the chemical and physical properties.

Moreover, peat is a material of fossil origin from a non renewable resource, extracted from a very fragile natural system, the peat bogs. In order to increase the inoculant quality and efficiency, and to reduce costs and environmental impacts, alternative carrier materials have been studied (Ben Rebah *et al*., 2007; Albareda *et al.,* 2008), including single and composite polymer formulations (Dommergues *et al*., 1979; Jawson *et al*., 1989; Denardin & Freire, 2000; Deaker *et al*., 2007), which have already been evaluated as rhizobial carriers (Dommergues *et al*., 1979; Denardin and Freire, 2000; Sarr *et al*., 2005), and associative bacteria (Bashan and Gonzales, 1999). Starch is a mixture of amylopectin (at about 75%) and amylose (at about 25%), directly extracted from plant material such as corn and wheat grains (Parker & Ring, 2001). Carboxymethyl cellulose (CMC) is a cellulose-derived ester, originated by the reaction of cellulose with sodium hydroxide and with sodium monochloroacetate, which results in a long chain of anhydroglucose which in turn generates a highly hygroscopic and viscous polymer, nontoxic to humans (Sanz *et al*., 2005). Starch and CMC are immiscible due to the high molecular weight of both. Nevertheless, their molecular similarity results in a partial affinity and contributes to the stability of the formed gel (Suvorova *et al*., 1999), with appropriate rheological and chemical characteristics for rhizobial storage. Furthermore, an advantage of CMC and starch blend, as inoculant carrier material, is that they are cheap and already used in other industrial areas, such as in food and pharmaceutical industries.

The survival of *Azospirillum* in number of locally available material such as lignite, pressmud, charcoal, soil and coffee waste and found to be better than other carriers including peat for the survival of *Azospirillum* upto 200 days the rate of decline in *Azospirillum* population was least in pressmud (Kumar Rao *et al*., 1982). Hence, it is essential to select suitable carrier materials to prepare biofertilizers with diazotrophs for sugarcane. Keeping all this views in mind the present study was undertaken to find out suitable carrier materials for production of eco-friendly biofertilizers with indigenous diazotrophic bacteria to be used for sugarcane production.

MATERIALS AND METHODS

The experiment was conducted at soil microbiology laboratory, Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University for sixty days during 1 October to 30 November 2012. Two promising diazotrophic bacterial strains isolated from sugarcane rhizosphere viz., *Bacillus cereus* (BUSo 13), *Acinetobacter calcoaceticus*(BUSo 9) and one strain of *Rhizobium* sp.(BULs 6) isolated from grasspea root nodule were used to conduct the experiment. *Rhizobium* strain (BULs 6) was considered as standard strain. Four liquid polymeric blend viz., CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ ZnO (1% w/w), CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ MgO (1% w/w), CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ ZnO (1% w/w), CMC-1.54 gL^{-1} +Starch-1.02 gL^{-1}+ MgO (1% w/w) and three solid carrier materials viz., peat Inoculant, pressmud inoculant and 50% peat + 50% pressmud were used as carrier materials to conduct the experiment. The experiment was laid out in a Completely Randomized Design (CRD) with four replications having 21 treatment combinations. For liquid carrier material 50 mL test tube and for solid carrier materials 100 g size poly ethylene bags were used to store carrier materials after inoculation of respective diazotrophs. The experimental test tubes and poly ethylene bags were arranged into 4 blocks representing replications. Each block consisted of 16 test tubes and 12 poly ethylene bags. All the test tubes were placed on two stainless steel wire rack. These racks and polyethylene bags with solid carrier materials were placed on a concrete rack at Soil Microbiology Laboratory, Department of Soil Science, BSMRAU in normal room temperature during the experiment period. The treatment combinations of the experiment were as follows:

T_1- CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ ZnO (1% w/w) + *Bacillus* sp.
T_2- CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ MgO (1% w/w) + *Bacillus* sp.
T_3- CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ ZnO (1% w/w) + *Bacillus* sp.
T_4- CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w) + *Bacillus* sp.
T_5- peat soil + *Bacillus* sp.
T_6- pressmud + *Bacillus* sp.
T_7- 50% peat+ 50% pressmud + *Bacillus* sp.
T_8- CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ ZnO (1% w/w) + *Acinetobacter* sp.
T_9- CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ MgO (1% w/w) + *Acinetobacter* sp.
T_{10}- CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ ZnO (1% w/w) + *Acinetobacter* sp.
T_{11}- CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w) + *Acinetobacter* sp.

T_{12}- peat soil + *Acinetobacter* sp.
T_{13}- pressmud + *Acinetobacter* sp.
T_{14}- 50% peat+ 50% pressmud + *Acinetobacter* sp.
T_{15}- CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ ZnO (1% w/w) + *Rhizobium* sp.
T_{16}- CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ MgO (1% w/w) + *Rhizobium* sp.
T_{17}- CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ ZnO (1% w/w) + *Rhizobium* sp.
T_{18}- CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w) + *Rhizobium* sp.
T_{19}- peat soil + *Rhizobium* sp.
T_{20}- pressmud + *Rhizobium* sp.
T_{21}- 50% peat+ 50% pressmud + *Rhizobium* sp.

CMC: Carboxy Methyl Cellulose, a polymer.

Preparation of liquid polymeric blends

Liquid polymeric blends were prepared by mixing respective polymers and compatibilizing agent (ZnO or MgO) in distilled water. To prepare these compositions, the reagents and the right water proportion were blended, and 5 mL of the blends were transferred to trial test tubes and autoclaved (Fernandes-Júnior *et al.*, 2009).

Picture 1. Experiment on carrier materials

Preparation of solid carrier materials

For solid inoculant collected peat and pressmud were dried, grined and passed through a 20 mesh sieve. The pH of the carrier materials was adjusted to 7.0 by adding $CaCO_3$. Forty gram of solid carrier materials were taken in a polyethylene bags and the bags were sealed with electric sealer machine and then autoclaved.

Preparation of bacterial culture

Bacterial broth was centrifuged at 10,000 g for 10 min. The supernatant was discarded, and the pellet resuspended in 25 mL of sterile distilled water. Bacterial concentration were adjusted using a spectrophotometer at 540 nm and 1 ml of suspension containing 4.5 x 10^6 cells mL^{-1} cells were inoculated.

Inoculation of diazotrophic bacterial culture in carrier materials

Two milliliters of bacterial cell suspension were filled into the sterilized polymer blend tubes, resulting in a concentration of 4.5 x 10^6 cells mL^{-1} were used to conduct the experiment. The inoculants were stored at room temperature (20-26^{0}C) for sixty days. The solid carrier materials were inoculated with a diluted culture of respective bacteria. An amount of 30 ml diluted culture aseptically injected to polyethylene packet containing solid carrier materials with the help of a syringe and mixed thoroughly. Initial population of bacteria in solid carrier materials were 4.5 x 10^6 cells g^{-1}. Then the solid inoculants were stored at room temperature for sixty days.

Enumeration of diazotrophic bacteria

Enumeration of bacteria in both liquid and solid carrier materials were done in 15 days interval by taking three samples from each treatment up to sixty days. To quantify the diazotrophs population in liquid carrier materials a cell suspension aliquot (1 mL) was diluted serially and for solid carrier materials 10 g carrier materials were taken in Erlenmeyer flask containing 95 ml sterile distilled water and then it was shaken for 10 minutes after that it was diluted serially. The dilutions from 10^{-2} to 10^{-8} were plated in nutrient agar medium by the drop plate method (Miles and Misra, 1938).

The collected data were compiled and tabulated in proper form and were subjected to statistical analysis by using the computer package Statistix 10 program for Windows Version. Computation and preparation of graphs were done by the use of Microsoft Excel 2003 program.

RESULTS AND DISCUSSION

Interaction effect of carrier materials (C) x days (D) on survival of diazotrophs

Results presented in Table 1 shows that population of different diazotrophs differed significantly due to interaction of carrier materials (C) days (D). The highest population of *Bacillus* sp., *Acinetobacter* sp. and *Rhizobium* sp. (2.50 x 10^7, 1.70 x 10^7 and 1.22 x 10^7 cfu ml^{-1}, respectively) were obtained from interaction of liquid carrier material C_4 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w)) D_3 (30 days) which was statistically similar to interaction effect of solid carrier material C_7 (50% peat + 50% pressmud) D_3 (30 days) in case of population of Rhizobium sp. (1.03 x 10^7 cfu ml^{-1}). carrier material C_4 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w)) D_3 (30 days) which was statistically similar to interaction effect of solid carrier material C_7 (50% peat+ 50% pressmud) D_3 (30 days) in case of population of Rhizobium sp. (1.03 x 10^7 cfu ml^{-1}).

Table 1. Interaction effect of carrier materials (C) x days (D) on survival of diazotrophs

Interaction (C x D)	*Bacillus* sp. (cfu ml^{-1})	*Acinetobacter* sp. (cfu ml^{-1})	*Rhizobium* sp. (cfu ml^{-1})
$C_1 \times D_1$	4.50 $x10^6$ gh	4.50 $\times 10^6$ ghi	4.50 $\times 10^6$ hi
$C_1 \times D_2$	3.83 $x10^6$ hij	3.17 $\times 10^6$ jk	3.33 $\times 10^6$ jk
$C_1 \times D_3$	1.20 $\times 10^6$ n	2.17 $\times 10^6$ l	2.83 $\times 10^6$ k
$C_1 \times D_4$	4.67 $\times 10^5$ p	1.33 $\times 10^6$ m	1.03 $\times 10^6$ mn
$C_1 \times D_5$	3.33 $\times 10^5$ q	7.33 $\times 10^5$ n	8.33 $\times 10^5$ n
$C_2 \times D_1$	4.50 $x10^6$ gh	4.50 $\times 10^6$ ghi	4.50 $\times 10^6$ hi
$C_2 \times D_2$	5.83 $\times 10^6$ fg	5.17 $\times 10^6$ gh	5.17 $\times 10^6$ gh
$C_2 \times D_3$	3.17 $\times 10^6$ jkl	6.83 $\times 10^6$ ef	5.83 $\times 10^6$ fg
$C_2 \times D_4$	7.33 $\times 10^5$ o	3.83 $\times 10^6$ ij	3.33 $\times 10^6$ jk
$C_2 \times D_5$	4.33 $\times 10^5$ pq	2.03 $\times 10^6$ l	2.83 $\times 10^6$ k
$C_3 \times D_1$	4.50 $\times 10^6$ gh	4.50 $\times 10^6$ ghi	4.50 $\times 10^6$ hi
$C_3 \times D_2$	4.17 $\times 10^6$ hi	3.83 $\times 10^6$ ij	3.83 $\times 10^6$ ij
$C_3 \times D_3$	2.67 $\times 10^6$ l	3.00 $\times 10^6$ k	3.17 $\times 10^6$ jk
$C_3 \times D_4$	4.67 $\times 10^5$ p	1.87 $\times 10^6$ l	1.50 $\times 10^6$ l
$C_3 \times D_5$	3.33 $\times 10^5$ q	9.00 $\times 10^5$ n	1.20 $\times 10^6$ m
$C_4 \times D_1$	4.50 $\times 10^6$ gh	4.50 $\times 10^6$ ghi	4.50 $\times 10^6$ hi
$C_4 \times D_2$	1.70 $\times 10^7$ b	1.22 $\times 10^7$ b	9.67 $\times 10^6$ bc
$C_4 \times D_3$	2.50 $\times 10^7$ a	1.70 $\times 10^7$ a	1.22 $\times 10^7$ a
$C_4 \times D_4$	7.83 $\times 10^6$ de	8.83 $\times 10^6$ cd	7.83 $\times 10^6$ cde
$C_4 \times D_5$	5.00 $\times 10^6$ gh	6.83 $\times 10^6$ ef	6.17 $\times 10^6$ fg
$C_5 \times D_1$	4.50 $\times 10^6$ gh	4.50 $\times 10^6$ ghi	4.50 $\times 10^6$ hi
$C_5 \times D_2$	7.17 $\times 10^6$ def	7.17 $\times 10^6$ def	6.83 $\times 10^6$ ef
$C_5 \times D_3$	8.83 $\times 10^6$ d	10.33 $\times 10^6$ bc	8.83 $\times 10^6$ bcd
$C_5 \times D_4$	3.00 $\times 10^6$ kl	5.17 $\times 10^6$ gh	5.83 $\times 10^6$ fg
$C_5 \times D_5$	1.70 $\times 10^6$ m	4.83 $\times 10^6$ ghi	4.17 $\times 10^5$ o
$C_6 \times D_1$	4.50 $\times 10^6$ gh	4.50 $\times 10^6$ ghi	4.50 $\times 10^6$ hi
$C_6 \times D_2$	6.83 $\times 10^6$ ef	5.83 $\times 10^6$ fg	5.83 $\times 10^6$ fg
$C_6 \times D_3$	4.17 $\times 10^6$ hi	8.17 $\times 10^6$ cde	7.17 $\times 10^6$ def
$C_6 \times D_4$	1.30 $\times 10^6$ n	4.17 $\times 10^6$ hi	4.33 $\times 10^6$ hi
$C_6 \times D_5$	7.67 $\times 10^5$ o	3.17 $\times 10^6$ jk	3.33 $\times 10^6$ jk
$C_7 \times D_1$	4.50 $\times 10^6$ gh	4.50 $\times 10^6$ ghi	4.50 $\times 10^6$ hi
$C_7 \times D_2$	1.22 $\times 10^7$ c	9.67x 10^6 bc	7.17 $\times 10^6$ def
$C_7 \times D_3$	1.43 $\times 10^7$ bc	1.22 $\times 10^7$ b	1.03 $\times 10^7$ ab
$C_7 \times D_4$	4.33 $\times 10^6$ hi	6.83 $\times 10^6$ ef	6.83 $\times 10^6$ ef
$C_7 \times D_5$	3.50 $\times 10^6$ ijk	5.83 $\times 10^6$ fg	5.17 $\times 10^6$ gh
CV (%)	1.01	1.01	0.89

The effect of C_4 x D_3 was followed by interaction of liquid carrier material C_4 x D_2 (15 days) (9.67 x 10^6 cfu ml^{-1}).Whereas the interaction effect of liquid carrier material C_4 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w) x D_3 (30 days) was followed by interaction of liquid carrier material C_4 x D_2 (15 days) (1.70 x 10^7 cfu ml^{-1}) and solid carrier material C_7 (50% peat+ 50% pressmud)x D_3 (30 days) (1.43 x 10^7 cfu ml^{-1}) in case of population of *Bacillus* sp. In case of population of *Acinetobacter* sp. the interaction effect of liquid carrier material C_4(CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w) x D_3 (30 days) was followed by interaction of solid carrier material C_7 (50% peat+ 50% pressmud) x D_3 (30 days), liquid carrier material C_4xD_2 (15 days) (both1.22 x 10^7 cfu mL^{-1}) and solid carrier material C_7 (50% peat+ 50% pressmud) x D_2 (15 days) (9.67x 10^6 cfu mL^{-1}). These results corroborates with the findings of Fernandes *et al*. (2012) who reported that the rhizobial survival was higher in cowpea seeds inoculated with MgO compatibilized polymeric formulations, compared to standard peat-based inoculant and polymeric formulations compatibilized with ZnO.

Survival of diazotrophic bacteria in different carrier materials

Results presented in Table 2 reveal that population of diazotrophs differed significantly due to different carrier materials. The highest population of *Bacillus* sp., *Acinetobacter* sp. and *Rhizobium* sp. (1.19 x 10^7, 9.87 x 10^6 and 8.07 x 10^6, respectively) were obtained in liquid carrier material C_4 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w) which was followed by solid carrier material C_7 (50% peat+ 50% pressmud) (7.77 x 10^6 , 7.81 x 10^6 and 6.80 x 10^6 cfu ml^{-1}, respectively). The lowest diazotrophs population was found in liquid carrier material C_1(CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ ZnO (1% w/w) (2.07 x 10^6, 2.38 x 10^6, 2.50 x 10^6 cfu ml^{-1}, respectively). The third highest diazotroph population was obtained in T_5 (Peat soil) (5.04 x 10^6, 6.40 x 10^6 and 5.28 x 10^6 cfu ml^{-1}, respectively). Other carrier materials showed inferior performance regarding diazotrophs population compared to carier materials C_4, C_7 and C_5. This was possibly due to complex molecular forms of CMC and starch blend compatibilized with MgO (1% v/v) along with sufficient and optimum nutrient supply for diazotrophs survival which might have take much time for degradation by diazotrophs, thus it provides higher populations of diazotrophs.

Table 2. Survival of diazotrophic bacteria in different carrier materials

Carrier Material	Diazotroph population (cfu ml^{-1})		
	Bacillus sp. (cfu ml^{-1})	*Acinetobacter* sp. (cfu ml^{-1})	*Rhizobium* sp. (cfu ml^{-1})
C_1	2.07 x 10^6 g	2.38 x 10^6 g	2.50 x 10^6 g
C_2	2.93 x 10^6 e	4.47 x 10^6 e	4.33 x 10^6 e
C_3	2.43 x 10^6 f	2.82 x 10^6 f	2.84 x 10^6 f
C_4	1.19 x 10^7 a	9.87 x 10^6 a	8.07 x 10^6 a
C_5	5.04 x 10^6 c	6.40 x 10^6 c	5.28 x 10^6 c
C_6	3.51 x 10^6 d	5.17 x 10^6 d	5.03 x 10^6 d
C_7	7.77 x 10^6 b	7.81 x 10^6 b	6.80 x 10^6 b
LSD (0.05)	0.0239	0.0246	0.0215

C_1= CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ ZnO (1% w/w), C_2= CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ MgO (1% w/w), C_3= CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ ZnO (1% w/w), C_4= CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w), C_5= Peat soil, C_6= Pressmud and C_7=50% peat+ 50% pressmud.

This was also true for solid carrier material containing 50% peat soil + 50% pressmud because it is high in nutrients content and has complex form of natural medium. This result corroborates with the findings of Fernandes Júnior *et al*. (2009) who reported that carboxymethyl cellulose (CMC) / starch blend is a polymer composition that has shown the ability to sustain a high rhizobial concentration up to six months of storage, and the inoculant produced with these carriers shows the same performance to peat inoculants in greenhouse conditions. Silva *et al.* (2009) reported that these blends are also feasible to produce inoculants containing associative diazotrophic bacteria to important grasses such as sugarcane.

Survival of diazotrophs in carrier material C_1 (CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ ZnO (1% w/w)) at different days

Results presented in Figure 1 shows that population of diazotrophs at different days differed significantly due to carrier material C_1 (CMC-1.28 gL^{-1}+ Starch-1.28 gL^{-1}+ ZnO (1% w/w). The population of all diazotrophs in carrier matterial C_1 decreased from the initial population and were lowest at 60 days. This might be due to less amount of CMC and ZnO as compatibilizing agent which might hampered diazotrophs survival and resulted declined population through the storage period.

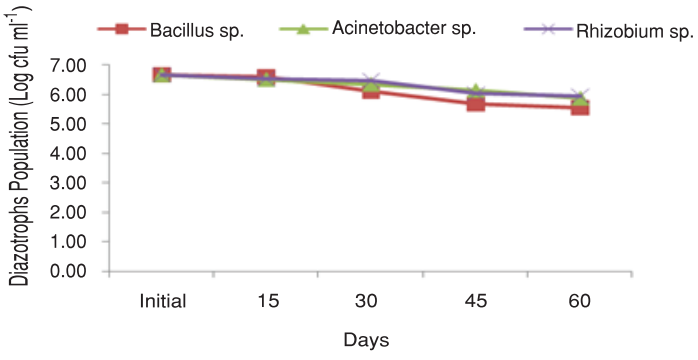

Figure 1. Survival of diazotrophs in carrier material C_1 (CMC-1.28 gL^{-1}+ Starch-1.28 gL^{-1}+ ZnO (1% w/w)) at different days.

Survival of diazotrophs in carrier material C_2 (CMC-1.28 gL^{-1}+Starch-1.28 gL^{-1}+ MgO (1% w/w) at different days

Results presented in Figure 2 reveal that population of diazotrophs at different days differed significantly due to carrier material C_2 (CMC^{-1}.28 gL^{-1}+ Starch-1.28 gL^{-1}+ MgO (1% w/w)). The population of all diazotrophs except *Bacillus* sp. in carrier matterial C_2 increases significantly up to 30 days and population of *Bacillus*

sp. declined from 15 days after that populations were declined from the initial levels and lowest at 60 days. This might be due to less amount of CMC though the compatibilizing agent is MgO which might hampered diazotrophs survival and resulted less population.

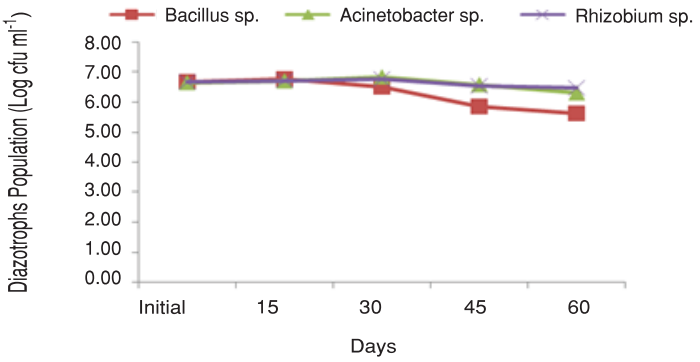

Figure 2. Survival of diazotrophs in carrier material C_2 (CMC-1.28 gL^{-1}+ Starch-1.28 gL^{-1}+ MgO (1% w/w)) at different days

Survival of diazotrophs in carrier material C_3 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ ZnO (1% w/w)) **at different days**

Results presented in Figure 3 shows that population of diazotrophs at different days differed significantly due to carrier material C_3 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ ZnO (1% w/w)). The population of all diazotrophs except *Bacillus* sp. in carrier matterial C_3 were remained as initial up to 30 days whereas, population of *Bacillus* sp. was remained as initial up to 15 days. After that the populations were declined and lowest at 60 days. This might be due to the compatibilizing agent is ZnO which might hampered survival of diazotrophs resulted less population.

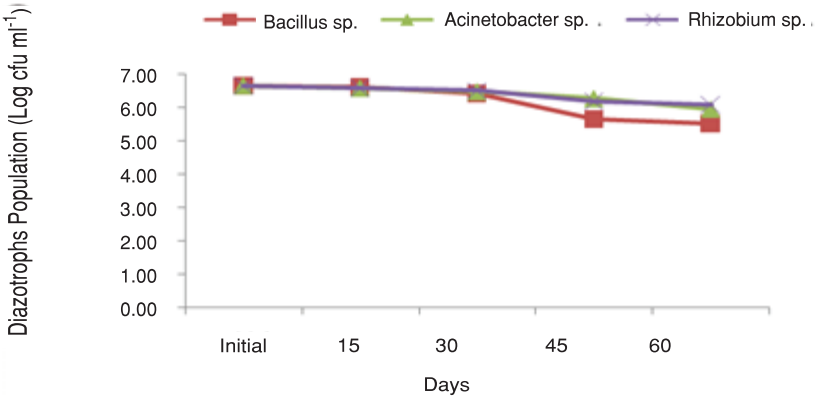

Figure 3. Survival of diazotrophs in carrier material C_3 (CMC-1.54 gL + Starch-1.02 gL^{-1}+ ZnO (1% w/w)) at different days

Survival of diazotrophs in carrier material C_4 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w) **at different days**

Results presented in Figure 4 reveal that population of diazotrophs at different days differed significantly due to carrier material C_4 (CMC-1.54 gL^{-1}+ Starch-1.02 gL^{-1}+ MgO (1% w/w)). The population of all diazotrophs in carrier matterial C_4 increases significantly up to 30 days then it was declined and lowest at 60 days but the population remained higher than the initial population (6.6 log cfu ml^{-1}) throughout the storage period of sixty days. This might be due to the higher amount CMC and lower amount of starch and the compatibilizing agent is MgO which might influence diazotrophs survival resulted higher populations. This result corroborates with the findings of Fernandes *et al*. (2012) who reported that the rhizobial survival was higher in cowpea seeds inoculated with MgO compatibilized polymeric formulations (CMC-1.54 gL^{-1} + Starch -1.02 gL^{-1} + MgO (1% w/w)) throughout the 35 days of storage.

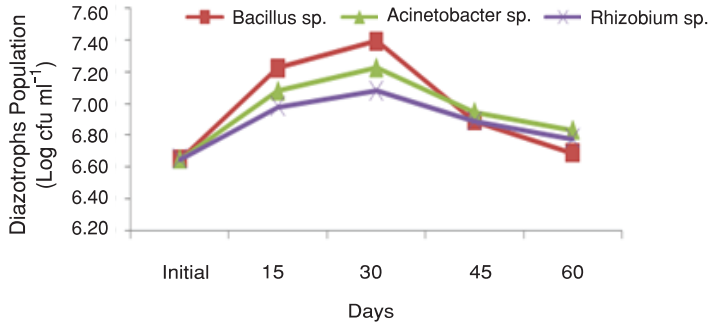

Figure 4. Survival of diazotrophs in carrier material C_4 (CMC-1.54 gL^{-1}+ Starch-1.02 gL^{-1}+ MgO (1% w/w)) at different days

Survival of diazotrophs in carrier material C_5 (Peat soil) at different days

Results presented in Figure 5 shows that population of different diazotrophs at different days differed significantly due to carrier material C_5 (Peat soil). The population of all diazotrophs in carrier matterial C_5 increases significantly up to 30 days then it was declined and it were continued upto 60 days but the populationsremained higher at 60 days than the initial levels. This might be due to sufficient carbon source ofpeat soil for diazotroph which may ledhigher population upto 60 days of storage than the initial levels. Ishwaran (1969) reported that Peat is generally as most dependable biofertilizer carrier because of its high content of organic matter and water holding capacity.

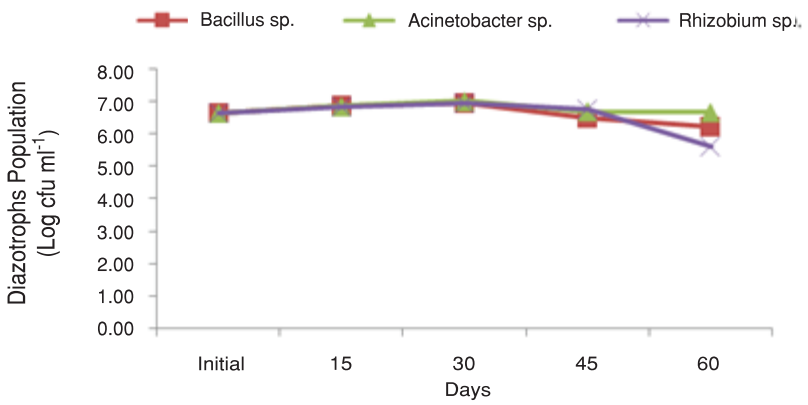

Figure 5. Survival of diazotrophs in carrier material 5 (Peat soil) at different days

Survival of diazotrophs in carrier material C_6 (Pressmud) at different days

Results presented in Figure 6 reveal that population of different diazotrophs at different days differed significantly due to carrier material C_6 (Pressmud).

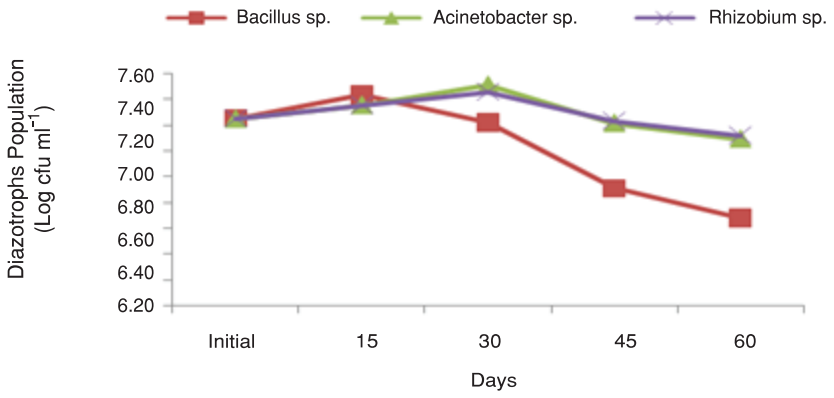

Figure 6. Survival of diazotrophs in carrier material C_6 (Pressmud) at different days

The population of all diazotrophs except *Bacillus* sp. in carrier matterial C_6 increases significantly up to 30 days whereas, population of Bacillus sp. increases upto 15 days. After that populations were declined and lowest at 60 days.The populationsof *Acinetobacter* sp. and *Rhizobium* sp. were remained above the initial levels (6.6 log cfu ml^{-1}) up to 30 dayswhereas; it was remained above the initial level upto 15 days in case of *Bacillus* sp. This might be due to rapid use of carbon from the pressmud by diazotrophs which mightledless survival in this material.

Survival of diazotrophs in carrier material C_7 (50% Peat soil+ 50% pressmud)at different days

Results presented in Figure 7 reveal that population of different diazotrophs at different days differed significantly due to carrier material C_7 (50% Peat soil+ 50% pressmud). The population of all diazotrophs in carrier matterial C_7 increases significantly up to 30 days then it was declined and lowest at 60 days.The population of *Bacillus* sp. were declined from the initial levels (6.6 log cfu ml^{-1}) at 45 days whereas; in case of *Acinetobacter* sp. and *Rhizobium* sp. it maintained the populations above the initial levels upto 60 days. This might be due to higher carbon source and nutrients in the combined materials which might enhance higher population of diazotrophs. This results was in accordance with that of Kumar Rao *et al*. 1982 who reported that the survival of *Azospirillum* in number of locally available material such as lignite, pressmud, charcoal, soil and coffee waste and found to be better than other carriers including peat.

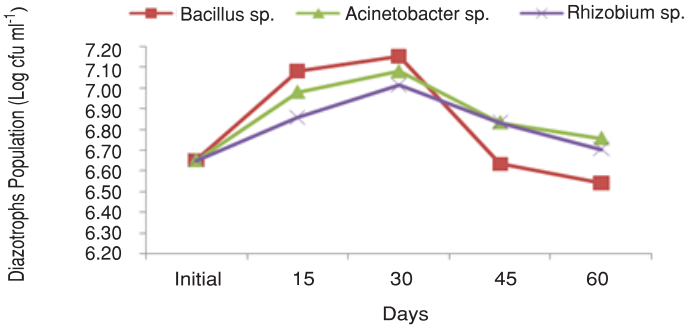

Figure 7. Survival of diazotrophs in carrier material C_7 (50% Peat soil+ 50% pressmud) at different days

Results reveal that the highest population of *Bacillus* sp., *Acinetobacter* sp. and *Rhizobium* sp. were obtained from liquid carrier material C_4 (CMC-1.54 gL^{-1} + Starch -1.02 gL^{-1}+ MgO (1% w/w)) which was followed by solid carrier material C_7 (50% peat+ 50% pressmud) (7.77 x 10^6 , 7.81 x 10^6 and 6.80 x 10^6 cfu ml^{-1}, respectively). The highest population of *Bacillus* sp., *Acinetobacter* sp. and *Rhizobium* sp. were obtained from interaction of liquid carrier material C_4 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w) x D_3 (30 days) which was followed by liquid carrier material C_4 x D_2 (15 days) and solid carrier material C_7 (50% peat+ 50% pressmud) x D_3 (30 days). The population of all diazotrophs in carrier matterial C_4 increases significantly up to 30 days then it was declined and lowest at 60 days but the population remained higher than the initial population (6.6 log cfu ml^{-1}) throughout the storage period. It was also revealed that the population of all diazotrophs in carrier matterial C_7 increases significantly up to 30 days then it was declined and lowest at 60 days. The population of *Bacillus* sp. were declined from the initial levels (6.6 log cfu ml^{-1}) at 45 days whereas; in case of *Acinetobacter* sp. and *Rhizobium* sp. it maintained the populations above the initial levels upto 60 days of storage period.

It may be concluded that the population of diazotrophs differed significantly due to interaction of carrier materials (C) × days (D). The highest population of *Bacillus* sp., *Acinetobacter* sp. and *Rhizobium* sp. were obtained from liquid carrier material C_4 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w) in 30 days storage period. It was found that population of diazotrophs differed significantly due to different carrier materials. The highest survival rate of nitrogen fixing bacteria viz., *Bacillus* sp., *Acinetobacter* sp. and *Rhizobium* sp. were found in carrier material C_4 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w)) which was followed by solid carrier material C_7 (50% Peat soil + 50% pressmud) throughout the storage period of sixty days. highest survival rate of nitrogen fixing bacteria viz., *Bacillus* sp., *Acinetobacter* sp. and *Rhizobium* sp. were found in carrier material C_4 (CMC-1.54 gL^{-1}+Starch-1.02 gL^{-1}+ MgO (1% w/w)) which is followed by solid carrier material C_7 (50% Peat soil + 50% pressmud) throughout the storage period of sixty days.

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