

Original Research Article

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A Comparative Study of Different Carriers for Shelflife of *Rhizobium* spp.as Bioinoculants

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In the present investigation three carriers, charcoal, mushroom compost, vermicompost were evaluated for the production of bioinoculants. The bacteria used for bioinoculants development was recommended *Rhizobium* spp. (M1). The *Rhizobium* strains were inoculated in all the three carriers singly and in combination with different carriers. The bacterial population was determined in each carrier up to 3 months of storage. The mushroom compost show the highest bacterial population and bacterial population was uniform throughout the months of storage, whereas after 15 days of incubation the bacterial population in vermicompost was increased and decreased in the charcoal. Considering these results, the mushroom compost as carrier could increase the survival of bacteria and might be as a bioinoculants.

Introduction

Application of biofertilizer for crop production is environmental friendly and sustainable for ecological system. Biofertilizer are low cost, effective and renewable source of plant nutrients to supplement chemical fertilizers (Boraste *et al.*, 2009). Biofertilizer consists of carriers and the microorganisms (Shariati *et al.*, 2013). Several types of biofertilizer have been developed from bacteria, particularly

Rhizobium spp., *Azospirillum* spp., and *Azotobacterspp.*, and used in production of various plants (Mala, 2003; Narula, 2000; Rai, 2006). These beneficial organisms are applied in the form of microbial inoculants (Karunai *et al.*, 2013).

The inoculants can be prepared from several types of carriers such as peat, charcoal, farm yard manure, lignite, alginate, etc., Nowadays; several types of agricultural waste like maize stubble, plant compost, mushroom

waste, rice straw, oil palm and bunch can be composted and used as bio inoculant carrier. This system helps reducing the pollutants, saving energy, decreasing cost of production, and utilizing natural resources to the benefit (Phiromtan *et al.*, 2013).

Earthworm's vermicompost give very high food productivity comparable to or even better than the chemical fertilizers with significantly higher nutritional quality which also improving the physical, chemical and biological properties of soil. Vermicomposting is highly nutritive and a powerful plant growth promoter and protector and has scientifically proven to be a miracle plant growth promoters.

Vermicompost is a uniform and odourless material with good physical structure, abundant labile resources and high microbial activity (Pramanik *et al.*, 2007; Ngo *et al.*, 2011). It contains larger quantities of mineral and trace elements than traditional compost which are available for plant uptake (Tejada *et al.*, 2009; Arancon and Edwards, 2011). It also contains plant growth regulating substances such as auxins, gibberellins, cytokinins, fulvic and humic acids which are beneficial for plant performance (Zhang *et al.*, 2014). Spent mushroom substrate (SMS) has good physical properties; it includes the water holding capacity, soil pH, soil porosity, salt content i.e electrical conductivity. Addition of SMS will add great amount of macro nutrients (Kim *et al.*, 2011). The biological properties of SMS enhance its marketability as a soil conditioner (Brady and Wiel, 2004).

Spent compost is believed to be a source of humus formation and humus is provided to the plants with micronutrients improve the soil aeration, soil water holding Capacity and contributes the maintenance of soil structure (Kediri and Mustapha, 2010). By considering all good properties of mushroom compost, it

has been tested in the present study to use it as carrier for shelf life of *Rhizobium* spp. and compared with other carriers like charcoal and vermicompost.

Materials and Methods

Preparation of carriers

Charcoal, mushroom compost and vermicompost were used in this study. The raw material were ground, sieved with 0.5 cm mesh screen and dried in a hot air oven at 60°C for two days. The materials were autoclave at 121°C at a pressure of 15 lb for 30 minutes for two successive days.

Treatments

T1= Charcoal
T2 =Vermicompost
T3=Mushroom compost
T4=Charcoal+vermicompost
T5=Charcoal+mushroom compost
T6=Vermicompost+mushroomcompost
T7=Charcoal+vermicompost+mushroom compost

Procurement and preparation of bacterial inoculants

Recommended native isolates of *Rhizobium* spp. (M1) were obtained from the Pulses section, Department of Plant Breeding and Genetics, for the use in present study. The medium used for *Rhizobium* (M1) was Yeast Extract Mannitol (YEM) broth sterilized by autoclaving at 121°C at a pressure of 15 lb for 30 minutes. After sterilization the medium was kept at room temperature followed by inoculation with a loopful of *Rhizobium* culture from agar slant under aseptic conditions and kept in rotary shaker for 48 hr at 28°C. After incubation, 10 ml of the inoculums was transferred to 1000 ml of respective broth and kept in shaking incubator

for mass multiplication. 750 ml was mixed thoroughly with 1000g of each sterile carrier, packed in polyethylene bags, sealed and incubated for 7 days at $28 \pm 1^{\circ}$ C. For studying the shelf life of *Rhizobium* the packets was stored at 4° C temperatures.

Evaluation for shelf life of the *Rhizobium* sp. during storage

The shelf life of *Rhizobium* sp. was determined after the inoculums were subjected to different carriers. One grams of each sample was taken for estimating viable cells at every 15 days up to 3 months using dilution plating method on Yeast Extract Mannitol Agar (YEMA) and incubated at 28° C for 2 days. The number of apparent *Rhizobium* colonies after incubation from different treatments was counted and calculated into viable cells.

Counting colonies of *Rhizobium* spp. (M1)

The number of colonies were counted from YEMA plates after the incubation period of 48 hrs as colony forming units (cfu) per ml and expressed as cfu per gram of carrier material. The plate count was carried out in triplicates and final value of cfu was the average of three readings and converting cfu into log cfu/ gm of carrier by using following formula:

$$\text{Log } (a \times b^n) = \log a + n \log b$$

Where

a = Mean number of bacterial colonies

b^n = Dilution factor

Results and Discussion

In the present study, the shelf life of *Rhizobium* sp. as bio inoculants was studied

in different carrier based culture packets. The population of bacteria was determined by measuring the log cfu/gm up to 3 months.

The data presented in Table 1 indicates the survivability of *Rhizobium* in different carrier up to 3 months. Among all the tested carrier material, mushroom compost was found to best carrier among all the carrier materials and supported the significantly highest bacterial count 10.85 log cfu/ gm of carrier material after 3 months of storage and it was found significantly superior over all other combination at 90 days., whereas after 15 days of incubation the bacterial population in the charcoal was intensively declined and it was maintained at 5.1 log cfu/gm after 3 months of storage (Fig.1). While the population of bacteria in the vermicompost increased after 15 days and observed maximum 10.54 log cfu/gm of carrier at 90 days. So, mushroom compost found to be a good carrier for shelf life and might be used as bio inoculants. This may be due to more water holding capacity of carrier material.

These findings are correlated with Shitole *et al.*, (2014) who reported that spent mushroom substrate showed good carrier for shelf life and survival of *T. viride* (2×10^6 cfu/g) and *Rhizobium* (20.66×10^8 cfu/g) at 180 days as compared to other carrier combination. Similarly Rebah *et al.*, (2007) reported that some industrial and agricultural by-products (e.g. cheese whey, malt sprouts) contain growth factors such as nitrogen and carbon which can support growth of rhizobia. Other agro-industrial wastes (e.g. plant compost, filterter mud and fly-ash) can be used as a carrier for Rhizobial inoculant. More recently, waste water sludge a worldwide recyclable waste has shown good potential for inoculant production as a growth medium and as a carrier (dehydrated sludge).

Table.1 Population density (log cfu/gm) of *Rhizobium* of in different carriers

Treatments	0 days	15 days	30 days	45days	60days	75 days	90days
T1	9.43	9.59	9.28	8.42	7.39	7.24	5.21
T2	8.77	8.85	9.45	9.98	10.02	10.44	10.54
T3	9.67	9.92	10.03	10.33	10.67	10.78	10.85
T4	8.23	8.72	8.84	8.57	7.83	7.71	7.69
T5	8.12	8.69	8.95	8.79	8.60	8.54	8.32
T6	9.02	8.83	8.90	8.71	8.64	8.57	8.40
T7	9.28	8.97	8.76	8.64	8.84	8.65	8.14
C D (5%)	Inoculants: 0.58 storage period: 0.58 Ix S: NS						

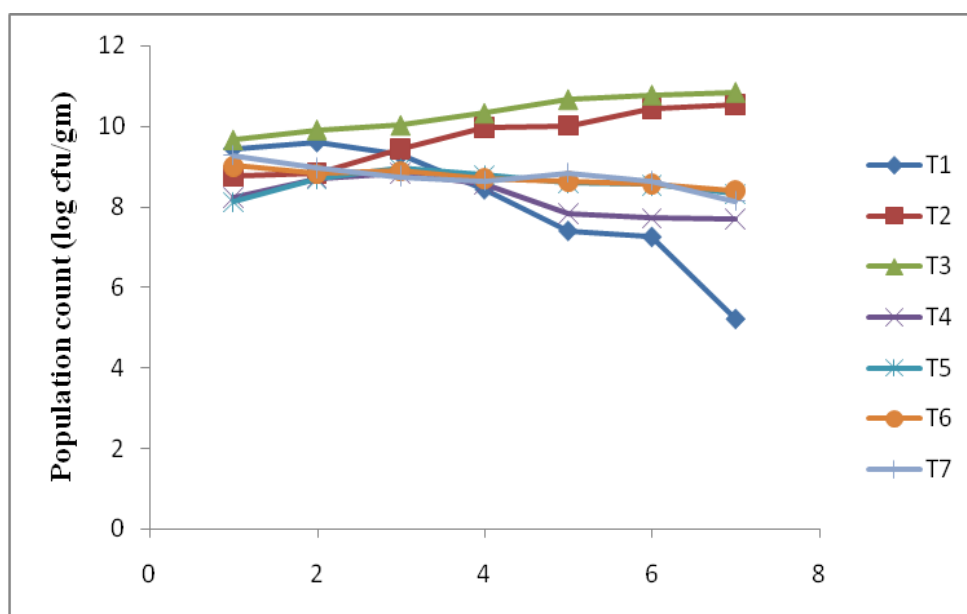


Fig. 1: Population density (log cfu/gm) of *Rhizobium* (M1) spp. in different carrier materials

Sekar and Karmegam (2010) reported that vermicasts from *E.euginiaeas* a carrier material which supports the survival of more than 1×10^7 g⁻¹ viable cell of *A.chroococum*, *B.megaterium* and *R. leguminosarum* till the end of 10th month which is longer than observed in lignite. Chao and Alexander (1984) concluded that bacterial retention on activated charcoal and peat carrier at 25⁰C was more than 4⁰C. Mendez and Videira (2005) stated that bacterial maintenance at 28⁰C for 41 days caused an increase in number of viable bacterial cells on all carriers so that the population reached nearly 10^9 bacteria per gram of carrier.

Use of organic sources of fertilizers improved the soil chemical properties through increasing the content of macro and micronutrients and organic carbon. This study recommended the mushroom compost as a carrier material because mushroom compost supported good survival of *Rhizobium* count.

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