COMPARATIVE STUDY OF CARRIER BASED MATERIALS FOR RHIZOBIUM CULTURE FORMULATION

Ashok Kumar Singh, Gauri, Rajendra Prasad Bhatt* and Shailja Pant

Institute of Biomedical and Natural Sciences,
Manduwala, Dehradun-248 007 India

Received: 23-08-2011
Accepted: 11-04-2012

ABSTRACT

In the present investigation four carriers – bagasillo, peat, charcoal and coal were evaluated for the production of bioinoculants. The bacteria used for bioinoculant development were Rhizobium trifolii (MTCC-905) and Rhizobium meliloti (MTCC-100). Both bacterial strains were inoculated in all the four carriers separately. The bacterial population was determined in each carrier up to six month storage. Bagasillo maintain maximum population count 9.39 and 9.40 log cfu/gm for Rhizobium trifolii (MTCC-905) and Rhizobium meliloti (MTCC-100) respectively while coal supported minimum population count 7.28 for Rhizobium meliloti and 7.58 for Rhizobium trifolii. Finally the impact of six month stored inoculants on plant productivity was determined. Bagasillo with Rhizobium meliloti enhanced the seedling biomass by 47% while with Rhizobium trifolii by 55%. Peat and charcoal with Rhizobium meliloti enhanced the seedling biomass by 34% and 28 % respectively and with Rhizobium trifolii by 45 % and 30 % respectively. Coal with Rhizobium meliloti showed 08 % increase of seedling biomass and with Rhizobium trifolii seedling biomass was enhanced by 10 %. The bagasillo-based inoculant was much better than any other carrier-based inoculant taken in the study in enhancing the seedling biomass and the nodule number. The present study suggested the use of bagasillo as an efficient and cheaper carrier material.

Key words: Bagasillo, Bio-inoculant, Rhizobium trifolii, Rhizobium meliloti.

INTRODUCTION

The staggeredly increasing world population demands sustainable crop production, which has become a threat by affecting total agricultural land area and extra burden on agriculture. The imprudent use of chemical fertilizers, pesticides and fungicides has resulted in the deterioration of soil health and also harmful effects on soil biota. Amongst soil biota, microorganisms play a significant role in regulating the dynamics of organic matter decompositing and the availability of plant nutrients such as nitrogen, phosphorous and sulphur. Thus it has led an emphasis on integrated management strategy to improve the interaction of the roots of plants with soil microorganisms (Mishra et al., 2006). Legumes are able to establish associations with different rhizobial species (Hara and Oliveira, 2005).

In case of bio-inoculation, the carriers used are biodegradable and the inoculum increases plant growth without affecting the environment adversely. The production and quality of rhizobial inoculants in developing countries is limited by technological limitations or the availability of suitable carriers (Khavazi et al, 2007). The carrier inoculant is a means of transport of living bacterial and fungal cultures to the fields. The viability of the inoculum in an appropriate formulation for a certain length of time is important for commercialization of the technology. The carrier with the ability to carry the inoculants will appear to be revolutionary for the agriculture industry. The present investigation has been aimed at the studies: To examine the growth pattern of legume inoculant on suitable carrier material and the effect of bio-fertilizers on morphological features and nodule number of host plants.

*Department of Botany and Microbiology, H.N.B. Garhwal University, Srinagar, Garhwal (Uttarakhand), India.
MATERIALS AND METHODS

This work was conducted in Microbiology Laboratory of Dolphin (PG) Institute of Biomedical & Natural Sciences Dehradun which is affiliated to H.N.B. University Srinagar Garhwal Uttarakhand in the year 2009. In the present study bacterial strains *Rhizobium trifolii* (MTCC-905) and *Rhizobium meliloti* (MTCC-100) were obtained from Microbial type culture collection (MTCC) of Institute of Microbial Technology (IMTECH), Chandigarh, India. Rhizobial strains were maintained at 4°C and cultured (30°C and 150 rpm for 24 h to get $1 \times 10^9$ cells/ml) on yeast extract mannitol (Himedia), and TY Media (Himedia), respectively. For the preparation of bioinoculants, locally available agricultural and industrial wastes were selected as carriers. These were bagasillo, peat, charcoal and coal.

Single strain legume inoculants were produced by batch culture using 10% sugar waste as a suitable culture medium (Singh et al, 2011) and formulated with suitable carrier materials. The solid carriers were mixed with the same volume (w/v) of distilled water with continuous stirring to form a paste and pH was determined by a digital pH meter. The moisture content of each carrier was determined on a wet and dry weight basis. For determining water holding capacity, 100 g of oven dried carrier was kept in 500 ml beaker and water added until the carrier became saturated. The slurry was transferred in a pre-weighed measuring cylinder, the mouth of which was covered with a sieve. The water was allowed to drain overnight (from the carrier) after which the measuring cylinder was weighed with the contents. The water holding capacity was reported on dry weight basis of the carrier (Somsegaran and Hoben 1994).

Periodically, inoculants were sampled and viable rhizobia were counted by dilution plate technique using YEMA media (Somsegaran and Hoben, 1985). The alfalfa (*Medicago sativa*) and barseem (*Trifolium alexandrinum*) seeds were rinsed in 95% ethanol, followed by immersion in 0.2% HgCl$_2$, acidified with 0.5% HCl for 3 minutes. The seeds were then washed thoroughly with sterile water and spreaded directly on 1% agar plates. The plates were inverted to provide the seedlings with uniform straight growth.

The pot experiment was conducted in 1.5 kg capacity plastic pots filled with sterile acid washed river sand and fine granular CaCO$_3$ (2 : 1) mixture. *Rhizobium* culture ($10^9$cfu g$^{-1}$ soil) was mixed with the soil in each pot. Two days old seedlings (10 seedlings / pot) were transplanted into each pot. The pots were irrigated with nitrogen free plant growth medium. Plants were cultured in a growth chamber under controlled condition of 16 hours light and 8 hours darkness cycles with 60% relative humidity. Two weeks after transplantation, the seedlings were thinned and maintained five plants per pot up to six weeks. The pots were irrigated with a nitrogen free nutrient solution of pH 6.8 as and when required to maintain a maximum water holding capacity of nearly 70%. After 6 weeks, plants were harvested from the pots and dissected. Roots, shoots and nodules were collected. Shoots, roots and nodules were separately oven dried at 65°C for determination of their dry weights. Data analysis was done using SPSS version 12.0.

RESULTS AND DISCUSSION

The pH of all the four carriers was monitored to be within the optimum range of 6.8-7.0. Bagasillo has the maximum inherent moisture content and water holding capacity, followed by peat, charcoal and coal (Table 1).

<table>
<thead>
<tr>
<th>Name of carrier</th>
<th>pH</th>
<th>Inherent moisture content (%)</th>
<th>Water holding capacity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagasillo</td>
<td>7.0</td>
<td>13.6</td>
<td>590</td>
</tr>
<tr>
<td>Peat</td>
<td>6.8</td>
<td>8.6</td>
<td>435</td>
</tr>
<tr>
<td>Charcoal</td>
<td>7.0</td>
<td>5.35</td>
<td>230</td>
</tr>
<tr>
<td>Coal</td>
<td>7.0</td>
<td>5.3</td>
<td>225</td>
</tr>
</tbody>
</table>

Survival of *Rhizobium meliloti* and *Rhizobium trifolii* in different carrier based inoculants at 28±2°C storage temperature

The population of bacteria was determined by measuring the log 10 cfu/gm monthly till 06 months. The initial increase in population count up to 02 months was recorded in all carrier materials. A gradual decline in population was observed with increased storage time might be due to effect of
desiccation of the inoculants. Rhizobial count was adversely affected during storage by low moisture contents in the carrier materials (Brockwell et al 1987).

Bagasillo maintained maximum population count 9.39 and 9.40 log cfu/gm for *Rhizobium meliloti* (MTCC–100) and *Rhizobium trifolii* (MTCC–905) respectively after 180 days of storage at room temperature, followed by peat which supported 9.25 and 9.20 log cfu/gm for *Rhizobium meliloti* (MTCC–100) and *Rhizobium trifolii* (MTCC–905) respectively (Fig. 1 and 2). Bagasillo maintained higher population count for both rhizobium strains probably because bagasillo had more available nutrients than the other carriers. Bagasillo had an additional advantage of mixing with broth culture before incubation. Bagasillo also had the maximum inherent moisture content and water holding capacity compared to the other carriers used in this study. A carrier must display high water holding capacity and retention characteristics, display chemical and physical uniformity and be non toxic to inoculants.

### TABLE 2: Effect of different carrier materials inoculated with *Rhizobium meliloti* on growth and nodulation of *Medicago sativa*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nodule No./Plant</th>
<th>Nodule Fresh Weight (mg/Plant)</th>
<th>Nodule Dry Weight (mg/Plant)</th>
<th>Shoot Dry Weight (g/Plant)</th>
<th>Root Dry Weight (g/Plant)</th>
<th>Total Weight (g/Plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.84 ± 0.030</td>
<td>0.21 ± 0.041</td>
<td>1.06 ± 0.072</td>
</tr>
<tr>
<td>Bagasillo</td>
<td>11.33 ± 1.527</td>
<td>2.29 ± 0.120</td>
<td>0.96 ± 0.061</td>
<td>1.7 ± 0.2</td>
<td>0.3 ± 0.055</td>
<td>2.0 ± 0.255</td>
</tr>
<tr>
<td>Peat</td>
<td>8.66 ± 1.527</td>
<td>2.09 ± 0.076</td>
<td>0.83 ± 0.064</td>
<td>1.36 ± 0.152</td>
<td>0.25 ± 0.036</td>
<td>1.61 ± 0.140</td>
</tr>
<tr>
<td>Charcoal</td>
<td>6.66 ± 1.527</td>
<td>1.82 ± 0.150</td>
<td>0.72 ± 0.045</td>
<td>1.16 ± 0.158</td>
<td>0.31 ± 0.020</td>
<td>1.48 ± 0.137</td>
</tr>
<tr>
<td>Coal</td>
<td>5 ± 1</td>
<td>1.02 ± 0.06</td>
<td>0.43 ± 0.051</td>
<td>0.93 ± 0.045</td>
<td>0.26 ± 0.020</td>
<td>1.16 ± 0.04</td>
</tr>
</tbody>
</table>

Results are mean of three replicates ± SD. Results are significantly different at P = 0.05.

### TABLE 3: Effect of different carrier materials inoculated with *Rhizobium trifolii* on growth and nodulation of *Trifolium alexandrinum*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nodule No./Plant</th>
<th>Nodule Fresh Weight (mg/Plant)</th>
<th>Nodule Dry Weight (mg/Plant)</th>
<th>Shoot Dry Weight (g/Plant)</th>
<th>Root Dry Weight (g/Plant)</th>
<th>Total Weight (g/Plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.9 ± 0.10</td>
<td>0.24 ± 0.025</td>
<td>1.14 ± 0.125</td>
</tr>
<tr>
<td>Bagasillo</td>
<td>13.66 ± 1.527</td>
<td>2.81 ± 0.116</td>
<td>1.07 ± 0.015</td>
<td>2.16 ± 0.208</td>
<td>0.39 ± 0.015</td>
<td>2.56 ± 0.215</td>
</tr>
<tr>
<td>Peat</td>
<td>10.33 ± 1.527</td>
<td>2.31 ± 0.196</td>
<td>0.96 ± 0.050</td>
<td>1.76 ± 0.152</td>
<td>0.32 ± 0.041</td>
<td>2.09 ± 0.194</td>
</tr>
<tr>
<td>Charcoal</td>
<td>8.33 ± 1.527</td>
<td>2.02 ± 0.050</td>
<td>0.79 ± 0.070</td>
<td>1.33 ± 0.152</td>
<td>0.32 ± 0.015</td>
<td>1.65 ± 0.168</td>
</tr>
<tr>
<td>Coal</td>
<td>5.66 ± 2.081</td>
<td>1.10 ± 0.080</td>
<td>0.43 ± 0.051</td>
<td>1.06 ± 0.121</td>
<td>0.26 ± 0.025</td>
<td>1.29 ± 0.175</td>
</tr>
</tbody>
</table>

Results are mean of three replicates ± SD. Results are significantly different at P = 0.05.
FIG. 1: Effect of different inoculated carrier based materials on population counts (cfu g⁻¹) of *Rhizobium meliloti*.

FIG. 2: Effect of different inoculated carrier based materials on population counts (cfu g⁻¹) of *Rhizobium trifolii*. 
strains and support large populations (Mohamed and Abdel Moniem 2010) Similar results were reported by Stephens and Rask. (2000).

Charcoal supported next to peat i.e. 8.95 and 8.99 for Rhizobium meliloti (MTCC–100) and Rhizobium trifolii (MTCC–905) respectively. Coal proved to be the inferior of all carriers. In coal there was a decline in population count of both fast growing strains. Coal supported 7.28 for Rhizobium meliloti and 7.58 for Rhizobium trifolii. Although charcoal could also support high population density, but was lower than peat. The charcoal (Sparrow and Ham 1983) has adequate carrier for rhizobium but is inferior to peat, probably due to low moisture holding capacity. In the present study coal proved to be the most inferior of all the carriers. Coal does not have the good chemical and physical attributes that bagasillo had. The inherent moisture content and water holding capacity of coal is also low. Coal was not recommend (Halliday and Graham 1978) as an inoculant carrier since it tended to aggregate into hard lumps during storage and to resist wetting at the time of seed inoculation.

**Effectiveness of bagasillo based inoculants with Rhizobium meliloti (MTCC–100) and Rhizobium trifolii (MTCC–905)**

After bioinoculant treatment, enhancement in the growth of both the plants was clearly seen when compared to control. Non treated plants had total weight (g/plant) 1.06 for Medicago sativa and 1.14 for Trifolium alexandrinum and no nodule was found on their roots. The treatment with bagasillo based formulations showed maximum enhancement in the vegetative growth parameters in both the leguminous plants after six weeks. Bagasillo with *Rhizobium meliloti* enhanced the seedling biomass by 47% while with *Rhizobium trifolli* by 55% (Table 2. and 3.). Peat and charcoal with *Rhizobium meliloti* enhanced the seedling biomass by 34% and 28 % respectively and with *Rhizobium trifolii* by 45 % and 30 % respectively. Coal with Rhizobium meliloti showed 08 % increase of seedling biomass and with Rhizobium trifolii seedling biomass was enhanced by 10 %. More numbers and dry weight of nodules in barseem and lucerne plants were reported (Sharma and Verma 1979) when inoculated with lignite based inoculum. Similarly (Pandey and Maheshwari 2007) observed considerable increase in plant biomass, nodule number and weight, and number of pods as compared with individual trials and with the control of the wheat-bran-based multispecies consortium on the growth of pigeon pea.

**CONCLUSION**

This study recommends the byproducts of sugarcane industry, bagasillo as a carrier material because bagasillo supported good survival of both fast growing species of rhizobia i.e. *Rhizobium meliloti* (MTCC-100) and *Rhizobium trifolii* (MTCC-905). The bagasillo inoculants were virtually free from contamination and results of pot experiments indicated that it was more effective than other carriers used in this study. To improve nodulation and biological nitrogen fixation using rhizobial inoculants may prove to be an economical and environmental friendly approach for better yield of leguminous crops.

**REFERENCES**


