EFFECT OF *PSEUDOMONAS* ON VIABILITY OF MICROBIAL (*RHIZOBIAL*) INOCULANTS DURING STORAGE

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ABSTRACT

Two carriers of biological origin were taken and studied that are sheep dropping and goat dropping. Physico-chemical analysis of some carrier of biological origin, which used in *Rhizobium* culture preparation were done. On the basis of physico-chemical properties it can be concluded that S\(_D\) is more suitable carrier than G\(_D\). For evaluation of growth of *Rhizobia* on carriers, two sets were taken *Rhizobial* growth on 100 mesh size S\(_D\) and G\(_D\) carriers at 28\(^\circ\)C and 50\% W.H.C. *Rhizobial + Pseudomonas* growth on 100 mesh size S\(_D\) and G\(_D\) carriers at 28\(^\circ\)C and 50\% W.H.C.

For first set, it was observed on 100 mesh size carriers *rhizobial* population is comparative less for both carriers than their mixed inoculation set. The suitability order for 100 mesh size carriers is S\(_D\) > G\(_D\), from Fig. 1 also similar trend could be seen. The other conclusion which can be drawn from second set observations is, *rhizobium* growth is more when a mixture *Rhizobium & Pseudomonas* was used as inoculants. It is indicated from second set’s observations that G\(_D\) which gives less *rhizobial* population in first set have comparative more *rhizobial* growth as is clear from Fig. 2 that its efficiency increases. It may be +ive influence of Psuedomonas bacteria on population of *rhizobia*, which ultimately increase nodule numbers and weight of plant.

**Key words:** *Pseudomonas, Rhizobial, Inoculant.*

INTRODUCTION

Symbiosis plays an important role in productive agriculture particularly in context of production of pulses and other leguminous crops. *Rhizobium* alone help the production agriculture since eternity. Utilization of atmospheric nitrogen by crops continue to help the humanity and in this century considerable improvement in the technology of utilization of atmospheric nitrogen has made Bio-fertilizer industry eventually to compete with fertilizer

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industry. Bio-fertilizers are vital components of sustainable agriculture. Rhizobia play an important role in nitrogen fixation. Previously peat is considered as an ideal carrier for Rhizobial inoculants, but good quality peat is not available in India. Survival of Rhizobium in coal based type of inoculants was done by various scientists\textsuperscript{1-3}. Later improved type inoculants were developed\textsuperscript{4}. So some carriers of biological origin (sundried, sheep and goat droppings, their manures) were tried as carriers. It is assumed that decomposed product of organic matter is less complex and easily available for Rhizobial growth. Their physico-chemical characteristics were compared with peat which is supposed to be an ideal carrier for Rhizobial inoculants.

Bio-fertilizer help to provide nutrients to soil microorganisms which are required for plant growth. These are of low cost, their application is easy and does not cause pollution. It serve as bio-pesticide, and its viability is maintained for longer period which may increase sustainable agriculture. Now special emphasis is given to new concept i.e. inoculation by group of associated bacteria. The inoculants should be compatible with each other and will be able to grow simultaneously without any inhibition in growth. Positive result of mixed inoculation was found on bean and pea\textsuperscript{5}. There was an associative positive affect of rhizobium and PSB on yield and nutrient uptake by chickpea\textsuperscript{6}. Co-inoculation on soyabean was also tried\textsuperscript{7}. Combined inoculation was found to be superior than individual inoculation\textsuperscript{8}. Here in Beawar (Rajasthan) most of farmer grow gram crop. Rhizobium and phosphate solubilizing bacteria are important to plant nutrition and play a significant role as plant growth promoting rhizobacteria (PGPR) in bio-fertilization of crops. It was found Pseudomonas enhance growth of mustard\textsuperscript{9}. A study is proposed to conducted on a suitable sterilized carrier (100 mesh sheep and goat droppings) to investigate the effects on growth of rhizobial isolates for gram and pea, if they are used in combination of Pseudomonas (P solubilizer bacteria).

**EXPERIMENTAL**

**Methods and materials**

(a) **Preparation of carrier samples**: All the carriers sample sun dried on the roof top, then gently crushed with the help of wooden hammer, standard sieving were done of 80 and 100 mesh dimensions. The samples were kept in polythene bags

(b) **Methods of analysis**: Determination of physico- chemical properties namely pH, bulk density, particle density, W.H.C. percentage of organic matter, total Nitrogen, P\textsubscript{2}O\textsubscript{5} content were carried out by adopting appropriate standard methodologies.
Procedure for testing suitability of carriers

- Inoculated 50 mL sterilized Y.E.M. broth with \( R_G \) (gram) or \( R_P \) (pea) isolate and incubates for 7 days.
- Took 20 g of each carrier in beaker. Add to \( S_D \), \( G_D \), 1.5 g of \( \text{CaCO}_3 \) each. To \( S_M \), \( G_M \) were added 14 mL, 22 mL, of \( \text{N}/10 \text{ H}_2\text{SO}_4 \) respectively for, the purpose of neutralization.
- Sterilized these carries at 15 lbs pressure. For 4 hours on three alternate days. For maintenance of 50% waters holding capacity of sterilized material of \( S_D \), \( G_D \), 32.0, 34.4 mL of sterile water mixed, respectively. In which there was 2.5 mL as inoculum of \( R_G \) (g) or \( R_P \) (pea) isolate was added.
- Mixed each material by separate sterile glass rods, each inoculated carrier was divided into 7 equal parts, out of which 6 part of each carrier were packed in polythene bags and placed at 28°C.
- From 7th part of each withdrew one gm inoculated material and plated as usual for initial population of the inoculum. At subsequent interval of 10, 20, 30, 60 days the enumeration of \textit{Rhizobia} population is done using one packet of each at a time.

RESULTS AND DISCUSSION

Physico-chemical analysis of some carrier of biological origin carriers used in \textit{Rhizobium} culture prepration

(i) As shown in Table 1 there is an increase in bulk density from \( G_D \) to \( S_D \), it can be justified on the basis of organic matter %, as the organic matter % decreases the material becomes denser\(^{10}\).

(ii) Porosity percentage can be correlated with organic matter percentage, as organic matter % decreases it decreases, which is clear by porosity % of carriers shown in Table 1.

(iii) General trend regarding W.H.C., as organic matter % increases, it increases. It is observed W.H.C. of \( G_D \) \( S_D \).

(iv) The organic matter % of \( G_D \) is less than \( S_D \). as indicated in Table 1.

(v) Nitrogen percentage of \( G_D \) is less than \( S_D \). But C/N ratio for \( G_D \) is more than; \( S_D \). Positives effect of Nitrogen sources on \( \text{N}_2 \) fixation was observed\(^{11}\). Slow
growing strains of *Rhizobium* were found to be stimulated by mixture of amino acids\(^{12}\). Addition of ammonium sulphate was found to improve *rhizobial* counts in carrier materials\(^{13}\). It was also reported C/N ratio 17 to 50 is appropriate to *rhizobial* growth.

(vi) P\(_2\)O\(_5\) percentage is more G\(_D\) for than S\(_D\).

(vii) So on the basis of physicochemical properties it can be concluded that S\(_D\) (100 mesh size) is more suitable carrier than G\(_D\) 100 mesh size).

Table 1: Physico-chemical analysis of some carrier of biological origin carriers used in *Rhizobium* culture preparation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mesh size</th>
<th>G(_D)</th>
<th>S(_D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density</td>
<td>100</td>
<td>0.37</td>
<td>0.35</td>
</tr>
<tr>
<td>Particle Density</td>
<td>100</td>
<td>1.87</td>
<td>1.49</td>
</tr>
<tr>
<td>Porosity %</td>
<td>100</td>
<td>74.95</td>
<td>80.91</td>
</tr>
<tr>
<td>W.H.C. %</td>
<td>100</td>
<td>305.5</td>
<td>329.12</td>
</tr>
<tr>
<td>Organic matter %</td>
<td>100</td>
<td>61.0</td>
<td>77.5</td>
</tr>
<tr>
<td>N%</td>
<td>100</td>
<td>2.05</td>
<td>3.42</td>
</tr>
<tr>
<td>P(_2)O(_5)%</td>
<td>100</td>
<td>0.48</td>
<td>0.2</td>
</tr>
<tr>
<td>C / N ratio</td>
<td>100</td>
<td>17.3</td>
<td>13.17</td>
</tr>
<tr>
<td>Ash %</td>
<td>100</td>
<td>32.00</td>
<td>22.5</td>
</tr>
<tr>
<td>pH</td>
<td>100</td>
<td>6.1</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Evaluation of growth of *Rhizobium & Rhizobium* + PS bacteria on cow urine treated & untreated sterilized carriers of biological origins to check the viability of *rhizobia* during storage of carriers

In Table 2 Population data of *Rhizobial* isolate on S\(_D\) & G\(_D\) which were biological carriers of 100 mesh size at 28\(^\circ\)C and 50% W.H.C. has been given after 10, 20, 30, 60 days.

Comparison of wS\(_D\) & G\(_D\) up to 60 days S\(_D\) proves better than G\(_D\), organic matter content, W.H.C. of S\(_D\) is more than G\(_D\), simultaneously aeration is more in S\(_D\) which are essential requirement for suitable carrier as is seen in Fig. 1. One more drawback of goat dropping is its susceptibility to contamination, so *rhizobial* population decreases abruptly.
For S\textsubscript{D} upto 10 days there is an abrupt increase in rhizobial population, then gradually it decreases. After 60 days it proves even better than goat dung as is clear in Table 2. The suitability order is S\textsubscript{D} > G\textsubscript{D} from Fig. 1 similar trend could be seen finally it may be concluded from the above observations that the sheep dung of 100 mesh size is more suitable carrier and can be used efficiently as rhizobial inoculant.

**Table 2: Population data of *Rhizobial* isolate on different biological carriers of 100 mesh size at 28°C and 50% water holding capacity.**

<table>
<thead>
<tr>
<th>Carriers</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep dropping</td>
<td>5.0 x 10^5</td>
<td>6600 x 10^5</td>
<td>4000 x 10^5</td>
<td>65 x 10^5</td>
<td>15 x 10^5</td>
</tr>
<tr>
<td>Goat dropping</td>
<td>2.5 x 10^5</td>
<td>3000 x 10^5</td>
<td>1820 x 10^5</td>
<td>40 x 10^5</td>
<td>4 x 10^5</td>
</tr>
</tbody>
</table>

![Fig. 1: Population data x 10^5 of *Rhizobial* isolate on different biological carriers of 100 mesh size at 28°C and 50% water holding capacity](image)

Table 3 indicates population data of *rhizobial* isolate in which inoculant is a mixture of *rhizobial* isolate + *pseudomonas* isolate on sterilized sheep dropping and goat dropping as carriers material of 100 mesh size at 28°C and 50% water holding capacity is maintained by sterilized water. Data shows after 10 days there is sudden increase in population of *rhizobia* 10^4 to 10^8, both in S\textsubscript{D} and G\textsubscript{D} which is maintained upto 20 days. Then after 30 days it decreases upto 10^7. Carriers in which only *Rhizobium* inoculant is used it was observed
though they are inoculated with high population of *rhizobia* there is gradual decrease in population more rapidly than of mixed inoculation. It indicates -

**Table 3: Population data of *Rhizobial* isolate + *Pseudomonas* isolate on different biological carriers of 100 mesh size at 28°C and 50% water holding capacity.**

Population (after days)

<table>
<thead>
<tr>
<th>Carriers</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep dr.</td>
<td>3.0 x 10⁴</td>
<td>85000 x 10⁴</td>
<td>40000 x 10⁴</td>
<td>6500 x 10⁴</td>
<td>1000 x 10⁴</td>
</tr>
<tr>
<td>Goat dr.</td>
<td>3.5 x 10⁴</td>
<td>45000 x 10⁴</td>
<td>12000 x 10⁴</td>
<td>4000 x 10⁴</td>
<td>420 x 10⁴</td>
</tr>
</tbody>
</table>

- Mixed inoculation works which was confirmed by pot experiments, number of nodules as well as plant weight is more in the plants of pot where seeds were inoculated by mixed culture’s broth. It may be of + ve influence of Psuedomonas bacteria on population of *rhizobia* which ultimately increase nodule numbers and weight of plant.

- Phosphate solubilizing effect of Psuedomonas bacteria play an important role in rise of *rhizobial* population.

**Fig. 2**

- If we compare experiments, which were conducted without Psuedomonas bacteria, and the population data of *rhizobial* isolate in which inoculant was a mixture of *rhizobial* isolate + *pseudomonas* isolate on sterilized sheep dropping
and goat dropping as carriers materials of 100 mesh size at 28°C and 50% water holding capacity, we found more promising results as seen in Fig. 2. Even in GD there is abrupt rise in rhizobial population up to two months.

- It may be + ve influence of Psuedomonas bacteria on population of rhizobia which ultimately increase nodule numbers and weight of plant in which co-inoculation was carried out.

REFERENCES


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