Collection of nodules and plant sample for *Rhizobium* population of Gird

In the present study, survey of the native *Rhizobium* sp population was undertaken in the gird region of Madhya Pradesh, where non–traditional legume crops plant *Cowpea* (*Vigna unguiculata*), *Guar* (*Cyamopsis tetragonoloba*) and *Moth bean* (*Vigna aconitifolia*) are cultivated in kharif season. Systematically, we have visited the field of Gwalior, Morena, Bhitarwar, Shivpurl, Guna, Bhind and Dabra Gird region of Madhya Pradesh in the month of September to October 12 and 13. Fifteen villages for *cowpea*, fifteen villages for *guar*, fifteen villages for *moth bean* were selected, names of places are given in table 1. Geographical situations are shown in the map of Madhya Pradesh and Gird region of district Gwalior which are taken into consideration in this study.

During survey, it was observed that most of the samples of root nodules of *cowpea* were globular, pink and big in size indicating the presence of effective *Rhizobium*, Few *guar* or *cluster bean* and *moth bean* root nodules samples were big, pinkish and in groups however we also found few samples with either absence of nodules or presence of only small size white nodules indicating either absence or very poor population of *Rhizobium*. The details samples of *cow pea*, *guar* and *moth bean* with the numbers of root nodules per plant is given in table 1.
AGRO-CLIMATIC ZONES OF MADHYA PRADESH
GIRD REGION

Fig 5 : Agro-Climatic Zones of Madhya Pradesh
### TABLE 1: Details of sample place of cow pea, Guar or cluster bean and moth bean, and number of nodules

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Crop</th>
<th>Place of sample collection</th>
<th>Nos of Nodules</th>
<th>Type of Nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cowpea</td>
<td>Ghati Gaon, Gwalior, MP India</td>
<td>11</td>
<td>#About 50% nodules were globular large and pink rest was small white</td>
</tr>
<tr>
<td>2.</td>
<td>Cowpea</td>
<td>Mohna Gaon, Gwalior, MP India</td>
<td>12</td>
<td>#Almost 75% nodule were globular and pink</td>
</tr>
<tr>
<td>3.</td>
<td>Cowpea</td>
<td>Baroley Gaon, Gwalior, MP India</td>
<td>10</td>
<td>#50% were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>4.</td>
<td>Cowpea</td>
<td>Chinor Gaon, Dabra MP, India</td>
<td>11</td>
<td>#50% were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>5.</td>
<td>Cowpea</td>
<td>Chhimak Gaon, Dabra MP, India</td>
<td>5</td>
<td>Very poor small white nodules</td>
</tr>
<tr>
<td>6.</td>
<td>Cowpea</td>
<td>Amrol Gaon, Dabra MP, India</td>
<td>11</td>
<td>7 were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>7.</td>
<td>Cowpea</td>
<td>Magroni Gaon, Shivpuri MP, India</td>
<td>12</td>
<td>#50% were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>8.</td>
<td>Cowpea</td>
<td>Deegod Gaon, Shivpuri MP, India</td>
<td>13</td>
<td>#All were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>9.</td>
<td>Cowpea</td>
<td>Payaga Gaon, Shivpuri MP, India</td>
<td>10</td>
<td>Very poor small white nodules</td>
</tr>
<tr>
<td>10.</td>
<td>Cowpea</td>
<td>Piprauda Gaon, Guna, MP, India</td>
<td>13</td>
<td>7 were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>11.</td>
<td>Cowpea</td>
<td>Vinayak khedi Gaon, Guna, MP, India</td>
<td>8</td>
<td>Only 3 were globular medium &amp; pink nodules</td>
</tr>
<tr>
<td>12.</td>
<td>Cowpea</td>
<td>Aron District, Guna, MP India</td>
<td>10</td>
<td>#60% were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>13.</td>
<td>Cowpea</td>
<td>Saktpur, Guna, MP, India</td>
<td>7</td>
<td>Only 3 were globular medium &amp; pink nodules</td>
</tr>
<tr>
<td>14.</td>
<td>Cowpea</td>
<td>Roopsahakapura Gaon, Bhind MP India</td>
<td>13</td>
<td>#All were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>15.</td>
<td>Cowpea</td>
<td>Saroda Dist, Ashoknagar, MP, India</td>
<td>9</td>
<td>50% were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>16.</td>
<td>Guar</td>
<td>Jhau khadi Gaon Bhitarwar, MP India</td>
<td>20</td>
<td>#Nodules in group but only very few are pink</td>
</tr>
<tr>
<td>17.</td>
<td>Guar</td>
<td>Bhejana Gaon Bhitarwar MP India</td>
<td>18</td>
<td>#Effective pinkish large size nodules</td>
</tr>
<tr>
<td>18.</td>
<td>Guar</td>
<td>Jheel Bareda Gaon Bhitarwar, MP India</td>
<td>22</td>
<td>#Nodules in groups and pinkish</td>
</tr>
<tr>
<td>19.</td>
<td>Guar</td>
<td>Wagh Wai Gaon Bhitarwar, MP India</td>
<td>20</td>
<td>Very poor small size nodules</td>
</tr>
<tr>
<td>20.</td>
<td>Guar</td>
<td>Tiktoli Gurjar Gaon, Morena, MP India</td>
<td>20</td>
<td>Very poor small white nodules</td>
</tr>
<tr>
<td>21.</td>
<td>Guar</td>
<td>Sumavali Gaon, Morena, MP India</td>
<td>19</td>
<td>7 were true large &amp; pink nodules</td>
</tr>
<tr>
<td>No.</td>
<td>Description</td>
<td>Location</td>
<td>Count</td>
<td>Description</td>
</tr>
<tr>
<td>-----</td>
<td>--------------------------------------------------</td>
<td>---------------------------------</td>
<td>-------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>22.</td>
<td>Guar</td>
<td>Ghuraiya Basai Morena, MP India</td>
<td>16</td>
<td>#50% were true large &amp; pink nodules</td>
</tr>
<tr>
<td>23.</td>
<td>Guar</td>
<td>Baroley Gaon, Gwalior, MP India</td>
<td>22</td>
<td>#All were true large &amp; pink nodules</td>
</tr>
<tr>
<td>24.</td>
<td>Guar</td>
<td>Ghati Gaon, Gwalior, MP India</td>
<td>20</td>
<td>Very poor small white nodules</td>
</tr>
<tr>
<td>25.</td>
<td>Guar</td>
<td>Mohna Gaon, Gwalior, MP India</td>
<td>13</td>
<td>7 were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>26.</td>
<td>Guar</td>
<td>Aron District, Guna, MP India</td>
<td>20</td>
<td>Only 3 were globular medium &amp; pink nodules</td>
</tr>
<tr>
<td>27.</td>
<td>Guar</td>
<td>Saktpur Gaon, Guna, MP, India</td>
<td>20</td>
<td># 60% were true large &amp; pink nodules</td>
</tr>
<tr>
<td>28.</td>
<td>Guar</td>
<td>Payaga Gaon, Shivpuri M.P, India</td>
<td>18</td>
<td>Only 3 were medium &amp; pink nodules</td>
</tr>
<tr>
<td>29.</td>
<td>Guar</td>
<td>Piprauda Gaon, Guna, MP, India</td>
<td>18</td>
<td>All were true effective large &amp; pink nodules</td>
</tr>
<tr>
<td>30.</td>
<td>Guar</td>
<td>Saroda Dist, Ashoknagar, MP, India</td>
<td>14</td>
<td>50% were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>31.</td>
<td>Moth Bean</td>
<td>Phoop Gaon, Bhind, MP India</td>
<td>14</td>
<td># 70% were true large &amp; pink nodules</td>
</tr>
<tr>
<td>32.</td>
<td>Moth Bean</td>
<td>Parsono Gaon, Bhind MP India</td>
<td>20</td>
<td>Nodules in group but only very few are pink</td>
</tr>
<tr>
<td>33.</td>
<td>Moth Bean</td>
<td>Heeratalpura Gaon, Bhind, MP India</td>
<td>13</td>
<td>7 were true globular large &amp; pink nodules</td>
</tr>
<tr>
<td>34.</td>
<td>Moth Bean</td>
<td>Saktpur Gaon, Guna, MP India</td>
<td>20</td>
<td>Only 3 were globular medium &amp; pink nodules</td>
</tr>
<tr>
<td>35.</td>
<td>Moth Bean</td>
<td>Kachodhara, Bhind, MP India</td>
<td>18</td>
<td>All were true effective large &amp; pink nodules</td>
</tr>
<tr>
<td>36.</td>
<td>Moth Bean</td>
<td>Roopsahakapura Gaon, Bhind MP India</td>
<td>20</td>
<td># 60% were true large &amp; pink nodules</td>
</tr>
<tr>
<td>37.</td>
<td>Moth Bean</td>
<td>Saroda Dist, Ashoknagar, MP India</td>
<td>16</td>
<td>Only 3 were medium &amp; pink nodules</td>
</tr>
<tr>
<td>38.</td>
<td>Moth Bean</td>
<td>Gwalior Research Farm College of Agriculture Gwalior, MP, India</td>
<td>22</td>
<td># All were true large &amp; pink nodules</td>
</tr>
<tr>
<td>39.</td>
<td>Moth Bean</td>
<td>Bangala, Gaon, Bhind MP India</td>
<td>22</td>
<td>Nodules in group but only very few are pink</td>
</tr>
<tr>
<td>40.</td>
<td>Moth Bean</td>
<td>Bilhaura Gaon, Bhind MP India</td>
<td>20</td>
<td># Effective pinkish large size nodules</td>
</tr>
<tr>
<td>41.</td>
<td>Moth Bean</td>
<td>Rau, Indore, MP India</td>
<td>20</td>
<td>Very poor small size nodules</td>
</tr>
<tr>
<td>42.</td>
<td>Moth Bean</td>
<td>Saktpur, Guna, MP, India</td>
<td>20</td>
<td>Very poor small white nodules</td>
</tr>
<tr>
<td>43.</td>
<td>Moth Bean</td>
<td>Didhi, Gaon, Bhind MP India</td>
<td>19</td>
<td>7 were true large &amp; pink nodules</td>
</tr>
<tr>
<td>44.</td>
<td>Moth Bean</td>
<td>Baroli Gaon Ashoknagar, MP, India</td>
<td>20</td>
<td>Very poor small white nodules</td>
</tr>
<tr>
<td>45.</td>
<td>Moth Bean</td>
<td>Saroda Dist, Ashoknagar, MP, India</td>
<td>18</td>
<td>50% were true large &amp; pink nodules</td>
</tr>
</tbody>
</table>
TABLE 2 : Details of *Rhizobium* sp isolated from various collection site

<table>
<thead>
<tr>
<th>S. No</th>
<th>Place of sample collected</th>
<th>Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ghati Gaon, Gwalior, MP India</td>
<td>1 (Isolate CP-1)</td>
</tr>
<tr>
<td>2</td>
<td>Mohna Gaon, Gwalior, MP India</td>
<td>2 (Isolate CP-2 &amp; CP-3)</td>
</tr>
<tr>
<td>3</td>
<td>Baroley Gaon, Gwalior, MP India</td>
<td>1 (Isolate CP-4)</td>
</tr>
<tr>
<td>4</td>
<td>Chinor Gaon, Dabra MP, India</td>
<td>1 (Isolate CP-5)</td>
</tr>
<tr>
<td>5</td>
<td>Magroni Gaon, Shivpuri MP, India</td>
<td>1 (Isolate CP--6)</td>
</tr>
<tr>
<td>6</td>
<td>Deegod Gaon, Shivpuri MP, India</td>
<td>2 (Isolate CP-7 &amp;CP--8)</td>
</tr>
<tr>
<td>7</td>
<td>Aron District, Guna, MP India</td>
<td>2 (Isolate CP-9 &amp; CP-10)</td>
</tr>
<tr>
<td>8</td>
<td>Roopsahkapura Gaon, Bhind MP India</td>
<td>1 (Isolate CP -11)</td>
</tr>
<tr>
<td>9</td>
<td>Jhau khadi Gaon Bhitarwar MP, India</td>
<td>1 (Isolate CB-1)</td>
</tr>
<tr>
<td>10</td>
<td>Bhejana Gaon Bhitarwar MP India</td>
<td>1 (Isolate CB-2)</td>
</tr>
<tr>
<td>11</td>
<td>Jheel Bareda Gaon Bhitarwar MP India</td>
<td>2 (Isolate CB-3 &amp;CB-4)</td>
</tr>
<tr>
<td>12</td>
<td>Ghuraiya Basai Morena, MP India</td>
<td>2 (Isolate CB-5&amp;CB-6)</td>
</tr>
<tr>
<td>13</td>
<td>Baroley Gaon, Gwalior, MP India</td>
<td>1 (Isolate CB-7)</td>
</tr>
<tr>
<td>14</td>
<td>Saktpur, Guna, MP, India</td>
<td>1 (Isolate CB-8)</td>
</tr>
<tr>
<td>15</td>
<td>Phoop Gaon, Bhind, MP India</td>
<td>1 (Isolate MB-1)</td>
</tr>
<tr>
<td>16</td>
<td>Roopsahkapura Gaon, Bhind MP India</td>
<td>2 (Isolate MB-2 &amp; MB-3)</td>
</tr>
<tr>
<td>17</td>
<td>Gwalior Reasearch Farm College of Agriculture Gwalior, MP, India</td>
<td>2 (Isolate MB-4 &amp; MB-5)</td>
</tr>
<tr>
<td>18</td>
<td>Bilhzura Gaon, Bhind MP India</td>
<td>1 Isolate MB-6)</td>
</tr>
</tbody>
</table>
Isolation of *Rhizobium* sp from root nodules

On the basis of their morphology, sticky appearance and the production of mucous *Rhizobium* sp. were selected for further studies. These isolated strains were further cultured on YEMA medium containing 0.0025% (w/v) Congo red (figure 6,7,8). The strains which were not taking the red dyes were further used for nodulation ability by pot nodulation test for further confirming as *Rhizobium* sp.

The *Rhizobium* colonies were identified by their morphology, sticky appearance showing the production of mucous although at lower levels. All isolates were Gram negative and rod shape under microscope (figure 9). Initially there were 11 strains (CP1-11) isolated from *cow pea*, 8 strains from *cluster bean* (CB 1-8) and only 6 strains were isolated from *moth bean* (MB1-6) (Table 2).

Colony characters include Colony Size, Surface, Margin, Elevation, Optical features and Gram staining are given in Table 3.

**Pot Nodulation (Cross Nodulation)**

**Results of nodulation by *rhizobium* sp in pot nodulation test**: In pot nodulation experiments a total of 25 isolates i.e 11 strains from *cow pea*, 8 strains from *guar cluster bean* and 6 strains from *moth bean* were tested for nodulation ability as well as cross nodulation.

Observations made after 45 days, show that all the isolates were not able to form nodules. No nodulation was observed in control plant. *Cowpea Rhizobium* isolates CP - 2, 5, 6 and, 10 successfully produced large pink nodules while isolates 1, 3, 4, 7, 8, 9 and 11 failed to do so. Out of 8 isolates from *cluster bean* only two CB – 3,7 induced nodulation. Similarly in *moth bean* isolates MB 1 and 3 produced nodulation while 2, 4, 5, 6 could not nodule in pot experiment.

The isolated 4 strains of *cowpea* (Now called as R1, R2,R3 and R4), 2 strains of *cluster bean* (R5 ,R6) and 2 strains of *moth bean* (R7,R8) were considered as true *Rhizobium* and used for biochemical analysis.
Fig 6 : Colony morphology of *Rhizobium Cowpea*

Fig 7 : Colony morphology of *Rhizobium Guar or Cluster Bean.*

Fig 8 : Colony morphology of *Rhizobium moth bean*
Fig 9: Light microscopic view (40X) of Gram staining slide of *Rhizobium* sp.  
(A) Cowpea, (B) Guar or Cluster Bean and (C) Mothbean
Fig 10: Nodules of *cowpea* (A) with *Rhizobium* sp., (B) control without inoculation

Fig 11: Nodules of *Guar* or *cluster bean* (A) with *Rhizobium* sp., (B) Control without Inoculation

Fig 12: Nodules of *Moth bean* (A) with *Rhizobium* sp., (B) control without inoculation
Table 3: Colony Characteristics of \textit{Rhizobial} strains under study

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Colony Size</th>
<th>Surface</th>
<th>Margin</th>
<th>Elevation</th>
<th>Optical Features</th>
<th>Gram Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>Spread</td>
<td>Creamy</td>
<td>Smooth</td>
<td>Raised</td>
<td>Opaque</td>
<td>Negative</td>
</tr>
<tr>
<td>R-2</td>
<td>Medium</td>
<td>Shiny</td>
<td>Smooth</td>
<td>Raised</td>
<td>Opaque</td>
<td>Negative</td>
</tr>
<tr>
<td>R-3</td>
<td>Spread</td>
<td>Shiny</td>
<td>Smooth</td>
<td>Raised</td>
<td>Opaque</td>
<td>Negative</td>
</tr>
<tr>
<td>R-4</td>
<td>Medium</td>
<td>Shiny</td>
<td>Smooth</td>
<td>Raised</td>
<td>Translucent</td>
<td>Negative</td>
</tr>
<tr>
<td>R-5</td>
<td>Medium</td>
<td>Shiny</td>
<td>Smooth</td>
<td>Raised</td>
<td>Opaque</td>
<td>Negative</td>
</tr>
<tr>
<td>R-6</td>
<td>Spread</td>
<td>Creamy</td>
<td>Smooth</td>
<td>Raised</td>
<td>Opaque</td>
<td>Negative</td>
</tr>
<tr>
<td>R-7</td>
<td>Pinpoint</td>
<td>Shiny</td>
<td>Smooth</td>
<td>Raised</td>
<td>Opaque</td>
<td>Negative</td>
</tr>
<tr>
<td>R-8</td>
<td>Medium</td>
<td>Shiny</td>
<td>Smooth</td>
<td>Raised</td>
<td>Opaque</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Cross inoculation study showed that all 4 \textit{cowpea} strain found nodulation ability only in \textit{cowpea} crop and no nodule formation in \textit{cluster bean} and \textit{moth bean}. However both \textit{cluster bean} and \textit{moth bean} found nodulation ability in each other but no nodulation in \textit{cowpea}. \textit{Cowpea} nodules also have different morphology than \textit{cluster bean} and \textit{moth bean}. Either of strains isolated from \textit{cluster bean} or \textit{moth bean}, however, may be used for good nodulation in either crop.

Fig 13 : Cross inoculation of \textit{cowpea, guar & mothbean}
Fig 14: Pot Nodulation test (A) cowpea, (B) Guar or cluster bean , (C) mothbean
Fig 15: Roots of *Vigna unguiculata* showing nodules developed by symbiotic bacteria *Rhizobium* (R1, R2, R3 & R4 strain cowpea)

Fig 16: Roots of *Cyamopsis tetragonoloba* & *Vigna aconitifolia* showing nodules developed by symbiotic bacteria *Rhizobium* (R5, R6 strain guar & R7, R8 mothbean)
Enzymatic activity of *Rhizobial* strains:

Enzymatic activity in all the 8 (R1-R8) strains was tested and the results are presented in Table 5. It was observed that bubbles appeared within 20 seconds when 2-3 drops of 3% hydrogen peroxide was added to the isolates on glass slide indicating that all isolates gave positive test catalase activity. All isolates except R6 showed gelatinase enzyme negative test. *Rhizobium* isolates R2, R3, R6, R7 and R8 were protease positive, whereas R1, R4 and R5 showed negative results. *Rhizobium* isolates R2 and R4 showed amylase positive test gave clear zone alone line of growth and changes colour of the medium on flooding with iodine solution. whereas R1, R3, R5, R6, R7 and R8 showed negative test. All the isolates gave negative results for lipase, pectinase and chitinase activity while single isolates gave positive results for cellulose (R6) cellulase activity.

Table 4: Enzymatic activity of *Rhizobial* strains

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Catalase</th>
<th>Gelatinase</th>
<th>Protease</th>
<th>Amylase</th>
<th>Lipase</th>
<th>Cellulase</th>
<th>Pectinase</th>
<th>Chitinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-3</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-4</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-7</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-8</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Fig 17: Proteolytic activity of selected bacterial strains

Siderophore production and HCN production of selected *Rhizobial* strains:

Experimental results on Siderophore and HCN production are given in table 5. *Rhizobial* strain. R3, R8 alone gave positive results for Siderophore, production (Fig 18) whereas all isolates were negative for HCN production.

**Table 5: Siderophore production and HCN production of selected *Rhizobial* strains**

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Siderophore production</th>
<th>HCN production</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>R-4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-8</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Qualitative Phosphate solubilization by different *Rhizobial* strains:

On the basis of qualitative Phosphate solubilization by different Rhizobial strains, results showed that R2, R3, R5, R7 and R8 show good solubilization while strain R1, R4 and R6 showing very poor results.

Table 6: Qualitative Phosphate solubilization by different *Rhizobial* strains:

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Qualitative Phosphate Solubilization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>R-1</td>
<td>++</td>
</tr>
<tr>
<td>R-2</td>
<td>++</td>
</tr>
<tr>
<td>R-3</td>
<td>++</td>
</tr>
<tr>
<td>R-4</td>
<td>++</td>
</tr>
<tr>
<td>R-5</td>
<td>++</td>
</tr>
<tr>
<td>R-6</td>
<td>++</td>
</tr>
<tr>
<td>R-7</td>
<td>++</td>
</tr>
<tr>
<td>R-8</td>
<td>++</td>
</tr>
</tbody>
</table>
Quantitative average Phosphate solubilized by different strains:

Studies on quantitative average Phosphate solubilization was carried out using R2, R3, R5, R7 and R8 by spectrometric analysis and the average amount of Phosphate solubilized by each strains with age are given in table 7.

On first day maximum average Phosphate solubilization by *Rhizobial* strains was recorded as between 24.82 µg/ml (R2) and 87.97 µg/ml (R7). On 3rd day as 223.56 µg/ml (R2) followed by 148.85 µg/ml (R3) 143.68 µg/ml (R7) 97.72 µg/ml (R8) 81.47 µg/ml (R5) on 5th day. Maximum P-solubilization was recorded as 230.26 µg/ml (R2), 164.45µg/ml(R3), 139.10µg/ml (R7) 106.10µg/ml (R8) 79.65 µg/ml (R5). On 7th day. Amount of P-solubilized varied from 33.08 µg/ml (R5) to 106.99 µg/ml (R7) and from 46.01 µg/ml (R3) to 91.12µg/ml (R7) in 7th day cultures respectively. Average Phosphate solubilization capacity of strain R2 was maximum while minimum with R5 *Rhizobium* strains (table 8).

Phosphate solubilization by eight *Rhizobium* strains was observed on 1, 3, 5, 7, and 10th day after inoculation. In general as the strains showed gradual increase in solubilization of Phosphate up to 5th day.
Table 7: Quantitative average Phosphate solubilized by different strains

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; day</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; day</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>7&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>10&lt;sup&gt;th&lt;/sup&gt; day</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-2</td>
<td>24.82</td>
<td>223.56</td>
<td>230.26</td>
<td>97.30</td>
<td>85.13</td>
</tr>
<tr>
<td>R-3</td>
<td>29.12</td>
<td>148.85</td>
<td>164.45</td>
<td>67.43</td>
<td>46.01</td>
</tr>
<tr>
<td>R-5</td>
<td>35.68</td>
<td>81.47</td>
<td>79.65</td>
<td>33.08</td>
<td>62.20</td>
</tr>
<tr>
<td>R-7</td>
<td>87.97</td>
<td>143.68</td>
<td>139.10</td>
<td>106.99</td>
<td>91.12</td>
</tr>
<tr>
<td>R-8</td>
<td>29.49</td>
<td>97.72</td>
<td>106.10</td>
<td>81.17</td>
<td>54.59</td>
</tr>
</tbody>
</table>

Blank

R-2

R-3

R-5

R-7

R-8

R-4

Fig 20: Quantitative Phosphate Estimation by Rhizobial strains along with a low solubilizer (R-4)
Chart 1: Comparative Phosphate-solubilization by 5 Rhizobial isolates on 1\textsuperscript{st}, 3\textsuperscript{rd}, 5\textsuperscript{th}, 7\textsuperscript{th}, 10\textsuperscript{th} day

Motility Test of Rhizobial strains:

Data on Motility Test of selected Rhizobial strains is presented in Table 21. show that all isolates R1-R8 strains were motile in which R5 was highly motile with a zone size of 1.2 cm Strain R1 was less motile (zone size 0.5 cm) The zone of motility of R3, R7, R4, R2 strain was recorded as 1.0 cm 0.9 cm, 0.8 cm, 0.8 cm 0.7 cm, 0.6 cm respectively.

Table 8: Motility Test of Rhizobial strains

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Motility Test</th>
<th>Zone (in cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>+</td>
<td>0.5</td>
</tr>
<tr>
<td>R-2</td>
<td>+</td>
<td>0.6</td>
</tr>
<tr>
<td>R-3</td>
<td>+</td>
<td>0.8</td>
</tr>
<tr>
<td>R-4</td>
<td>+</td>
<td>0.7</td>
</tr>
<tr>
<td>R-5</td>
<td>+</td>
<td>1.2</td>
</tr>
<tr>
<td>R-6</td>
<td>+</td>
<td>1.0</td>
</tr>
<tr>
<td>R-7</td>
<td>+</td>
<td>0.8</td>
</tr>
<tr>
<td>R-8</td>
<td>+</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Quantitative estimation of Auxin production by Rhizobial strains

Results on quantitative analysis of auxin production (Fig 22) Isolated strain of Rhizobium R1- R8 recorded with different levels of auxin production (Table 9) Auxin production by different Rhizobial strains were estimated at 530 nm. Maximum auxin production of 42.37 µg/ml was shown by R1 strain followed on 24.53µg/ml (R6) 22.08ug/ml (R5) 15.54 µg/ml (R8) 14.59 µg/m R4, 14.36 µg/ml( R3 ) and 12.88 µg/ml (R2). Minimum auxin production was shown by 11.34 µg/ml (R7)

In between them R6-24.53µg/ml,R5- 22.08ug/ml,R8- 15.54 µg/ml ,R4- 14.59 µg/ml,R3 -14.36 µg/ml and R2- 12.88 µg/ml were as recorded.

Table 9: Quantitative estimation of Auxin production by Rhizobial strains

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Auxin production (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>42.37</td>
</tr>
<tr>
<td>R-2</td>
<td>12.88</td>
</tr>
<tr>
<td>R-3</td>
<td>14.36</td>
</tr>
<tr>
<td>R-4</td>
<td>14.59</td>
</tr>
<tr>
<td>R-5</td>
<td>22.08</td>
</tr>
<tr>
<td>R-6</td>
<td>24.53</td>
</tr>
<tr>
<td>R-7</td>
<td>11.34</td>
</tr>
<tr>
<td>R-8</td>
<td>15.54</td>
</tr>
</tbody>
</table>
Fig 22: Auxin Production test of *Rhizobial* strains

Chart 2: Quantification of Auxin production by *Rhizobial* strains
Biofilm production by *rhizobial* strains:

Maximum quantity of Biofilm produced by 17.796 µg/ml R1, R6 and R7 strains followed on R5, R4, R2 and R3 strains. Minimum Biofilm production was 11.940 µg/ml R8.

**Table 10: Biofilm Production by rhizobial strains**

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Biofilm production(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>17.796</td>
</tr>
<tr>
<td>R-2</td>
<td>15.768</td>
</tr>
<tr>
<td>R-3</td>
<td>15.444</td>
</tr>
<tr>
<td>R-4</td>
<td>16.104</td>
</tr>
<tr>
<td>R-5</td>
<td>16.800</td>
</tr>
<tr>
<td>R-6</td>
<td>17.772</td>
</tr>
<tr>
<td>R-7</td>
<td>17.268</td>
</tr>
<tr>
<td>R-8</td>
<td>11.940</td>
</tr>
</tbody>
</table>

**Chart 3: Quantitative biofilm production of *Rhizobial* Strains**
Quantitative estimation of EPS production:

Quantitative estimation of EPS production by Rhizobial strains were observed spectrometrically at 490 nm. Maximum EPS production was shown as 689.97µg/ml by R4 and minimum production was shown as 248.95µg/ml by R8. While with other Rhizobial strains recorded as 650.97µg/ml-R2, 420.27µg/ml-R3, 417.30 µg/ml-R7, 387.07 µg/ml-R5, 368.55µg/ml-R6 and 282.75µg/ml-R1.

Table 11: Quantitative estimation of EPS production

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>EPS production (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>282.75</td>
</tr>
<tr>
<td>R-2</td>
<td>650.97</td>
</tr>
<tr>
<td>R-3</td>
<td>420.27</td>
</tr>
<tr>
<td>R-4</td>
<td>689.97</td>
</tr>
<tr>
<td>R-5</td>
<td>387.07</td>
</tr>
<tr>
<td>R-6</td>
<td>368.55</td>
</tr>
<tr>
<td>R-7</td>
<td>417.30</td>
</tr>
<tr>
<td>R-8</td>
<td>248.95</td>
</tr>
</tbody>
</table>
Fig 23: Quantification of EPS, produced by 48 hours old culture

Chart 4: Quantitative EPS production of Rhizobial Strains
There details of variable responses, were represented by different isolates are given below.

**Temperature tolerance by different *Rhizobial* strains at (10\(^0\)C, 15\(^0\)C, 30\(^0\)C 40\(^0\)C and 42\(^0\)C)**

Temperature tolerances of the cultures of different *Rhizobial* strains, tested at 10\(^0\)C on 1\(^{st}\), 3rd 5\(^{th}\), 7\(^{th}\) and 10\(^{th}\) day of culture, are given in Table 12. All strains showed better growth in first 24 hrs. (1\(^{st}\) day). Strain R4, R5 and R7 continued to show same response up to 10\(^{th}\) day while others (R1, R2, R3, R6 and R8) showed decrease in performance with moderate growth.

**Table 12: Temperature Tolerance of different *Rhizobial* strains at 10\(^0\)C**

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Temperature Tolerance at 10(^0)C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>R-1</td>
<td>+++</td>
</tr>
<tr>
<td>R-2</td>
<td>+++</td>
</tr>
<tr>
<td>R-3</td>
<td>+++</td>
</tr>
<tr>
<td>R-4</td>
<td>+++</td>
</tr>
<tr>
<td>R-5</td>
<td>+++</td>
</tr>
<tr>
<td>R-6</td>
<td>+++</td>
</tr>
<tr>
<td>R-7</td>
<td>+++</td>
</tr>
<tr>
<td>R-8</td>
<td>+++</td>
</tr>
</tbody>
</table>

+= Poor growth; ++= Moderate growth; +++= better growth and – = Absence of growth.

Temperature tolerance of different *Rhizobial* strains, tested at 15\(^0\)C on 1\(^{st}\), 3rd 5\(^{th}\), 7\(^{th}\) and 10\(^{th}\) day of culture, are given in Table 13. All strains showed better growth in first 24 hrs. (1\(^{st}\) day). Strain R1, R4, R5 & R7 continued to show same response up to 10\(^{th}\) day while others (R2, R3, R6 and R8) showed decrease in performance with moderate growth.
Table 13: Temperature Tolerance by different *Rhizobial* strains at 15°C.

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Temperature Tolerance at 15°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>R-1</td>
<td>+++</td>
</tr>
<tr>
<td>R-2</td>
<td>+++</td>
</tr>
<tr>
<td>R-3</td>
<td>+++</td>
</tr>
<tr>
<td>R-4</td>
<td>+++</td>
</tr>
<tr>
<td>R-5</td>
<td>+++</td>
</tr>
<tr>
<td>R-6</td>
<td>+++</td>
</tr>
<tr>
<td>R-7</td>
<td>+++</td>
</tr>
<tr>
<td>R-8</td>
<td>+++</td>
</tr>
</tbody>
</table>

+= Poor growth; ++= Moderate growth; +++= better growth and – = Absence of growth.

Temperature tolerances of different *Rhizobial* strains, tested at 30°C, on 1st, 3rd, 5th, 7th and 10th day of culture, are given in Table 14. All strains showed better growth in first 24 hrs. (1st day) Strain R1, R4, R5 & R7 continued to show same response up to 10th day while others (R2, R3, R6 and R8) showed decrease in performance with moderate growth.

Table 14: Temperature Tolerance by different *Rhizobial* strains at 30°C.

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Temperature Tolerance at 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>R-1</td>
<td>+++</td>
</tr>
<tr>
<td>R-2</td>
<td>+++</td>
</tr>
<tr>
<td>R-3</td>
<td>+++</td>
</tr>
<tr>
<td>R-4</td>
<td>+++</td>
</tr>
<tr>
<td>R-5</td>
<td>+++</td>
</tr>
<tr>
<td>R-6</td>
<td>+++</td>
</tr>
<tr>
<td>R-7</td>
<td>+++</td>
</tr>
<tr>
<td>R-8</td>
<td>+++</td>
</tr>
</tbody>
</table>
Temperature tolerances of different Rhizobial strains at 40°C, performed on 1st, 3rd 5th 7th and 10th day. Results are given in Table 15. Results showed that R2, R3, R4, R7 and R8 showed maximum growth, R5, R6 showed moderate growth and R1 showed no growth as observed on 1st day, whereas R4, R7 showed maximum growth. R2, R3, R6 and R8 showed moderate growth and R1 showed no growth as observed on 3rd day. R4 showed maximum growth in all the days, whereas R2, R3 and R7, showed moderate growth, R6 showed minimum growth, R1 showed no growth on 5th day and R4 showed maximum growth, R3 showed minimum growth and R1, R2, R5, R6, R7 and R8 showed no growth as observed on 7th day. All isolates except R4 showed no growth on 10th day.

Table 15: Temperature Tolerance of different Rhizobial strains at 40°C

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Temperature Tolerance at 40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>R-1</td>
<td>-</td>
</tr>
<tr>
<td>R-2</td>
<td>+++</td>
</tr>
<tr>
<td>R-3</td>
<td>+++</td>
</tr>
<tr>
<td>R-4</td>
<td>+++</td>
</tr>
<tr>
<td>R-5</td>
<td>++</td>
</tr>
<tr>
<td>R-6</td>
<td>++</td>
</tr>
<tr>
<td>R-7</td>
<td>+++</td>
</tr>
<tr>
<td>R-8</td>
<td>+++</td>
</tr>
</tbody>
</table>

+= Poor growth; ++= Moderate growth; +++= better growth and – = Absence of growth.

Temperature tolerances of different Rhizobial strains at 42°C were performed on 1st, 3rd 5th 7th and 10th day. Results are given in Table 16. Results showed that R2, R3, R4, R7 and R8 showed maximum growth. R5, R6 showed moderate growth and R1 show no growth as observed on 1st day. R4 and R7 showed maximum growth, R2, R3, R6 and R8 showed moderate growth and R1 showed no growth on 3rd day. R4 showed maximum growth whereas R2, R3 and R7 showed moderate growth, R6 minimum growth and R1 showed no growth on 5th day. R4 showed maximum growth, R3 minimum growth and R1, R2, R5, R6, R7 and R8 showed no growth on 7th day. All isolates except R4 showed no growth as observed on 10th day.
Table 16: Temperature Tolerance of different *Rhizobial* strains at 42°C.

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Temperature Tolerance at 42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>R-1</td>
<td>-</td>
</tr>
<tr>
<td>R-2</td>
<td>+++</td>
</tr>
<tr>
<td>R-3</td>
<td>+++</td>
</tr>
<tr>
<td>R-4</td>
<td>+++</td>
</tr>
<tr>
<td>R-5</td>
<td>++</td>
</tr>
<tr>
<td>R-6</td>
<td>++</td>
</tr>
<tr>
<td>R-7</td>
<td>+++</td>
</tr>
<tr>
<td>R-8</td>
<td>+++</td>
</tr>
</tbody>
</table>

+= Poor growth; ++= Moderate growth; +++= better growth and – = Absence of growth.
Fig 24: Isolate R5 strain from Guar or Cluster bean exhibited very poor growth at 40°C and 42°C temperature.

Fig 25: Isolate R4 strain from Cowpea was able to grow at 40°C and 42°C temperature.
PH Tolerance by different Rhizobial strains:
None of the strains survived at pH 3.0 whereas all the strain (R1- R8) showed growth at pH 7.0 and 11.0 up to 10th day of culture. The performance was better irrespective of the type of strain at pH (7.0 or 11.0) or age of culture (1-10 days).

Salt (NaCl) Tolerance by different Rhizobial strains
The growth response of eight Rhizobium strains in NaCl (salt tolerance) at 100 mM, 250 mM and 500 mM concentration are shown in Table 18. Results clearly indicated that all the strains were not able to grow at 100m during 1st to 10th day, whereas irrespective of the strains, culture at higher concentration i.e. 250 mM and 500mM were better growth from 1st day to 10th day.

The growth response in 1M NaCl concentration however, was different from strain to strain and drastically decreased with increase in age. Strains R2,R3 and R4 showed poor growth at 3rd day and the growth was inhibited with increase in the age, while strain R1 and R6 showed moderate growth as observed up to 3rd day thereafter growth ceased. Growth of R5, R7 and R8 was observed up to 7th day in 1M NaCl medium.

Drought Tolerance by different Rhizobial strains at 30%, 45% 60% PEG concentration
Tolerance to drought by the Rhizobial strain was studied by culturing in PEG supplement medium. The drought tolerance of R1-R8 strains, in 1st, 3rd, 5th, 7th and 10th day culture was recorded. Results of drought tolerance by different Rhizobial strains at 30% PEG, 45% PEG and 60% PEG concentrations. Results are presented in Table 19. All strains were tolerant to 30% PEG concentration. The growth was moderate with all the eight strains.

Similar results were recorded at 45% PEG concentration with R3 and R4 strains, while R1, R5, R6 survived up to 5th day and R8 up to 7th day on 45% PEG concentration.

These observations clearly showed the tolerance of all strains were poor to moderate at 60% PEG concentrations up to 3rd day, thereafter the growth was inhibited. Only R4 and R8 strain showed growth up to 5th day. None of the strains could survive after 5th day in the 60% PEG medium.
Table 17: pH Tolerance of different *Rhizobial* strains

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>pH Tolerance</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; day</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; day</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>7&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>10&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; day</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; day</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>7&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>10&lt;sup&gt;th&lt;/sup&gt; day</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>R-5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+= Poor growth; ++= Moderate growth; +++= better growth and – = Absence of growth.
Table 18: Salt (NaCl) Tolerance of different Rhizobial strains

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Salt Tolerance (Culture age in day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100mM</td>
</tr>
<tr>
<td></td>
<td>1st</td>
</tr>
<tr>
<td>R-1</td>
<td>-</td>
</tr>
<tr>
<td>R-2</td>
<td>-</td>
</tr>
<tr>
<td>R-3</td>
<td>-</td>
</tr>
<tr>
<td>R-4</td>
<td>-</td>
</tr>
<tr>
<td>R-5</td>
<td>-</td>
</tr>
<tr>
<td>R-6</td>
<td>-</td>
</tr>
<tr>
<td>R-7</td>
<td>-</td>
</tr>
<tr>
<td>R-8</td>
<td>-</td>
</tr>
</tbody>
</table>

+= Poor growth; ++= Moderate growth; +++= better growth and – = Absence of growth.
Table 19: Drought Tolerance by different *Rhizobial* strains at 30%, 45% and 60% PEG concentration

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Drought Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30% PEG</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>R-1</td>
<td>+++</td>
</tr>
<tr>
<td>R-2</td>
<td>+++</td>
</tr>
<tr>
<td>R-3</td>
<td>++</td>
</tr>
<tr>
<td>R-4</td>
<td>+++</td>
</tr>
<tr>
<td>R-5</td>
<td>+++</td>
</tr>
<tr>
<td>R-6</td>
<td>+++</td>
</tr>
<tr>
<td>R-7</td>
<td>+++</td>
</tr>
<tr>
<td>R-8</td>
<td>+++</td>
</tr>
</tbody>
</table>

+= Poor growth; ++= Moderate growth; +++= better growth and – = Absence of growth.
Utilization of different Carbon sources by *Rhizobial* strains

Ability to utilize different carbohydrates as carbon sources was tested by supplementing Stab glucose, sucrose, Galactose, Maltose, Fructose and Arabinose supplement in M9 minimal medium.

All the strains could accept Galactose as carbon source. R1, R2, R4, R5, R6, R7 and R8 except R3 strain. *Rhizobium* strain could utilize glucose, sucrose, Maltose and Fructose as carbon source. Arabinose utilization was shown by R1 and R2 only.

**Table 20: Utilization of different Carbon sources by *Rhizobial* strains**

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Utilization of Different Carbon Sources (20 %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td>R-1</td>
<td>+</td>
</tr>
<tr>
<td>R-2</td>
<td>+</td>
</tr>
<tr>
<td>R-3</td>
<td>-</td>
</tr>
<tr>
<td>R-4</td>
<td>+</td>
</tr>
<tr>
<td>R-5</td>
<td>+</td>
</tr>
<tr>
<td>R-6</td>
<td>+</td>
</tr>
<tr>
<td>R-7</td>
<td>+</td>
</tr>
<tr>
<td>R-8</td>
<td>+</td>
</tr>
</tbody>
</table>

+= Positive test and – = Negative test.
Utilization of different Nitrogen sources by *Rhizobial* strains:

The utilization of different Nitrogenous sources such as Ammonium chloride, Ammonium bis citrate, potassium nitrate and Magnesium nitrate by Rhizobial strain was tested on supplementation in M9 minimal medium. Results recorded in table 21.

All strains viz R1, R2, R4, R5, R6, R7 and R8 except R3 strain could accept Ammonium chloride, potassium nitrate and Magnesium nitrate as nitrogen source, while R2, R6, R7 and R8 strains utilized Ammonium bis citrate as Nitrogen source.

**Table 21: Utilization of different Nitrogen sources by *Rhizobial* strains**

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Utilization of Different Nitrogen Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ammonium chloride</td>
</tr>
<tr>
<td>R-1</td>
<td>+</td>
</tr>
<tr>
<td>R-2</td>
<td>+</td>
</tr>
<tr>
<td>R-3</td>
<td>-</td>
</tr>
<tr>
<td>R-4</td>
<td>+</td>
</tr>
<tr>
<td>R-5</td>
<td>+</td>
</tr>
<tr>
<td>R-6</td>
<td>+</td>
</tr>
<tr>
<td>R7</td>
<td>+</td>
</tr>
<tr>
<td>R-8</td>
<td>+</td>
</tr>
</tbody>
</table>

+= Positive test and -= Negative test.
Heavy metal Tolerance by different *Rhizobial* strains:

Heavy metal tolerance is a common characteristic of *Rhizobium*. Heavy metal tolerance by different Rhizobial strain with different metal such as lead acetate, copper sulphate, nickel sulphate, mercuric chloride and sodium Meta arsenate is presented in Table 22. All isolates were found 100% resistant to lead acetate and copper sulphate at 2.5 mg/ml and 1.0mg/ml where as they were sensitive to mercuric chloride; Nickel sulphate and sodium Meta arsenate at 0.1 mg/ml, 0.5 mg/ml and 0.5mg/ml.

**Table 22: Heavy metal Tolerance by different *Rhizobial* strains**

<table>
<thead>
<tr>
<th>Strain. No.</th>
<th>Lead Acetate</th>
<th>Mercuric Chloride</th>
<th>Copper Sulphate</th>
<th>Nickel Sulphate</th>
<th>Sodium Meta Arsenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-3</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-4</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-6</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-7</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-8</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+= Positive test and – = Negative test.
Antibiotic Tolerance by different *Rhizobial* strains:

All strains were found to have resistance to Tetracycline at 7.5 µg ml⁻¹ concentration and sensitive to Rifampicin at 35 µg ml⁻¹. Further, strain R1, R2, R7 and R8 were sensitive to Streptomycin at 75 µg ml⁻¹, whereas R6, R7 and R8 to Ampicillin. R1 alone showed sensitivity to Kanamycin at 5.0 µg ml⁻¹ and R3 for Neomycin at 10 µg ml⁻¹.

Table 23: Antibiotic Tolerance by different *Rhizobial* strains

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Streptomycin</th>
<th>Ampicillin</th>
<th>Kanamycin</th>
<th>Rifampicin</th>
<th>Neomycin</th>
<th>Tetracycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>R-4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-6</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-7</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R-8</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+= Positive test and – = Negative test.
Biocontrol activity of rhizobial strains against phytopathogenic fungi *Fusarium oxysporum*, *Fusarium qubens*, *Rhizoctonia solanii* and *Sclerectonia sclerotiorum*

Results of Biocontrol activity of rhizobial strains against phytopathogenic fungi *Fusarium oxysporum*, *Fusarium qubens*, *Rhizoctonia solanii* and *Sclerectonia sclerotiorum* are presented in table 24.

Out of the eight rhizobial strains tested for Biocontrol activity against four fungal pathogens only R6 strain, isolated from Guar or cluster bean showed positive result against *Rhizoctonia solanii* and *Sclerectonia sclerotiorum*. The inhibition zone recorded as 5 mm and 8 mm respectively (Fig 26 and 27).

Table 24: Biocontrol activity of rhizobial strains against phytopathogenic fungi *Fusarium oxysporum*, *Fusarium qubens*, *Rhizoctonia solanii* and *Sclerectonia sclerotiorum*

<table>
<thead>
<tr>
<th>Strain. No.</th>
<th>Fungal Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>F.oxysporum</em></td>
</tr>
<tr>
<td>R-1</td>
<td>-</td>
</tr>
<tr>
<td>R-2</td>
<td>-</td>
</tr>
<tr>
<td>R-3</td>
<td>-</td>
</tr>
<tr>
<td>R-4</td>
<td>-</td>
</tr>
<tr>
<td>R-5</td>
<td>-</td>
</tr>
<tr>
<td>R-6</td>
<td>-</td>
</tr>
<tr>
<td>R-7</td>
<td>-</td>
</tr>
<tr>
<td>R-8</td>
<td>-</td>
</tr>
</tbody>
</table>

+= Positive test and – = Negative test.
Fig 26: Biocontrol activity against *Rhizoctonia solanii*

Statistical analysis was performed by one way Anova (analysis of variance) calculator using (p < 0.05) free statistical software (turner).
ERIC-PCR

Polymerase chain reactions based on enterobacterial repetitive intergenic Consensus derived primer (ERIC-PCR) to discriminate between eight strains of *Rhizobium* were conducted by comparing the banding patterns obtained.

A total of eight amplification out of them were observed with monogenic band of about 100 bp in size. R1 strain showed a single monogenic band. R2, R3 and R5 strain appear to be similar while other R4, R6, R7 and R8 showed heterogenic bands, indicating that the *Rhizobium* isolates of three non–traditional leguminous vary genetically. Out of 4 isolates from cowpea, R2 & R3 appeared similar while R1 & R4 show deviation where as 2 isolates from cluster bean R5 & R6 and 2 isolates from moth bean R7 & R8 showed similar deviations from one another.

**Fig 28 :** ERIC-PCR of Rhi