Application Guideline for Rhizobial Biofertilizer Technologies

የኢትዮጵያ የግብርና ምርምር ኢንስቲትዩት
Ethiopian Institute of Agricultural Research
Table of Contents

Table of Contents ........................................................................................................................................ iii
Introduction .................................................................................................................................................. 1
Rhizobial biofertilizers ................................................................................................................................. 2
Agricultural and Environmental Benefits of rhizobial biofertilizers ....................................................... 3
Utilization of rhizobial biofertilizers .......................................................................................................... 4
Transportation and storage of biofertilizers ............................................................................................... 6
  Quality characteristics of rhizobial biofertilizers .................................................................................... 7
  Material and facilities for seed dressing .................................................................................................. 9
  Application rate of rhizobial biofertilizers ............................................................................................ 9
  Seed dressing and planting ..................................................................................................................... 10
Compatibility of rhizobial biofertilizers with agricultural inputs ............................................................ 13
Evidence of effective inoculation .............................................................................................................. 14
Introduction

Though agriculture is the most important source of livelihood in Ethiopia, the overall food production is far from self-sufficient. In 2005, humanitarian appeal was made for 8.6 million people, 11 percent of the total population. This, therefore, necessitates the intense use of rehabilitation and sustainable management system that can accommodate increased crop production and soil protection to secure food demand and improve malnutrition problem of resource poor smallholder farmers of the country.

Biological Nitrogen Fixation is a reliable option that can enhance yield sustainably. There are several types of biofertilizers which tremendously contribute to plant growth and development. Some of the most commonly used ones are nitrogen fixing, phosphate solubilizing, growth promoting and decomposers. Nitrogen fixing (rhizobial) biofertilizers are, however, most highly exploited across the globe as well as in Ethiopia. The legume-rhizobia (a symbiotic bacterium capable of invading and eliciting root or stem nodules on leguminous plants to convert atmospheric nitrogen (N$_2$) into ammonia (NH$_3$) in plant roots) symbiosis plays a very important role in a productive and sustainable agriculture. These technologies can deliver enormous benefits through judicious use of fertilizers, example, phosphorus and exploitation of the genetic diversity and symbiotic effectiveness of the hosts (leguminous plants) and their corresponding endosymbionts (rhizobia).

Several demonstration works confirmed that leguminous crops show remarkable growth and yield response to rhizobial biofertilizer use in different agro-ecologies in Ethiopia. However, because of absence of standardized and user-friendly guideline, irregularities in use and inconsistencies in rhizobial inoculant effectiveness have been recorded. Therefore, the objective of this guideline is to deliver agricultural extension workers and smallholder farmers with standardized and simplified application procedures of rhizobial biofertilizer.
**Bio-fertilizers**: preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants’ uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil.

**Rhizobia**: symbiotic bacteria capable of invading and eliciting root or stem nodules on leguminous plants to convert atmospheric nitrogen (N\(_2\)) into ammonia (NH\(_3\)) in plant roots.

**Nodule**: Circular/irregular/cylindrical root outgrowths in which symbiotic atmospheric N\(_2\) fixing rhizobia is taking place.

**Rhizobium**: is a soil habitat bacterium, which able to colonize the legume roots and fixes the atmospheric nitrogen symbiotically.

**Strain**: bacteria having its own identity

### Rhizobial Biofertilizers

Rhizobial biofertilizers are selected strains of beneficial soil microorganisms cultured in laboratory and packed in a carrier or without carrier. Despite these fertilizers improve nutrient availability, enhance pest tolerance and stimulate plant growth, nitrogen fertilization of the rhizosphere remains to most prevalent. They are host specific (Table 1). Carrier-based rhizobial biofertilizers are coated on legume seeds before planting to enhance growth and yield of legume crops and provide nitrogen and organic carbon for subsequent or associated crops. Incorporating legume crop residues will make this effect even more significant. Rhizobial dressed seeds must be planted in moist soil as soon as possible. Rhizobial biofertilizers can improve and sustain soil fertility and soil health when used as part of a long-term rotation system.
Table 1. Grain legume crop species and rhizobia specificity

<table>
<thead>
<tr>
<th>Crop</th>
<th>Type of inoculant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faba bean</td>
<td><em>Rhizobium</em></td>
</tr>
<tr>
<td>Field pea</td>
<td><em>Rhizobium</em></td>
</tr>
<tr>
<td>Chick pea</td>
<td><em>Mesorhizobiumcicer</em></td>
</tr>
<tr>
<td>Soybean</td>
<td><em>Bradyrhizobiumjaponicum</em></td>
</tr>
<tr>
<td>Lentil</td>
<td><em>Rhizobium</em></td>
</tr>
<tr>
<td>Alfalfa</td>
<td><em>Ensifermeliloti</em></td>
</tr>
<tr>
<td>Common bean</td>
<td><em>Rhizobium</em></td>
</tr>
<tr>
<td>Cowpea</td>
<td><em>Bradirhizobiumelkanii</em></td>
</tr>
<tr>
<td>Groundnut</td>
<td><em>Rhizobium spp</em></td>
</tr>
</tbody>
</table>

**Agricultural and Environmental Benefits of Rhizobial Biofertilizers**

**Soil health and fertility improvement:** The continuous use of rhizobial biofertilizers in cropping systems help to improve soil fertility status through improving soil nitrogen levels, soil health (by promoting the growth of other beneficial soil microorganisms such as fungi, actinomycetes, bacteria etc if the roots of the legumes are left in the ground). It also increases soil organic matter as root and leaf drop and systematically worked into the soil.

**Yield improvement:** Rhizobial biofertilizers help enhance production and productivity of grain legume crops. It improves grain or biomass yield up to 10% (particularly with 100 kg Di Ammonium Phosphate ha⁻¹) in any cropping system through
boosting plant growth promoting enzymes, hormones and auxins and increasing yields leads to higher income that leads to greater margins when favorable markets exist for the farm produces. It also improves protein quality of grain legume crops. Moreover, the residual effect of rhizobial biofertilizers help ultimate yield increment up on control of striga by increasing soil nitrogen and increasing nutrient availability due to higher stover and straw yield.

**Economic benefit:** despite the introduction of minimal labor cost in inoculating the seeds before planting, farmers often obtain direct and indirect cost savings. The use of rhizobial biofertilizers reduces the cost of production of legume crops by supplementing inorganic nitrogen fertilizer. For example, the use of 500 g of rhizobial biofertilizer which is enough to dress seed required to plant a hectare of land is 10 times cheaper than 50 kg of urea that serve the same. The small pack sizes (sachets) make it cheap and easy to transport and store compared with inorganic fertilizers. It is an approved input for organic farming; this means that products may get premium price especially for exportable legume crops.

**Environmental benefit:** this technology reduces soil and water pollution compared to chemical fertilizers from manufacturing to its use.

**Utilization of Rhizobial Biofertilizers**

The effectiveness of rhizobial biofertilizers usage is generally governed by four factors. These are microbial quality of rhizobial biofertilizer (rhizobial factor), handling and transport condition (logistic factor), application care (human factor), and soil condition (edaphic factor). The quality of rhizobial and other biofertilizers need to be as to the standard (Ethiopian
Biofertilizer Standard, 2016). When someone uses rhizobial biofertilizer, he/she has to apply the right type, the right rate, right crop at the right time.

The nature of the soil highly determines the final output of rhizobial biofertilizer use. Soil conditions such as soil acidity, soil salinity, and soil water logging as well as soil fertility conditions like total soil nitrogen, soil organic carbon, soil available phosphorus, boron and molybdenum contents highly determines the efficacy of these biofertilizers under farmers field condition. High pH (saline) and low pH (acidic) soils suppress the survival and reproduction capacity of rhizobia. Hence, efficacy of rhizobial biofertilizers will be poor unless reclamation is made to neutralize the soil. Their efficacy of rhizobial biofertilizers is inversely proportional to soil total N and organic carbon. That is why researchers often advise not to apply rhizobial biofertilizer on farm yards and farms supplied with compost, farmyard manure or vermicompost sources.

- It will be crucial to use the right inoculant for the right legume (host legume against rhizobia specificity)
- Highly acidic soils (pH < 5.5) should be treated with lime (based on exchangeable acidity) before biofertilizer application.
- Waterlogged soils should be drained before applying rhizobial biofertilizers.
- Seeds can be co-dressed with fungicides, insecticides and rhizobia. Dressing should be done in the order of insecticides, fungicides and then rhizobia.
- Soils with high heavy metals and salt content are unsuitable for the bacteria to thrive.
- Inoculation of legume seeds each season is advisable to maintain active and adequate rhizobial population.
- Rhizobial biofertilizers should be applied earlier than the date of expiry.
• Soil with signs of low TN give better response to legume inoculation. Apart from soil test, these can be determined based on levels of previous crop yields and deficiency symptoms observed on them. Crops display different nitrogen deficiency symptoms (Figure 1).

![N deficiency symptom on maize](image1)
![N deficiency symptom on soybean](image2)
![N deficiency symptom on barell](image3)
![N deficiency symptoms on wheat field](image4)

Figure 1: Deficiency symptoms of nitrogen element on different crops

### Transportation and Storage of Biofertilizers

Any distributor, user or retailer that engages in rhizobial biofertilizers business should take the following fundamental cares:

a) Transportation temperature should be regulated if beyond the range of 1°C to 28°C. In very hot areas, consider transporting in cold vans/ice box.

b) Users/suppliers do not have to expose to direct sunlight or vehicle engine heat.

c) Users/suppliers do not have to remove or open the overcoat unless
ready for use.
d) Users/suppliers do not have to transport together with chemicals (e.g. pesticides).

Farmers also should consider the following precautions during transportation and application of rhizobial biofertilizers to home or to farm:

a. Store at room temperature up to $28^\circ$C, and not lower than $10^\circ$C to keep organisms potent. In very hot areas, storing in an earthen pot partially buried in the soil will be very advisable.
b. Exposing to direct sunlight (Illustration 1) or keeping in the kitchen or near fires is forbidden.
c. Storing close to chemicals is not advisable (Illustration 2). The chemicals may kill the N-fixing bacteria.

Illustration 1: Exposure to direct sunlight or heat    Illustration 2: Storage close to chemicals

Quality characteristics of rhizobial biofertilizers

The ultimate quality of rhizobial biofertilizer technology is measured by its infectiveness (ability to bear nodules) and effectiveness (ability to produce nitrogen) on the target legume crop. Some of the most determinant conditions that highly dictate the quality of rhizobial biofertilizer products are elaborated as follows:

- Carrier material should be finer (able to pass through 106
micrometer mesh size) for easy adherence to the seed surface. In addition, it has to be free of microbial contamination, have enough organic carbon to sustain the bacteria and being without harmful effect on human health. The most commonly used carrier materials for powdered biofertilizers are lignite, charcoal, vermicompost and peat. The pH of the carrier materials prepared for biofertilizers production should be adjusted around neutral or 7.

- The number of efficient and viable bacteria cells at 15 days after production and at 2 weeks before expiry date should be greater than $10^8$ and greater than or equal $10^6$ colony forming unit (CFU)/g$^{-1}$ of rhizobial biofertilizers, respectively.

- Rhizobial biofertilizers package should be aired and watertight and double-bagged to protect the inoculums from direct sunlight. The inner plastic has preferably been transparent and heat resistant (up to 121°C). Whereas, the outer packet should be bright and opaque to reflect sunlight (but not black that absorbs heat and large amount of ultraviolet rays).

  The outer package should bear the following labels in:

  a. Product name,
  b. Usage instructions in understandable language to the end-user,
  c. Target legume,
  d. Production and expiry date,
  e. Strain code of the rhizobial biofertilizer,
  f. Bacteria population at time of manufacture in CFU g$^{-1}$ of rhizobial biofertilizers and
g. Manufacturer details: name, contacts, location.
Material and facilities for seed dressing

There are many rhizobial biofertilizer types on the market. Some are the powdered and others are liquid and usually come with instructions on how to use. Users should follow the manufacturers’ instructions to obtain the desired output from rhizobial biofertilizer products. The following are materials and facilities required to dress seeds with powdered rhizobial biofertilizer at farm:

- **Water**: help moisten the outer surface of legume seeds
- **Sticker**: table sugar can be solubilized to the measured water so that the solution can be sticky enough.
- **Measuring spoon**: helps to measure sugar
- **Container**: objects for mixing the seed with the sugar solution such as a bucket or plastic bag.
- **Shaded area**: tree canopy or umbrella should be available to keep inoculated seeds away from direct sunlight.

Application rate of rhizobial biofertilizers

Similar to the inorganic fertilizers, rhizobial biofertilizers do also have application rate for different legume crops. The minimum number of rhizobia cells that a seed should carry determines application rate of rhizobial biofertilizers at field. Application rate, in turn, decicate the number of rhizobia cells per gram of the inoculant as well as the seed surface area. By accounting the international recommendations (7 and 12gm/kg for small size and large size seeded legume crops, respectively) and the existing blanket recommendation rate in Ethiopia (125gm per
quarter of a hectare), users are advised to employ the following rhizobial biofertilizers rates (Table 2).

Table 2: Rate of rhizobial biofertilizers for different legume types and seed size categories

<table>
<thead>
<tr>
<th>Amount of biofertilizers (gm)</th>
<th>Faba bean</th>
<th>Soybean</th>
<th>Lentil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quantity of seeds (kg)</td>
<td>Area (ha)</td>
<td>Quantity of seeds (kg)</td>
</tr>
<tr>
<td>125</td>
<td>18</td>
<td>0.125</td>
<td>20</td>
</tr>
<tr>
<td>250</td>
<td>36</td>
<td>0.25</td>
<td>40</td>
</tr>
<tr>
<td>500</td>
<td>72</td>
<td>0.5</td>
<td>80</td>
</tr>
</tbody>
</table>

Note: Faba bean, soybean and lentil are supposed to represent large, medium and small seeded legume crops, respectively.

**Seed dressing and planting**

So far in Ethiopia, the formulation of rhizobial biofertilizers is in powder form and this needs to be dressed to the seeds before planting. The following steps, therefore, display the procedure of seed dressing and then planting:

**Step 1**: Prepare clean seed lot sufficient to plant 0.25 hectare and transfer it to a container.

![Illustration 4: Seed weighing](image)

**Step 2**: Prepare 300 ml of clean water in plastic bottle.
Step 3: Add two tablespoons of table sugar to the water and mix thoroughly to get an even solution of the sugar. This solution is called the sticker.

Step 4: Add the sticker to the seed, mix until all the seeds are evenly coated and properly drain excess sticker solution from the seed before adding biofertilizer.
Step 5: Inspect the inner transparent bag for any fungal growth (*shagata*); once you checked no more fungal growth, mix the entire content very well until all the clods are broken and open the biofertilizer sachet under shade. Pour equivalent amount of biofertilizer (Table 2) onto the moistened seeds.

![Illustration 8: Transferring rhizobial biofertilizer to the moistened seeds](image)

**Step 6:** Mix seed and biofertilizer until all the seeds are uniformly coated. Be careful not to split the seeds or peel the outer coat by using excessive force.

![Illustration 9: Mixing the seeds with rhizobial biofertilizers](image)
**Step 7:** Keep dressed seeds away from direct sunlight.

![Illustration 10: Putting dressed seeds under tree shade](image)

**Step 8:** Plant dressed seeds and cover with soil immediately.

![Illustration 11: Planting and covering dressed seeds](image)

The effectiveness of rhizobial fertilizer is highly influenced by the duration of planting. The shorter the planting duration of dressed seeds the better the response inoculant is. Longer durations will cause moisture loss and hence removal of inoculant from seed coat. Farmers are advised to dress seed lots piece by piece. Dressed seeds are strictly not allowed to stay overnight.

**Compatibility of rhizobial biofertilizers with agricultural inputs**

Currently, legume farmers apply several agricultural inputs to enhance productivity. Lime, chemical fertilizers, organic fertilizers, and agro-chemicals are some at Ethiopian condition. Rhizobia hardly overcome...
acids to acidic niches unlike fungi. Hence, farmers growing legumes on acidic soils should first apply recommended lime rate before panting. Organic fertilizers like farm yard manure, compost and vermicompost are primarily applied to supply nitrogen to the crops. So, in case of application of sufficient amount organic fertilizers such as homestead areas, rhizobial biofertilizer application would be less important. Application of inorganic fertilizers is mandatory except nitrogen containing fertilizers. Phosphatic, molybdenium and cobaltic fertilizers have synergistic interaction with Rhizobial biofertilizers. Phosphorus fertilization could increase N-fixation up 6% for faba bean (Balesh Tulema et al 1999).

The practice of using inorganic nitrogen (18 kg N/ha) along with rhizobial biofertilizers should be based on soil test information and economic feasibility. Field observation work at Holetta confirmed that pre-emergent herbicides application such as dual gold did not harm rhizobial inoculants performance since spacial and temporal mismatch b/n the chemical and the bacteria. Likewise, apronstar and imidalem dressing did not significantly affected rhizobial inoculant performances on chickpea on potted soil. Co-application of apronstar dressing and mancozeb spray did not also affect performance of rhizobial inoculants (FB1035 and FB 1017) at Holetta nitosols on faba bean.

According to ICRISAT (2010), when one farmer needs to co-apply insecticide, fungicide and rhizobial biofertilizer, he has to apply first the insecticide, following the fungicide and last the inoculant.

**Evidence of effective inoculation**

Effectiveness of rhizobial inoculation can be manifested on nodule status (size & position), vegetative growth, seed yield as well as N content of tissue. Different grain legume crops do have different manifestations for effectiveness of inoculation. The following are indexes of the presence of effective inoculation of rhizobia lbiofertilizers at field.
1. Nodules should be visible one month after planting. If there are no nodules after one month of planting, corrective measures like top-dressing with urea should be taken immediately.

<table>
<thead>
<tr>
<th>Illustration 12: Inoculated and nodulated legume (a) and uninoculated and non-nodulated legume (b)</th>
</tr>
</thead>
</table>

2. Concentration of nodules on the upper crown root.

<table>
<thead>
<tr>
<th>Illustration 13: Occurrence of nodules on upper crown root</th>
</tr>
</thead>
</table>

3. Nodule size, color and number at flowering
   - Size – larger and firm nodules formed
   - Color – pink/reddish brown when dissected
   - Nodules concentrated on the upper main root with few on the lateral roots.

<table>
<thead>
<tr>
<th>Illustration 12: Occurrence of larger and reddish brown colored nodules</th>
</tr>
</thead>
</table>

4. Plant vigor
   - Color – deep green leaves
   - Height – tall and thick stems with many branches
   - Broader leaves and effective canopy cover
   - Increased number of flowers and pods set per plant and increased number of seeds in each pod

<table>
<thead>
<tr>
<th>Illustration 15: Appearance of vigor plant stand</th>
</tr>
</thead>
</table>
Rhizobial biofertilizers help improve grain as well biomass yield of the grain legume as well as the following crops. However, the grain yield response to be obtained varies with the type of grain legume crop and the location. In results obtained so far, soybean > common bean > faba bean > and chickpea. Table 3 shows how location and legume species affect legume response.

Table 3. Rhizobia inoculant grain yield enhancing effect

<table>
<thead>
<tr>
<th>Region</th>
<th>District</th>
<th>Grain legume</th>
<th>Inoculant yield Advantage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oromiya</td>
<td>Girar Jarso</td>
<td>Faba Bean</td>
<td>10-16</td>
</tr>
<tr>
<td>South</td>
<td>Gumer</td>
<td>Faba Bean</td>
<td>21-23</td>
</tr>
<tr>
<td></td>
<td>Kacha Bira</td>
<td>Faba Bean</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Field Pea</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Hadero</td>
<td>Faba Bean</td>
<td>2</td>
</tr>
<tr>
<td>Amhara</td>
<td>Basonawerana</td>
<td>Faba Bean</td>
<td>26.3</td>
</tr>
</tbody>
</table>

Source: NSTC feedback report (2011), unpublished