Ethiopian Institute of Agricultural Research

Rhizobia-based bio-fertilizer

Guidelines for smallholder farmers

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Acknowledgment

This manual on rhizobia-based biofertilizers is the result of a growing need expressed by stakeholders to provide relevant and user-friendly extension support information for scaleup of rhizobium inoculant biofertilizers, as a promising intervention to increase smallholder productivity for legume crops within the context of integrated soil fertility management.

Under the auspices of the COMPROII project, managed by the International Institute for Tropical Agriculture (IITA) with technical support from the Africa Soil Health Consortium project, more than 16 different stakeholders participated in a four-day writeshop that saw the emergence of this manual. The writeshop was hosted by the Ethiopian Institute for Agricultural Research (EIAR) in Addis Ababa from 19 to 24 March 2014. The manual is aimed at extension support, agencies and personnel, private sector and other intermediaries that work with smallholder farmers to promote uptake and scaleup of the use of rhizobium inoculants biofertlizers as an appropriate technology.

It was prepared with participation and support of number of individuals and institutions. The following institutions deserve special mention for their contribution:

- Ethiopian Institute for Agricultural Research (EIAR)
- Africa 2000 Network (A2N)
- Africa Soil Health Consortium
- African Fertilizer and Agribusiness Partrnership (AFAP)
- Agricultural Transformation Agency (ATA)
- Amhara Agricultural Research Institute
- Amhara Bureau of Agriculture
- COMPROII project
- Haromaya University, Jimma University
- Hawassa University
- Menagesh Biotech P. L. C.
- Ministry of Agriculture (MoA)
- N2Africa
- Notore Chemical Industries Limited (Notore)
- Oromiya Agricultural Research Institute
- Oromiya Bureau of Agriculture
- Southern Nations, Nationalities and Peoples' Region Agricultural Research Institute
- Southern Nations, Nationalities and Peoples' Region Bureau of Agriculture
- Tigray Agricultural Research Institute
- Tigray Bureau of Agriculture

Acronyms

ATA	Agricultural Transformation Agency
CFU	Colony Forming Unit
DAP	Diammonium Phosphate
GTP	Growth and Transformation Plan
MoA	Ministry of Agriculture
NSTC	National Soils Testing Center

1. Introduction

Ethiopia, with a current estimated population of 77 million growing at a rate of 2.4%, has one of the world's highest incidences of malnutrition.

Though agriculture is the most important source of livelihood employing about 80% of the population, the overall food production is far from self-sufficient. In 2005, a humanitarian appeal for food was made for 8.6 million people, about 11% of the total population *(http://www.wfp.org/country)*.

Subsistence farming is the backbone of the economy. The scanty economic resources of Ethiopian farmers do not allow for mechanized agriculture or purchase of fertilizers. This, therefore, necessitates the intense use of rehabilitation and sustainable management system that can accommodate increased crop production and soil protection.

The legume-rhizobia (a symbiotic bacteria capable of invading and eliciting root or stem nodules on leguminous plants to convert atmospheric nitrogen into ammonia (NH_3) in plant roots) symbiosis plays a very important role in productive and sustainable agriculture. While research has indicated many promising avenues for introduction of nitrogen-fixing plants into cropping systems and for enhancement of the contributions from nitrogen-fixation, to date farmers have adopted few of these technologies.

It is important to note that most of the technologies that are likely to lead to improvements of nitrogen fixation in different cropping systems are well within the reach of research programs in developing countries. The technologies can deliver enormous benefits through judicious use of fertilizers, e.g. phosphorus and exploitation of the genetic diversity and symbiotic effectiveness of the hosts (leguminous plants) and their corresponding *endosymbionts* (rhizobia).

Several field demonstrations have confirmed that leguminous crops show remarkable growth and yield response to rhizobia inoculations in different agroecologies in Ethiopia. As a result, the use of rhizobia inoculants has shown spectacular growth in Ethiopia. Besides the existing commercial producers (National Soils Testing Center (NSTC) and Menagesha Biotech P.L.C.), additional biofertilizer production units are under construction, targeted by the government of Ethiopia to be ready by the end of the Growth and Transformation Plan (GTP). However, because of absence of standardized and user-friendly guidelines, irregularities in use and inconsistencies in rhizobia inoculant effectiveness have been reported. Therefore this manual seeks to deliver to farmers and extension workers a standardized and simplified rhizobial biofertilizer manual.

2. Description of terminologies

Biofertilizers: Preparations containing living cells or latent cells of efficient strains of microorganisms, that help plants' uptake of nutrients by their interactions in the rhizosphere when applied on seed or soil.

Nodule: Circular/irregular/cylindrical root outgrowths in which symbiotic atmospheric N_2-fixing rhizobia is taking place.

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. Rhizobia are able to colonize the legume roots and fix atmospheric nitrogen symbiotically.

Strain: Bacteria having its own identity.

3. Rhizobia inoculants

Rhizobia inoculants are selected strains of beneficial soil microorganisms cultured in a laboratory and packed in with or without a carrier. They are host-specific, low cost and an environmentally friendly source of nitrogen.

Rhizobia inoculants coated on legume seeds before planting 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.

Seeds coated with rhizobia inoculants should not come into contact with chemical nitrogen fertilizer. The coated seeds must be planted in moist soil as soon as possible.

Phosphatic fertilizers help the rhizobia inoculants work well with the legume. Rhizobia inoculants can improve and sustain soil fertility and soil health when used as part of a long-term rotation system. These inoculants help provide nitrogen but other nutrients should be added to crops in line with the recommendations for the area.

4. Benefits of using rhizobia inoculants

A. Soil improvement

The continuous use of rhizobia inoculants in cropping systems can help improve the soil fertility levels for subsequent crops planted in the same field. The technology, therefore, is good for Ethiopian soils where 85% are reported to have low levels of Nitrogen (Annex 3). Rhizobia inoculants improve soil fertility by:

- Maintaining or improving soil nitrogen levels through fixation.
- Promoting the growth of other beneficial soil microorganisms if the roots, stems and leaves of the legumes are left in the ground.
- Providing increased soil organic matter from root, stem and leaf drops when systematically worked into the soil.

B. Yield improvement

Rhizobia inoculants help enhance production and productivity of field crops in the following ways:

- Improves yield by up to 10% (particularly with 100 kg DAP/ hectare) in any cropping system, through:
    - Improving soil health.
    - Boosting plant growth-promoting enzymes, hormones and auxins.
    - Increased yields leads to higher income especially when favorable markets exist for the farm produces.
- Improves protein quality of crops
- The residual effect of rhizobial inoculants help ultimate yield increment by:
    - Helping to control striga by increasing soil nitrogen.
    - Increasing legume stover and straw yield. Leaving these crop residues in the field contributes to improved soil fertility.

C. Economic benefit

Despite the introduction of some labor cost in inoculating the seeds before planting, cost savings are made in the following ways:

- The use of biological nitrogen fixing translates into lower expenditure on nitrogen fertilizer in the long run. For example, the use of 500 g of a product, which is enough to treat 1 hectare, is 10 times cheaper than a 50 kg bag of Urea that does a similar job.
- 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 production may get a premium, especially for export.

D. Environmental benefit

This technology reduces environmental pollution compared with chemical fertilizers from manufacture to its use. It is also good for organic/sustainable farming.

5. Identification of nitrogen and other selected nutrient deficiencies

The use of rhizobia inoculant will not fix all problems associated with nitrogen deficiency in crop production. It can support the long-term improvement of soil fertility by helping legume crops fix nitrogen from the atmosphere at a fraction of the cost of applying the same amount of nitrogen from a nitrogen-based fertilizer.

Table 1: Deficiency symptoms of nutrient elements

Deficiencies of nutrient elements											
Symptoms	Deficiencies										
	N	P	K	Mg	Fe	Cu	Zn	B	Mo	Mn	OF
Yellowing of younger leaves					●					●	
Yellowing of middle leaves									●		
Yellowing of older leaves	●		●	●			●				
Yellowing between veins				●						●	
Old leaves drop	●										
Leaves curl over				●							
Leaves curl under			●			●					●
Younger leaf tips burn								●			
Older leaf tips burn	●						●				
Young leaves wrinkle/curl			●				●	●	●		
Necrosis			●	●	●		●			●	
Leaf growth stunted	●	●									
Dark green/purple leaf stems		●									
Pale green leaf colour	●										
Molting							●				
Spindly	●										
Soft stems	●		●								
Hard/brittle stems		●	●								
Growing tips die			●					●			
Stunted root growth		●									
Wilting						●					

Plate 1: Maize leaf with N deficiency symptom (increasing from right to left)

Plate 2: Soybean leaf without N-deficiency (left) and with N-deficiency (right)

Plate 3: Barley leaf with N-deficiency symptom (increasing from bottom to top)

6. Advice for farmers on selection and management of rhizobia inoculants:

A. Selecting the right rhizobia

It is crucial to use the right inoculant for the right legume.

Table 2: Rhizobia species in commercially available inoculants for legume crops in Ethiopia as at March 2014.

Crop	Type of inoculant (Rhizobia)
Faba bean	Rhizobium leguminosaru vicae
Field pea	
Chick pea	Mesorhizobium cicer
Soybean	Bradyrhizobium japonicum
Lentil	Rhizobium leguminosarum
Alfalfa	Ensifer meliloti
Common bean	Rhizobium leguminosarum phaseoli
Cowpea	Bradyrhizobium elkanii
Groundnut	Rhizobium spp

** New and improved strains of rhizobia are to be launched regularly as a result of ongoing research - so better products may be available in the future.*

B. General points to consider

- Rhizobia inoculants should be applied before the date of expiry – because they are a live product and they will die over time.
- Seeds pre-treated against pathogens need not be inoculated with rhizobia. If you must have to use such seeds, place the inoculant product in the seed hole in such a way that direct contact between inoculant and pre-treated seed will be avoided.
- Because effectiveness may deteriorate over time through population and genetic loss, inoculate legume seeds each season to maintain active population build up.

C. Storage

- Store at room temperature up to 28°C, and not lower than 10°C to keep organisms potent. In very hot areas, consider storing in an earthen pot partially buried in the soil.
- Do not expose to direct sunlight or store in the kitchen or near fires.
- Keep air tight and water tight. Do not open unless ready for use.
- Do not store close to chemicals (e.g. pesticides) - they may kill the N-fixing bacteria.
- Use before the expiry date.
- Use immediately after opening the package.

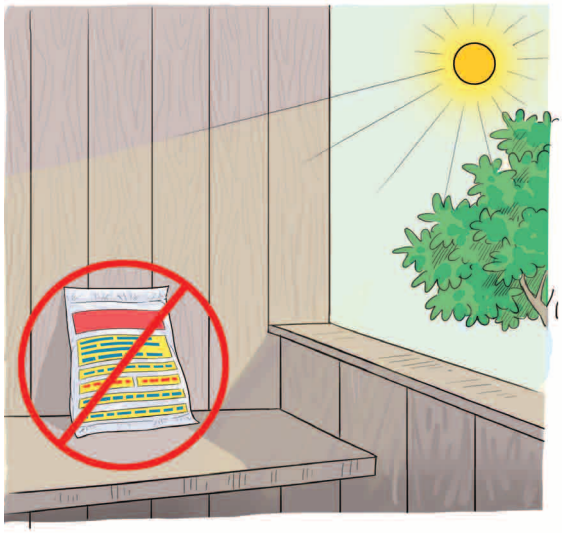


Illustration 1: Do not expose to direct sunlight or heat

Illustration 2: Do not store close to chemicals

D. Soil conditions

- Highly acidic soils will not respond well to rhizobia inoculant, and should be treated with lime before planting.
- Waterlogged soils should be drained before applying rhizobia inoculant.
- Soils with high heavy metal content are unsuitable for the bacteria to thrive.
- Soil with signs of low N content give better response to legume inoculation. Apart from soil testing, this can be determined based on levels of previous crop yields and deficiency symptoms observed on them. Crops display different N deficiency symptoms (see section 5).

7. Inputs required for inoculation of seed at the field

There are many inoculant products in the market. Some are powdered and others are liquid, and usually come with usage instructions. Users should follow the manufacturers' instructions for the best results. The following are generic steps on how to use the powdered inoculant for legume seeds just before planting. It is important to note that there may be some differences from the manufacturers' instructions.

Materials/facilities required to inoculate legumes using powdered inoculants:

- Rhizobia inoculant
- Water
- Sugar or sticking material like gum arabic *(mucha)* or lukewarm water if not using sugar solution
- Measuring spoon
- A container big enough to mix the inoculant with the seed to be inoculated.
- Shaded area should be available to keep inoculated seeds away from direct sunlight e.g. a tree, straw canopy or umbrella

8. Seed inoculation process

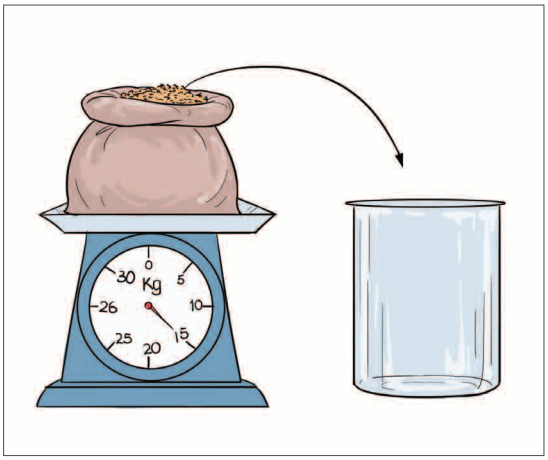
Users should inoculate only the amount of seed that can be planted within the same day. Use the information in Table 3 to calculate the amount of inoculants to use on the seed you could plant in a day, depending on the labour force available. When planting inoculated seeds, cover immediately with soil to protect the seeds from damaging effects of sunlight.

Table 3: Inoculant amount and quantity of seeds

Amount of inoculant (gram)	Quantity of seeds (kg) and area coverage (ha)					
	Faba bean	Area (ha)	Soybean	Area (ha)	Lentil	Area (ha)
125	18	0.125	20	0.25	21	0.25
250	36	0.25	40	0.5	42	0.5
500	72	0.5	80	1	84	1

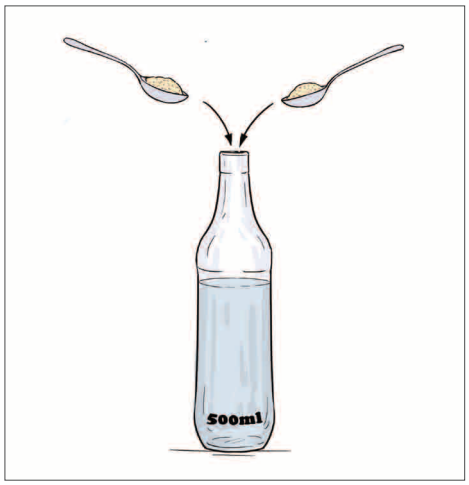
NB: The above three legumes represent larger (faba bean), medium (soybean) and smaller (lentil) seed size.

Step 1: Measure clean seed sufficient to be planted in at most the same day and transfer it to a container.



Step 2: Fill a 300 ml soda bottle with lukewarm water and transfer the water into 500 ml plastic bottle for easier mixing.

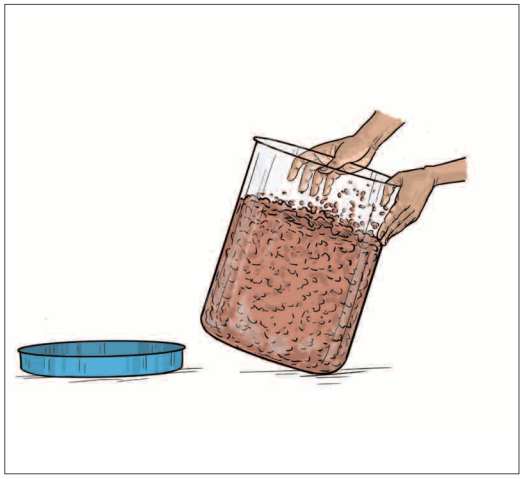
Step 3: Add two tablespoons of table sugar to the water and mix thoroughly to get an even solution of the sugar. This solution is called the sticker.



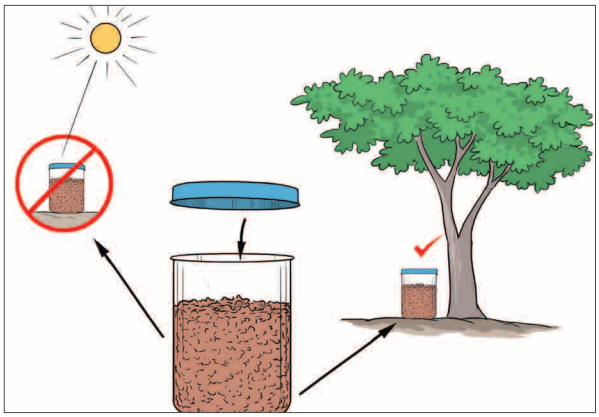
Step 4: Add some of sticker to the weighed amount of seed to be planted on the same day. Mix until all the seeds are evenly coated with the sticker.

Step 5: Inspect the inner inoculant transparent bag for any fungal growth *(shagata)*. If there is no foreign growth, shake the entire contents very well until all clods are broken. Open the inoculant sachet under the shade and pour the recommended amount onto the moistened seeds (see table 3).

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



Step 7: Cover or put inoculated seed under the shade, if necessary. Do not expose coated seeds to direct sunlight for a long time, else the N-fixing bacteria will die before the seed is planted.



Step 8: Plant seeds immediately after inoculation. The seed needs to be covered in soil immediately and not exposed to harmful sunlight

Mix and plant the next batch of seeds by repeating steps 1, 4 6, 7 and 8 until the whole field is planted.

9. Planting inoculated seed

Best environmental conditions for planting are:

- When the rains are well established in moist soil. Too wet or too dry conditions at planting retard germination process and therefore slow colonization of the roots by the bacteria in the inoculant.
- When the sunshine is not excessive, e.g. on cloudy days, early in the morning or late in the afternoon.

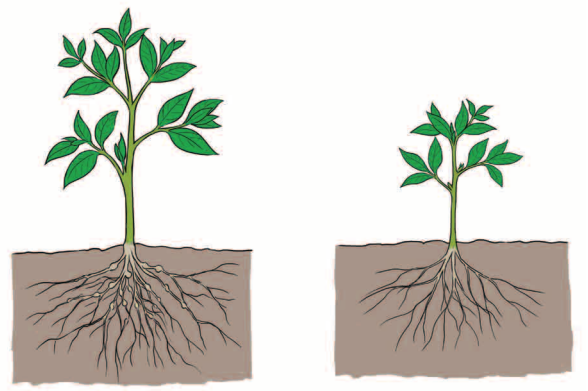
10. The role of chemical fertilizers

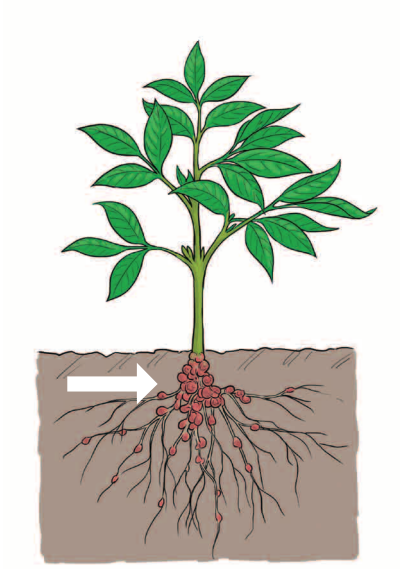
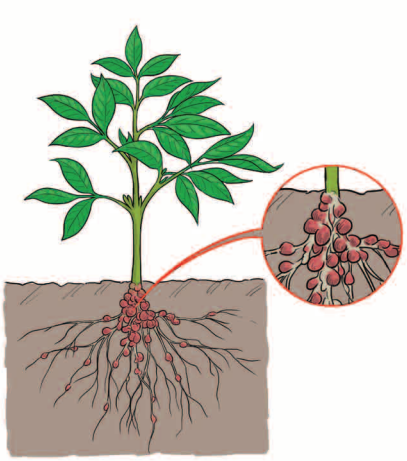
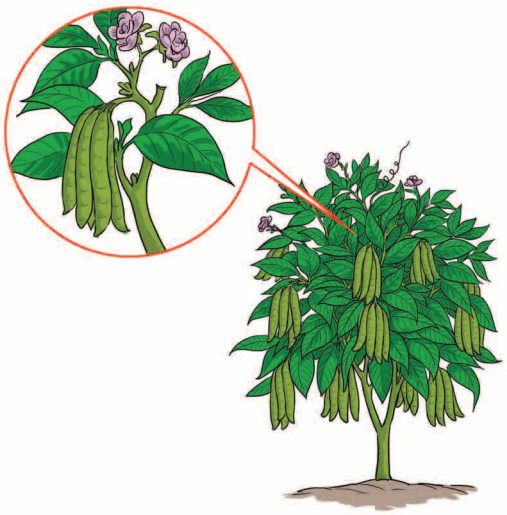
Small amounts of 'starter N' can be applied on legumes – the plants use this nitrogen before the nodules form and are able to fix nitrogen. Blended fertilizers (containing P and/or S) and some micronutrients can be applied to promote the right conditions for crop to benefit from N-fixing bacteria. Diammonium phosphate, DAP, which contains N and P, should be used at 100 kg per hectare for all legume crops.

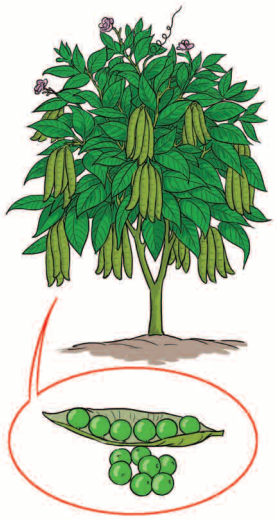
Where deficiency of sulphur (reduction in growth, branching and leaf size and general paling of younger leaves) and phosphorus (red pigmentation of leaf margins and stalk of both young and old leaves) is detected, NPS (18-23-5) at 100 kg per hectare is recommended for use on legumes. Other nutrient formulations can be beneficial but they should be applied based on soil test based fertilizers recommendations.

11. Evidence of effective inoculation

The following are signs suggesting the presence of effective inoculation of rhizobia on crops:

1. Nodules should be visible 1 month after planting.	

2. Concentration of nodules on the upper main root.	
3. Nodule size, colour and number at flowering • Size – larger and firm nodules formed • Colour – pink/reddish brown when cut opened.	
4. Plant vigour • Colour – dark 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	

5. Bigger plants and grain yield	

12. Management of legume inoculation

Legumes are “selfish” crops because they try to assimilate or transport almost all fixed nitrogen to the above ground biomass. If possible, stover should be left in the field after harvesting. If a non legume crop like maize is grown after nitrogen fixing legume, the non legume crop benefits from the nitrogen fixed by the legume and left in the soil.

At the same time if a legume is grown after a crop (e.g. maize) that was applied fertilizer containing phosphorus, the legume can benefit from the phosphorus left in the soil. Integrating use of legume inoculants in the cropping system improves productivity of the system as well as productivity.

Annex 1: Information for manufacturers

Quality characteristics of rhizobia inoculants

- Strain should be efficient in colonizing the root zone and form active nodules on the target crop.
- Carrier material should be fine (10^6 or one micrometer), stick properly to the seed surface, free of microbial contamination, have enough 'food' (organic carbon) to sustain the bacteria and without harmful effect on human health. e.g. lignite powder, vermicompost and peat of pH 7.
- Number of viable bacteria cells at 15 days after production should be greater than 10^8 colony forming unit (CFU/g of inoculant) and greater than or equal to 10^6 CFU/g at 2 weeks before the expiry date.

Packaging

- Package should be air and water-tight and double-bagged to protect the inoculum from direct sunlight.
- The inner plastic should preferably be transparent and heat resistant (up to 121°C), and the outer packet bright and opaque to reflect sunlight (UV rays) during transportation and storage. (It should not be black, as black absorbs heat and large amounts of UV rays).
- Should be packaged in sizes that allow the usage of all the inoculant once opened. The least size of packaging, 125 g sachet, is the most cost-effective for easy usage.
- The outer package should have the following information:
    a. Product name.
    b. Clear usage instructions in a language that is easily understood by the end-user.
    c. Target legume.
    d. Production and expiry date.
    e. Strain code of the inoculum.
    f. Bacteria population at time of manufacture indicated as CFU g-1 inoculant.
    g. Manufacturer details – name, contacts, location
    h. Batch/lot number
    i. Net weight
    j. Storage condition guide line

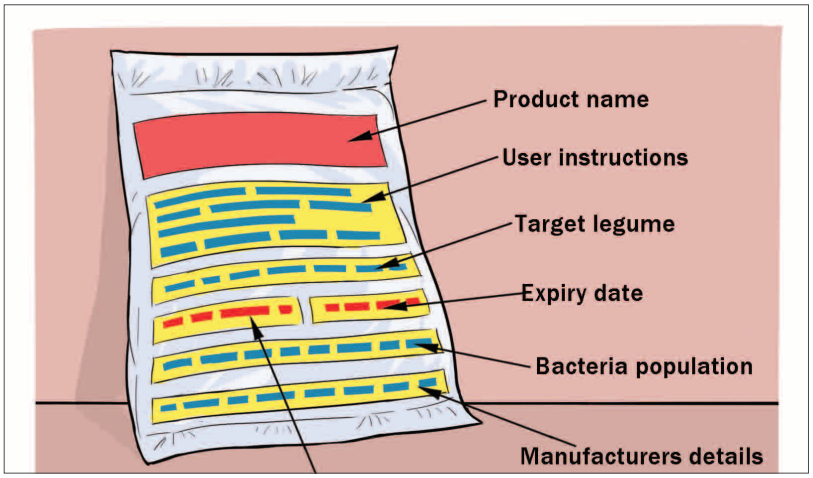


Illustration 3: Inoculant packet with labels to show the above points

Handling and storage of inoculants

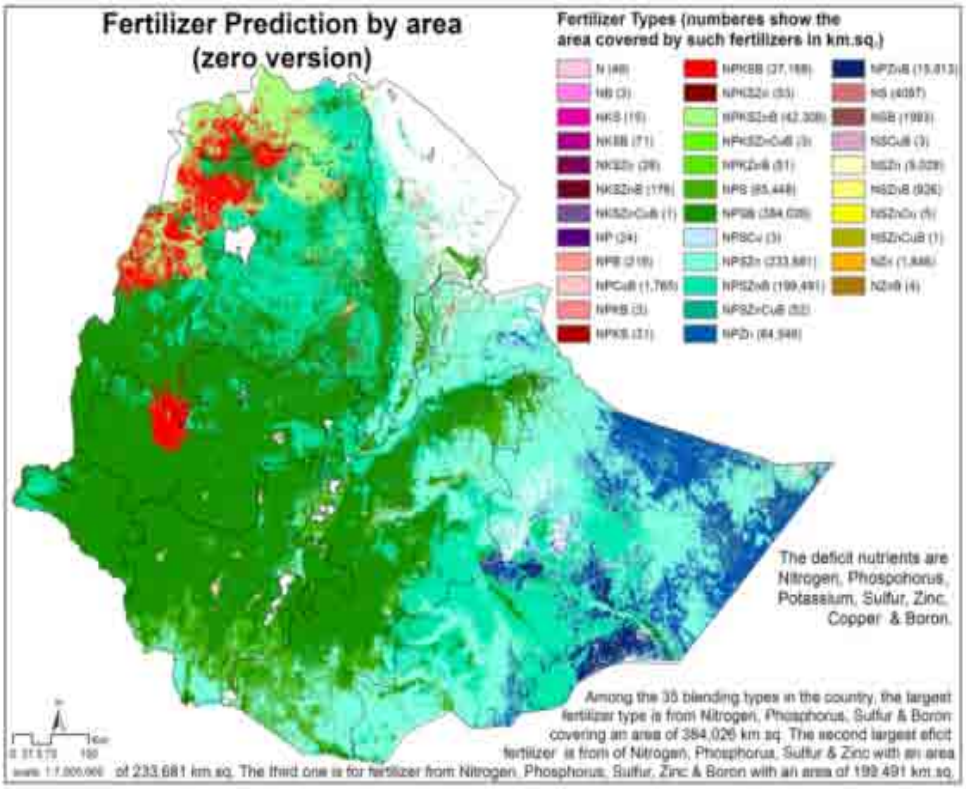
1. Transportation at temperatures between 10°C to 28°C to keep organisms alive. In very hot areas, consider transporting in cold vans/ice box.
2. Do not expose to direct sunlight or vehicle engine heat.
3. Do not transport together with chemicals (e.g. pesticides).

Annex 2: Information for retailers

1. Store at room temperature of up to 28°C, and not lower than 10°C to keep organisms potent. In very hot areas, consider storing in an earthen pot partially buried in the soil or in cold rooms for bulk storage.
2. Do not expose to direct sunlight or other heat sources.
3. Keep air tight and water tight. Do not open unless ready for use.
4. Do not store close to chemicals (e.g. pesticides). They may kill the N-fixing bacteria.
5. Do not stock longer than the expiry date.

Annex 3: Soil fertility map of Ethiopia

Source: Ethiosis



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