Africa 2000 Network (A2N) Uganda

Rhizobia-based bio-fertilizer

Guidelines for smallholder farmers

Table of Contents

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. From 14 to 17 July 2014, Africa 2000 Network Uganda (A2N Uganda) and its stakeholders met in Kampala, Uganda to adapt this manual for the Ugandan context. A2N is a grassroots non-governmental organization that helps rural farmers and their families benefit from a sustainable livelihood in agriculture. This manual will support A2N Uganda and its partners to promote uptake and scaleup of the use of rhizobium inoculants biofertilizers as an appropriate technology in Uganda.

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

- Africa Soil Health Consortium
- Bucadef
- Grameen Foundation
- JAAL Bugudu Agriculture
- Makerere University Kampala (MUK)
- Ministry of Agriculture, Animal Industry and Fisheries (MAAIF)
- Mukono ZARDI
- National Agrcultral Research Organization (NARO)
- Uganda Soil Health Consortium

Acronyms

Bucadef	Buganda Cultural and Development Foundation
CFU	Colony Forming Unit
DAP	Diammonium Phosphate
JAAL Bugudu Agriculture	Growth and Transformation Plan
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
MUK	Makerere University Kampala
Mukono ZARDI	Mukono Zonal Agricultural Research and Development Institute
NARO	National Agricultral Research Organization

1. Introduction

Uganda, with a current estimated population of 35 million growing at a rate of 3.4%, has high 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. Groundnut, soybean, common bean, cowpea and pigeonpea are the common legumes grown in Uganda - especially in the Western, Northern and Eastern regions.

Subsistence farming is the backbone of the economy. The limited economic resources of Ugandan farmers do not allow the use of a mechanized forms of agriculture or the spend money on 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 (N_2) 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 Uganda. As a result, the use of rhizobia inoculants has been showing spectacular growth in Uganda. Currently there are few commercial producers of rhizobia inoculants, with Makerere University being the main source of these rhizobia inoculants.

However, because of absence of standardized and user-friendly guidelines, irregularities in use and inconsistencies in rhizobia inoculant effectiveness have been recorded. Therefore this manual seeks to deliver to farmers and extension workers standardized and simplified information on the use and handling of rhizobial biofertilizer.

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 atmospheric nitrogen is fixed through the action of symbiotic bacteria called rhizobia.

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). 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 N_2.

Carrier-based rhizobia inoculants coated on legume seeds before planting enhance growth and yield of legume crops and provide N_2 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 N_2 fertilizer. The coated seeds must be planted in moist soil as soon as possible.

Phosphatic fertilizers are needed for rhizobia inoculants to work well with the legumes. Rhizobia inoculants can improve and sustain soil fertility and soil health when used as part of a long-term rotation system. These inoculants provide N_2 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 Ugandan soils where 85% are reported to have low levels of Nitrogen (Annex 3). Rhizobia inoculants improve soil fertility by:

- Maintaining soil nitrogen levels through fixation
- Promoting the growth of other beneficial soil microorganisms if the roots of the legumes are left in the ground.
- Providing increased soil organic matter from root and leaf drops when systematically worked into the soil.
- Helping to control striga in the cropping system through increased soil N (especially in areas where striga is prevalent)

B. Yield improvement

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

- Improves yield by up to 50% (particularly with 50 kg DAP/ acre) 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:
    - Increasing stover and straw yield in legume involving cropping systems.

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 N fertilizer in the long run. For example, the use of 250 g (@ UGSh 5,000) of a product, which is enough to treat 1 acre, is almost 20 times cheaper than a 50 kg bag of Urea that does a similar job (@ UGSh 100,000)
- 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.

How to know if your crops are nutrient deficient

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 (Annex 4).

5. Management of rhizobia inoculants: Advice for farmers

A. Selecting the right rhizobia

Legume crop species and rhizobia specificity - it is crucial to use the right inoculant for the right legume crop.

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

Crop	Type of inoculant (Rhizobia)
Groundnut	Rhizobium spp
Soybean	Bradyrhizobium japonicum
Common bean	Rhizobium leguminosarum phaseoli
Cowpea	Bradyrhizobium elkanii
Pigeonpea	Rhizobium leguminosaru vicae

** Some rhizobia have also been evolved for fodder legumes, e.g. Desmodium*

B. Applying inoculants

- 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, drop tiny moulds (balls) of the inoculant product in the seed hole without direct contact. High concentration of antibiotics or presence of pathogens is also not suitable.
- Because effectiveness may deteriorate over time through population and genetic loss, inoculate legume seeds each season to maintain active population build up.

Consider the following soil conditions when using inoculants:

1. Highly acidic soils will not respond well to rhizobia inoculant, and should be treated with lime before planting.
2. Waterlogged soils should be drained before applying rhizobia inoculant.

3. Soils with high heavy metal content are unsuitable for the bacteria to thrive.
4. 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 Annex 4).

C. Storage

1. 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.
2. Do not expose to direct sunlight or store in the kitchen or near fires.
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. Use before the expiry date.
6. Use immediately after opening the package.

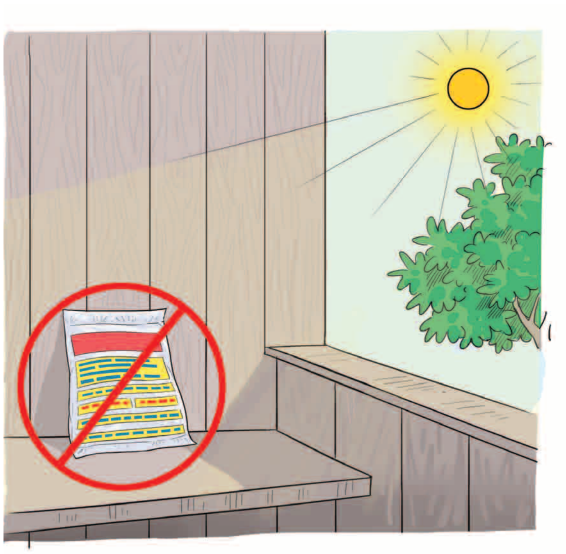


Illustration 1: Do not expose to direct sunlight or heat

Illustration 2: Do not store close to chemicals

6. Seed inoculation: Inputs required 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 (chlorine-free)
- Sugar
- 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

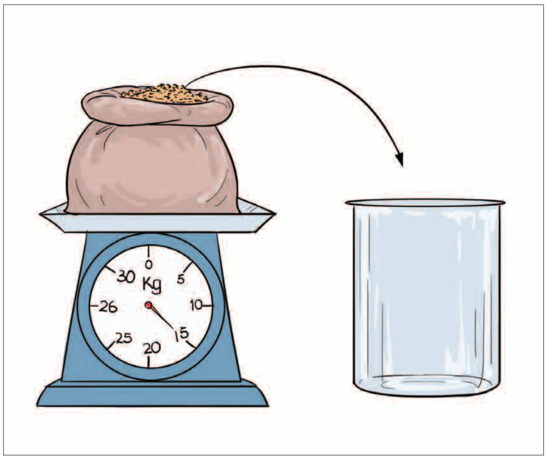
7. Seed inoculation process

Users should inoculate only the amount of seed that can be planted within an hour (the inoculant becomes less effective when exposed for too long in the open). Once this quantity is planted, more seed should be inoculated. Use the information in Table 3 to calculate the amount of inoculants to use on the seed you could plant in an hour, depending on the labour force available. When planting inoculated seeds, cover immediately with soil to protect the seeds from damaging effects of sunlight/UV (ultraviolet) rays.

Table 3: Inoculant amount and quantity of seeds

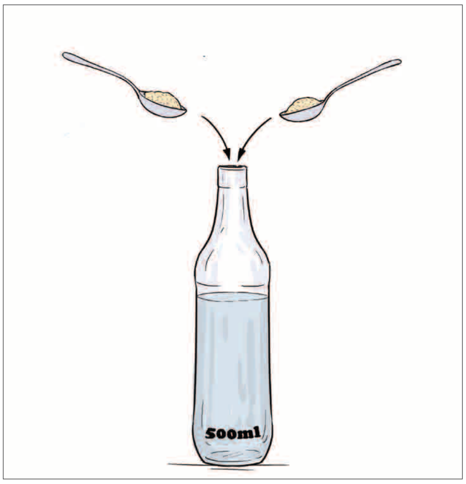
Amount of inoculant (gram)	Quantity of seed
125	12.5 kg of average-size seeds sufficient to plant on ½ acre
250	Average-size seeds sufficient to plant on 1 acre
500	Average-size seeds sufficient to plant on 2 acres

Step 1: Prepare clean seed lot sufficient to be planted within 4 hours for example 1 kg of seed (approximately 8-10 kg to plant on ½ acre) and transfer it to a container. In Uganda, 32 *tumpecos* is 16 kg.

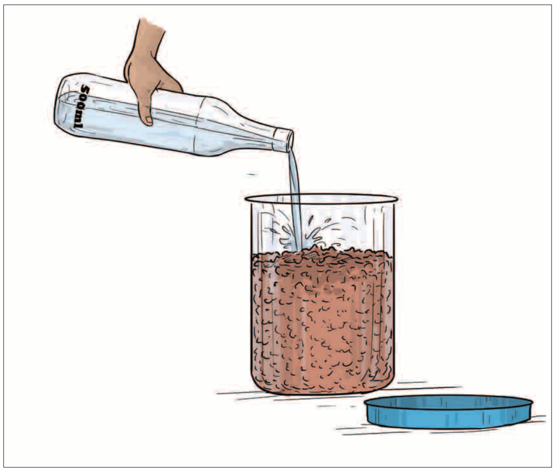


Step 2: Measure and transfer 300 ml of clean lukewarm water into 500 ml plastic bottle for easier mixing.

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.

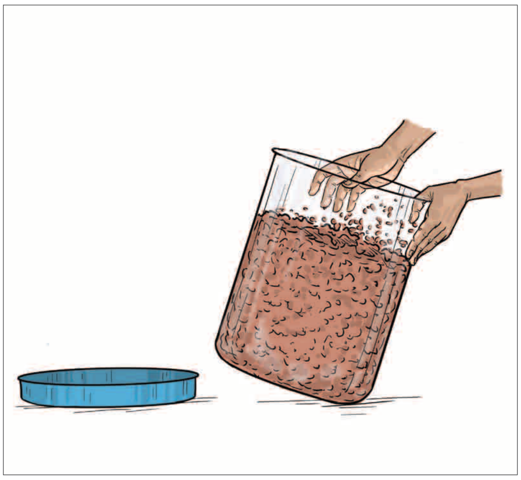


Step 4: Add some of sticker to the weighed amount of seed to be planted in about 1 hour (e.g.1 kg) and mix until all the seeds are evenly coated with the sticker.

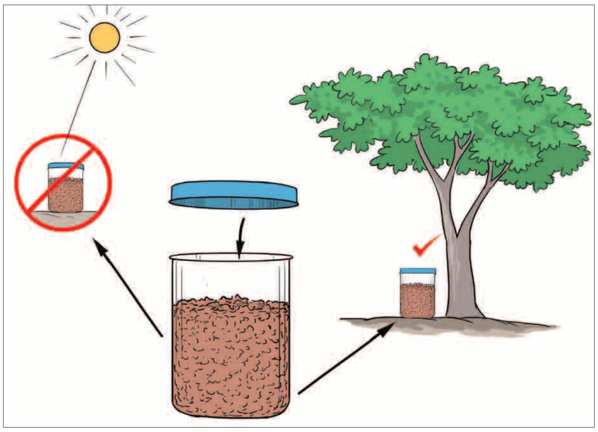


Step 5: Inspect the inner inoculant transparent bag for any fungal growth. If there is no foreign growth, shake the entire contents very well until all clods are broken. Open the inoculant sachet under the shade and pour the recommended amount onto the moistened seeds (usually the rates are 7g/ kg of seed for smaller seeds and 10g/ kg of seed for the larger legume seeds. This works out to around 125 g inoculant to seeds enough for planting on ½ acre).

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 planting.



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

8. 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. Cover the seed holes with soil immediately after planting to protect the N-fixing bacteria from UV rays.

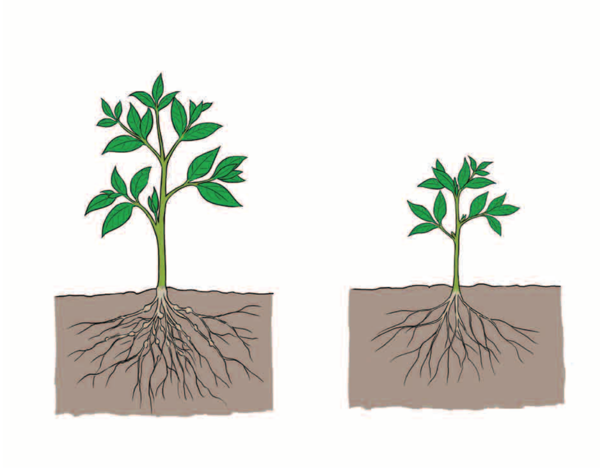
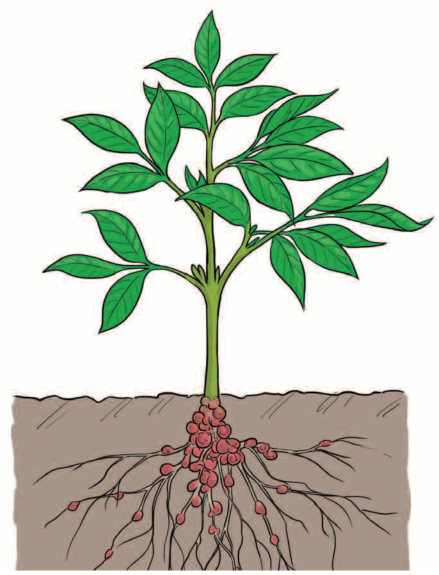
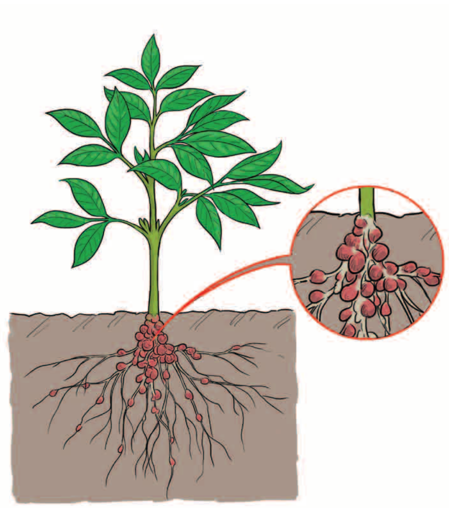
9. The role of chemical fertilizers

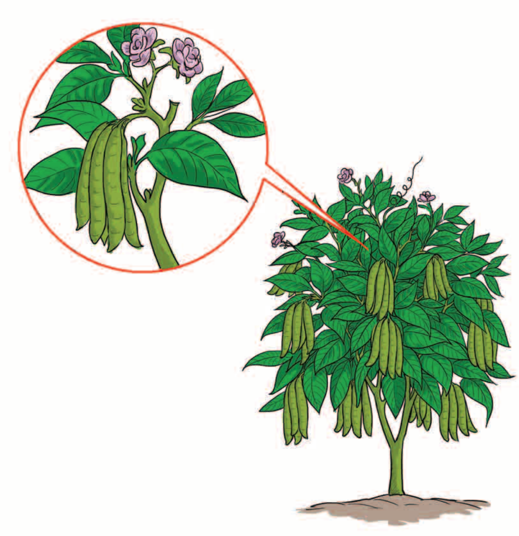
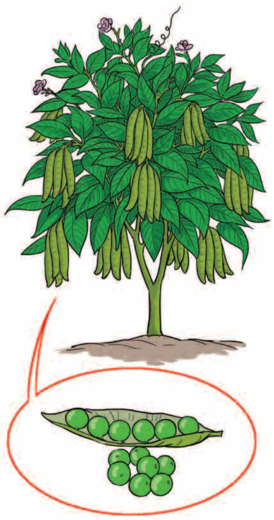
Starter N and blended fertilizers (containing P and/or S) and some micronutrients promote the right conditions for the N-fixing bacteria to multiply, colonize the root cells of the legume and to actively work to the benefit of the crop and the system. Di-Ammonium Phosphate (DAP) and phosphate fertiizers (TSP, SSP and compound fertilizers) are used at a blanket recommendation of 50 kg DAP per acre for all legume crops.

Where deficiency of sulphur is detected, (reduction in growth, branching and leaf size and general paling of younger leaves, red pigmentation of leaf margins and stalk of both young and old leaves), NPS (18-23-5) also at 50 kg per acre is recommended for use on legumes. Other nutrient formulations can be beneficial but they should be applied based on soil test based fertilizers recommendations.

10. 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. • Nodules concentrated on the upper main root with few on the lateral roots.	

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. Higher biomass and grain yield	

Annex 1: Information for manufacturers

Quality characteristics of rhizobia inoculants

- Strain should be efficient to colonize the root zone in order to form active nodules on the target crop.
- Carrier material should be fine (10^6 or one micrometer), easily stick 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 efficient and viable bacteria cells at 15 days after production should be greater than 10^8 colony forming unit (CFU/g of inoculant and at 2 weeks before expiry date should be greater than or equal 10^6 CFU/g of product).

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^{O}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 (it is enough for ½ acre) and transportation by smallholder farmers.
- The outer package should have the following information:
    a. Product name.
    b. Usage instructions in a language that is easily understood by the end-user.
    c. Target legume's name.
    d. Production and expiry date.
    e. Strain code of the inoculum.
    f. Bacteria population at time of manufacture indicated as CFU/g inoculant.
    g. Manufacturer details – name, contacts, location

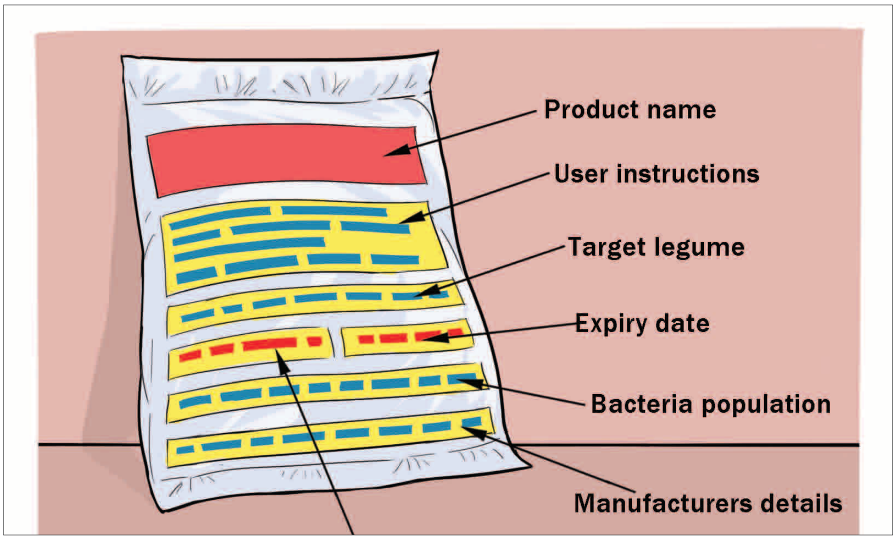


Illustration 3: Inoculant packet

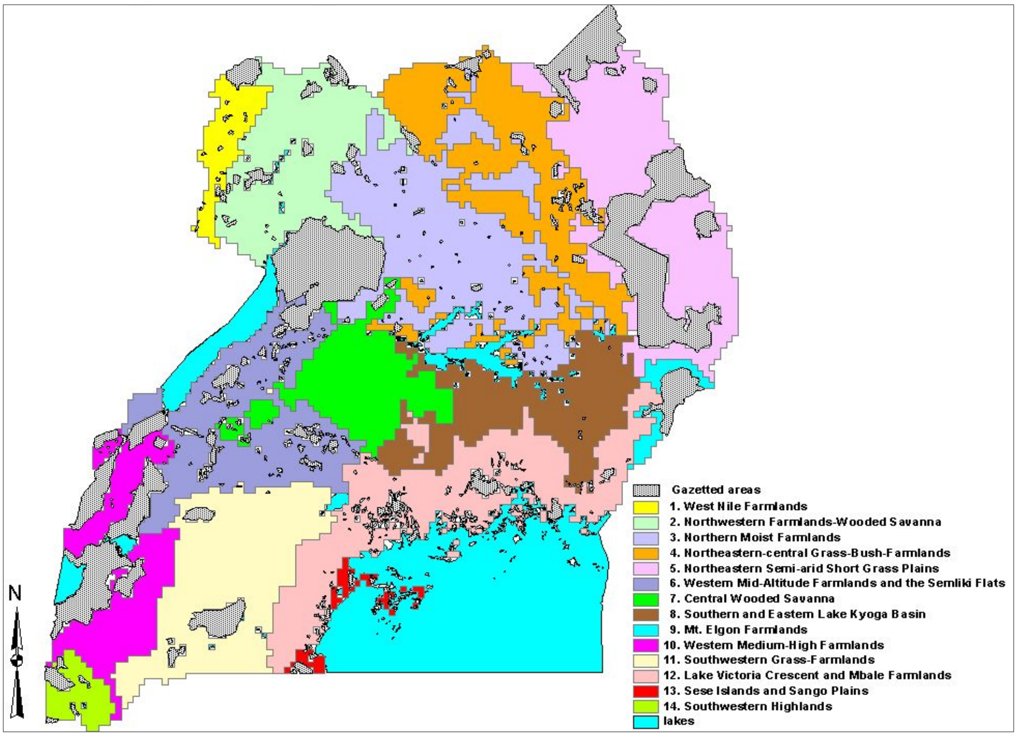
Handling and storage of inoculants

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 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: Agroecological zones in Uganda



Annex 4: Deficiency symptoms of nutrient elements

Deficiencies of nutrient elements										
Symptoms	Deficiencies									
	N	P	K	Mg	Fe	Cu	Zn	B	Mo	Mn
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: N-deficient maize (right)

Plate 2: N-deficient cotton

Plate 3: N-deficient soybean (right)

Plate 4: N-deficient barley (top)

Plate 5: N-deficient wheat field

Plate 6: N-deficient chick pea

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