

The Export of Lentil Rhizobium Inoculants to Nepal

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AGR 1110\*0104

## Part 1:

Product Information

Lentil Rhizobium inoculant is applied to the soil to allow plant roots to form nodules and help with the fixing of nitrogen (Erker and Brick, 2014). Rhizobia is a bacteria that infects the root hairs of plants by the binding of the rhizobia to the root hairs with the help of cellulose fibrils (Gage, 2004). The fibrils form caps on the root hairs, and allow the rhizobia to enter the root tissue. The root tip fibres are deformed, and a new root tip is created. The rhizobia form in tubules called infection threads that are a combination of plant and rhizobia structures (Figure 1). The deformed root hairs support the growth of the rhizobia and will eventually develop into

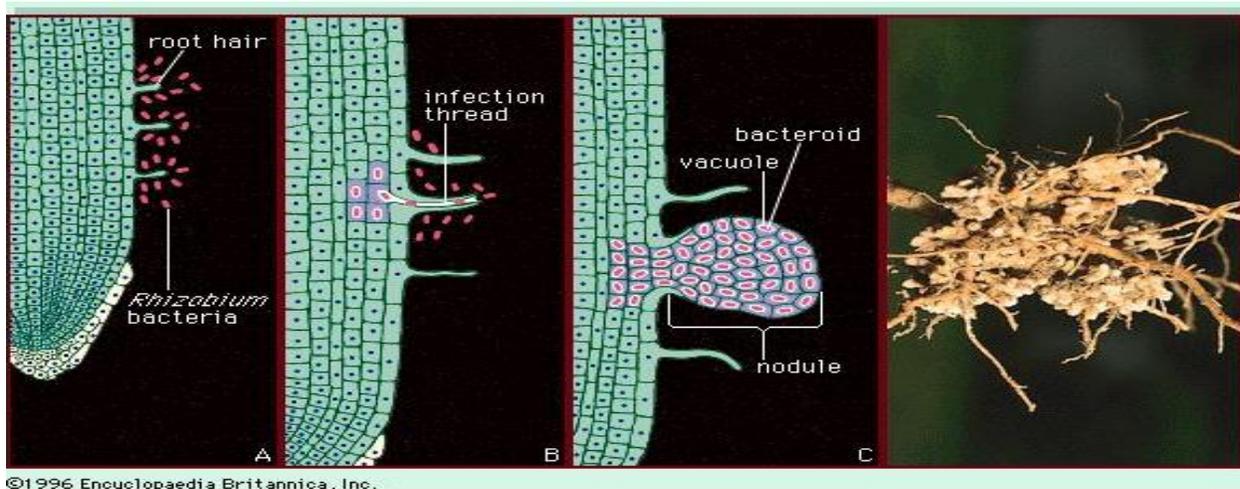


Figure 1: The formation of a nodule. In step A, the rhizobia surround the root hairs. In step B the infection thread is formed, and cells in the plant root become infected. In step C the infected cells form a nodule. Retrieved from <http://www.britannica.com/EBchecked/media/106760/The-roots-of-an-Austrian-winter-pea-plant-with-nodules>

Figure 2: Nodules formed by rhizobia. Retrieved from <http://www.britannica.com/EBchecked/media/106760/The-roots-of-an-Austrian-winter-pea-plant-with-nodules>

nodules (Figure 2) (Gage, 2004).

### Soil Benefits

The biggest benefit of the use of inoculants is the fixing of nitrogen (Stephens and Rask, 2000). Air is 70% nitrogen and the rhizobia in the legumes convert this to ammonium which is a form accessible for plant use (Erker and Brick, 2014). Plant roots have a symbiotic relationship with the rhizobia, as the rhizobia help them to multiply the root tissue (Erker and Brick, 2014). The importance of having a large root system is to support the plant, as well as obtain water and nutrients, in competition with other plants (Waisel, Eshel, Beckman, and Kafkafi, 2002).

### Production

The rhizobia production can be difficult, and slightly more time consuming than the production of other products. The rhizobia take 48 to 72 hours for a 20 to 100 x increase depending on the species of the rhizobia (Burton, 1984). The needs of the bacteria must be met during the production to develop an effective high quality product (Stephens and Rask, 2002). The bacteria cultures are started in test tubes, and transferred to Erlenmeyer flasks or Roux bottles (Burton, 1984). After a period of growth the bacteria are transferred to fermenters depending on the needs of the bacterium. The fermenters should not be mistaken for storage containers (Burton, 1984). A fermenter is a heated agitating container that optimizes the growth of the rhizobia (Gulati, 2004). The amount of bacteria cultures should not be more than the amount that can be combined with a carrier, as the carrier needs to support the life of the bacterium (Burton, 1984). Three major concerns during the processing of the rhizobium are; the quality of the carrier, having adequate numbers of rhizobium per unit of carrier, and the purity of the nitrogen for fixation (Stephens and Rask, 2000).

Three to five percent larger amount of bacteria are used when culturing a slow growing bacteria, to speed up the time of the production. Aiming to produce 1.3 to 1.5 times the required amount should be done as the yield is often less than the theoretical amount (Burton, 1984). A study by Singleton et al. (1997) found that worldwide, approximately 2000 tonnes of inoculant are produced annually. It is likely that this number has increased since this study was done. With 2000 tonnes of inoculant approximately 20 million hectares of legumes can be fertilized (Singleton et al., 1997).

### Forms of Inoculant

There are three main types of carriers. The first is peat. Peat is the oldest and one of the most commonly used forms of inoculant (O'Hara et al., 2012). The rhizobia bacteria are placed in the peat once it has been sterilized (O'Hara et al., 2012). The sterilization of the carrier greatly improves the shelf life of the rhizobium (Tittabutr et al., 2012). Rhizobia can survive in the peat for up to 18 months (O'Hara et al., 2012). The second most common type of carrier is granular. Granular inoculant is often made up of either peat or clay. The third most common type of inoculant is liquid. The rhizobia are suspended and protected in the liquid (O'Hara et al., 2012). Liquid inoculant can be poured into the furrow after it has been diluted with water (Erker and Brick, 2014). Inoculant in the liquid form can also be put directly on the seed (Jim Drury, personal communication, November 18, 2014).

### Packaging

Rhizobia have specific packaging requirements due to the fact that they are living organisms. The packaging must be sturdy enough that it can be printed on, but it must also be

breathable to allow for gas exchange of the rhizobium (Burton, 1984). The packaging must also allow for the retention of water, to aid the rhizobium in their survival (Burton, 1984).

### Requirements

In the soil the rhizobium also have specific requirements for their populations to flourish. Rhizobia are sensitive to pH (Figure 3) and to the salinity of the soil (Catroux, Hartmann, and Revellin, 2001). The moisture content of the soil may also affect the effectiveness of the rhizobium (Catroux, Hartmann, and Revellin, 2001).

Figure 3: The green areas, both light and dark, show the optimal pH were the rhizobium are the most effective. The yellow areas are the second most effective, the red areas are where the rhizobia are sensitive to the pH. Retrieved from: O'Hara G., Howieson J., Drew E., Ballard R., Herridge D., Gemmeil G., ... Ballard N, (2012). *Inoculating legumes: A practical guide*. Kingston ACT. Grains Research and Development Corporation.

Rhizobia	Host legume	pH 4	pH 5	pH 6	pH 7	pH 8
<i>Bradyrhizobium</i> spp.	Cowpea, mungbean, lupin, serradella	Light Green	Dark Green	Dark Green	Yellow	Red
<i>Bradyrhizobium japonicum</i>	Soybean	Yellow	Light Green	Dark Green	Dark Green	Yellow
<i>Rhizobium leguminosarum</i> bv. <i>trifolii</i>	Clovers	Yellow	Light Green	Dark Green	Dark Green	Dark Green
<i>Rhizobium leguminosarum</i> bv. <i>viciae</i>	Pea, faba bean, lentil, vetch	Red	Yellow	Dark Green	Dark Green	Dark Green
<i>Mesorhizobium ciceri</i>	Chickpea	Red	Yellow	Dark Green	Dark Green	Dark Green
<i>Sinorhizobium</i> spp.	Medics	Red	Red	Yellow	Dark Green	Dark Green

Lentil rhizobia inoculant is not a niche product. For any producer of lentils or soybeans the inoculant will help to improve yields, without the use of nitrogen based fertilizers (Stephens and Rask, 2000). Currently, the land in Nepal is fairly heavily fertilized (Raja Khanal, Personal Communication, October 3, 2014). The yields of their other crops would also improve, because the legumes will leave nitrogen that is available to the other plants that can be grown on the same piece of land. The inoculant would be available to many farmers, and they would all see benefits from the use of inoculants.

### Canadian Benefits

The major benefits to Canada would not be directly to a Canadian farmer. The benefits to the economy would be significant. In 2011 Canadian imports and exports totalled \$1.1 trillion (GC, 2012). The export of the lentil rhizobia inoculant would benefit the producers of the product, but would also benefit the people who supply them with their materials. These include companies who supply XiteBio with the equipment for the growth of the rhizobia, as well as anyone who supplies a carrier such as the peat or the liquid for the suspension of the rhizobia. In general, trade helps both the importing and exporting nation to better allocate their resources (Tupy, 2006). For example, a glass of water in the Sahara will make more of a difference than a glass of water in the rainforest (Tupy, 2006). More international trade will help Canada to better decide what resources are most necessary, and where it is possible to make the system more efficient.

### Canadian Companies

In Canada, there are a few companies who produce rhizobium inoculants in combinations of all three forms. Three companies based out of Saskatchewan are: Becker Underwood Canada,

Farmers of North America, and Novozymes BioAg (Government of Saskatchewan, 2012).

XiteBio Technologies Inc. is another company based out of Winnipeg Manitoba that focuses on the production of liquid inoculant (XiteBio, 2013).

XiteBio produces a product PeasRhizo for the inoculation of lentils and peas (Manas Banerjee, CEO of XiteBio, personal communication, November 21, 2014). This product comes in a liquid form, and is sold in cases containing 4, 3L jugs of inoculant (Manas Banerjee, CEO of XiteBio, personal communication, November 21, 2014). The cost of the rhizobia varies depending on the product cost, the amount of handling required, as well as the volume. The cost is also affected by any extra packaging required for shipping. XiteBio also carries other products that may be beneficial for export to Nepal such as SoyRhizo (XiteBio, 2013). SoyRhizo is primarily for soybean use, and promotes the wild strains of rhizobia as well as adding new bacteria to the soil (XiteBio, 2013). All of XiteBio's products are manufactured in Winnipeg Manitoba by XiteBio Technologies Inc. (Manas Banerjee, personal communication, November 22, 2014).

The liquid form is the easiest to apply (Hynes et al., 1995). It can be applied directly to the seed or in the furrow during planting (XiteBio, 2013). For on the seed application, the inoculant is applied at 2.5 fluid oz. per pound or 75mL for 25kg of seed. In the furrow application is slightly different. It is applied at 0.5 fluid oz. per 1000 feet or 15mL per 305m row (XiteBio, 2013). For the application of liquid inoculant the liquid should be prepared according to the manufacturer's directions, and can then be mixed directly with the seed at the ratios provided by the manufacturer (Jim Drury, personal communication, November 18, 2014). The inoculant can also be sprayed into the furrow after being mixed with water according to the

manufacturer's directions (Erker and Brick, 2014). The inoculant should be applied within 12 hours of mixing (XiteBio, 2013).

## Part 2

Nepal is located in Asia between India and China. It is a land locked country and transportation can be challenging. The Capital city of Nepal is Kathmandu, home to 1.02 million people, or approximately 3.3 percent of the population of Nepal (CIA, 2011). Temperatures in Kathmandu range from 10°C in January to 26°C in July (The Best Time to Visit Nepal, n.d.). Nepal is broken up into three main regions the mountain region where the Himalayas are located, the hill region, with steep hills and where some agriculture occurs, and the Terai region or plains region (Nepal –Agriculture, n.d.). The Terai region is where most of the agriculture occurs in Nepal. In the Terai region, temperatures can rise up to 45°C, and be tropical (The Best Time to Visit Nepal, n.d.). Eighty percent of the population in Nepal is involved in agriculture, and it makes up 41 percent of the GDP (Encyclopedia of the Nations, n.d.). The increase in population, with the use of the same agricultural practices has led to a food shortage in a nation that used to be an exporter of rice (Encyclopedia of the Nations, n.d.).

### Distribution

The lentil rhizobial inoculant would be distributed to the farmers of Nepal through a seed distributor such as SEAN Seed Service Centre Ltd (SSSC). This distributor is located in

Kathmandu and therefore is accessible to many people. The goal of SSSC is to promote seed in Nepal (SSSC, n.d.). Seed from a seed distributor will have more genetic diversity, and there will be less likelihood of the seed being diseased or carrying a fungal infection, that may put the whole crop in jeopardy.

There are some challenges involved with the transportation of the inoculant to Nepal from Winnipeg, as Nepal is a land locked country. The basic route of the inoculant for its export to Nepal will be: A truck would pick up the packaged inoculant at XiteBio Technologies Ltd. in Winnipeg Manitoba. From there it will travel to Thunder Bay Ontario, in order to be shipped to Calcutta India. From there the inoculant will be trucked to Nepal's capital Kathmandu, and sent to SEAN Seed Service Centre Ltd.

An alternate route that would be slightly more expensive would be to fly the inoculant from Winnipeg to Kathmandu. This would take less time, which could be beneficial since the rhizobia will expire approximately 8 months after the production. However this would increase the cost, and therefore make it more difficult for the Nepalese farmers to purchase the inoculant. The cost for the transport from Winnipeg Manitoba to Chennai India by ship is \$4352.00 USD (A1 Freight Forwarding, 2014). There would then be extra cost to ship the inoculant from Chennai India to Kathmandu Nepal.

### Challenges

The shipping of the inoculant can be difficult because the rhizobia are living. They must be kept in a temperature ranging from 4 to 25°C, and out of direct sunlight (XiteBio, 2013). The temperature of the average shipping container traveling a similar route can vary from -21°C up to 57° C (Weiskircher, 2008). These temperature fluctuations usually occur during transportation

on land. During ocean transportation, the temperature fluctuations are still great, but may occur over days as opposed to hours (Weiskircher, 2008). This means that the inoculant must be shipped in a refrigerated container, should they be shipped overseas. The refrigerated container would not be for the refrigeration of the inoculant but to maintain a more constant temperature. Lentil rhizobium is a living bacteria therefore the bacteria cells will die over time and when exposed to stresses such as heat, they will die more quickly (XiteBio, 2013). The average lifespan of the inoculant is 8 months so there is an expiry date to be conscious of during the shipping (Hynes et al., 1995).

### Benefits to Nepal

Benefits to Nepal are numerous. The use of inoculant can improve the yields by up to 5.4 bushels per acre. In the US that is a 17 percent increase, and it could be even greater in Nepal (XiteBio, 2013). The use of inoculant will not only improve the yields of the lentils, but also of any other crops that are planted in the same fields as part of the crop rotation. Crop rotation was used by the Roman Empire to improve their yields (Rosen, 2007). The lentils take the Nitrogen in the air and convert it into a form of ammonium for the plant to use (Erker and Brick, 2014). This form of nitrogen is used by many plants not just lentils. It will therefore reduce the need for nitrogen fertilizers, because when included in the crop rotation, it will allow other plants to use the nitrogen it left behind (Zahran, 2001). Consumers also benefit from trade. When specialized goods and services are imported, the production will become more efficient and therefore the cost to purchase the product will decrease (Bized, 2001). The cost for the lentil rhizobium inoculant will not decrease, but the yields of the lentils will increase. This means that the farmers will have more products to sell in the markets. In Nepal the farm size is also decreasing (Raja Khanal, personal communication, October 3, 2014). The farm size is decreasing due to the plots

of land becoming smaller and smaller. A father will divide up the farm between the children, and therefore the land that the children are allocated is smaller (Raja Khanal, personal communication, October 3, 2014). The increased yields from the use of the inoculant will allow the farmers to continue to provide their families with enough food, despite the decrease in the amount of land that is being cultivated.

### Disadvantages to the Nepalese

The use of the rhizobial inoculant reduces the need for nitrogen fertilizers (Erker and Brick, 2014). This would impact the current suppliers of the nitrogen fertilizers. The supplier's business would dramatically decrease because they would no longer be selling fertilizers for lentils. The manufacturers would also have less people purchasing the nitrogen fertilizers.

### Environmental Impact

The environmental impact will be greatly affected by the use of lentil rhizobia inoculants. There are pros and cons to both the use of inoculants and fertilizers (Figure 4). PeasRhizo is non-toxic to both animals and humans. The only side effect is that it may cause mild skin irritation after prolonged exposure (XiteBio, 2013).

Pros	Cons
<ul style="list-style-type: none"> <li>• Inoculants can increase the availability of nutrients to plants</li> <li>• Inoculants can help with the solubility of phosphorus, allowing plants absorb</li> </ul>	<ul style="list-style-type: none"> <li>• Fertilizers can leach into water sources</li> <li>• Fertilizer can cause algal blooms in water, using all of the oxygen and</li> </ul>

<p>it more easily as well as nitrogen</p> <ul style="list-style-type: none"> <li>• Rhizobia promote root growth allowing for more water and better absorption of nutrients already in the soil</li> <li>• Increased root growth leads to less soil erosion</li> <li>• Rhizobium inoculant costs less to apply than fertilizers</li> </ul>	<p>creating a dead spot where nothing can survive</p> <ul style="list-style-type: none"> <li>• Not all fertilizers can be absorbed by the plant. Some is leached away before it can be used</li> <li>• Inoculants may be more labour intensive due to the required coating of the seed before planting</li> <li>• Inoculants cannot be used with other disinfectants such as fungicide</li> <li>• After inoculation with a liquid inoculant, the seeds must be planted within 12 hours</li> </ul>
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Figure 3: The pros and cons of inoculants and fertilizers.

Information retrieved from: (Adesemoye, Torbert, and Kloepper, 2009), (XiteBio, 2013), and (Erker and Brick, 2014).

### Other Required Information

There are still a few unknowns to consider when inquiring about the export of lentil rhizobium inoculant. Rhizobia are sensitive to the salinity and pH of soil, and therefore, some testing would need to be done to determine the pH and salinity of the soils in the areas for which the rhizobia is to be used (Catroux, Hartmann, and Revellin, 2001). Inoculants do not perform

well when used in conjunction with other disinfectants, particularly fungicides (Erker and Brick, 2014). Studies would need to be done in regards to any disinfectants that are currently in use, as well as any replacements that could be considered.

### Competition

There are very few liquid fertilizers on the market as they are a fairly new invention (Guenther, 2013). There are a few peat and granular inoculants for export from the United States, but there were no liquid inoculants being shipped from India or China (Alibaba.com, n.d).

### Recommendations

It is recommended that despite the cost, the Nepalese farmers apply the inoculant according to the manufacturer's directions. If the lentil rhizobia inoculant is not applied as stated by the manufacturer, the yield increases will be minimal. It is also recommended that the product is packaged in smaller quantities. Seeds should be applied at 30-40 pounds per acre, for small varieties or 70-80 lbs per acre for larger varieties (MFARD, n.d.). A Nepalese farmer would need enough inoculant to treat between 12.12 pounds and 16.16 pounds for small varieties or 28.28 to 32.32 pounds for large varieties. One bottle of inoculant can inoculate 2400 pounds of seed, and therefore is far too great an amount for one farmer to use (Manas Banerjee, CEO XiteBio Technologies Inc., personal communication, November 21, 2014). It would be possible to have the seed distributor also separate the inoculant into smaller amounts, but the liquid inoculant should not be opened until ready for use (XiteBio, 2013). If the package was opened it would

need to be used shortly after opening, and SSSC may have a difficult time distributing the entire amount of inoculant in a few hours for the farmers to use.

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