



## BIOLOGICAL INOCULANTS FOR LEGUMES. TOP 5 PRINCIPLES FOR CHOOSING PRODUCTS FOR THEIR EFFECTIVE USE

**In the 1880s, scientists discovered the ability of leguminous plants to enrich soil with nitrogen. They determined that microorganisms forming symbiotic relationships with plants play a key role in this process. Within a decade, the first commercial inoculant was introduced. However, it took humanity nearly 100 years to recognize the importance of this discovery and begin its large-scale implementation in agricultural practices. The delay in demand for the new technology was due to a lack of understanding of the phenomenon and the low efficiency of microbial products and the technologies used for their production.**

Today, thanks to revolutionary advances in microbiology and biotechnology, agricultural technologies are increasingly incorporating biotechnological products. Even globally renowned multinational agrochemical companies are now involved in developing biofertilizers, which underscores the promising future of microbiological products.

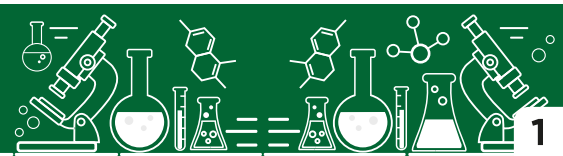
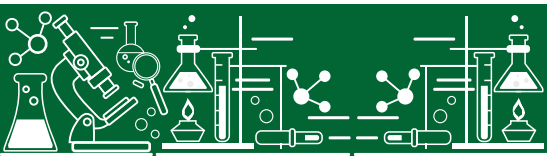
Nevertheless, despite significant progress in using beneficial microorganisms for plant nutrition, end-users are often disappointed with microbial products due to their low effectiveness. While some cases of yield loss result from the poor quality of inoculants, the majority stem from improper use of biofertilizers, such as a lack of understanding of their mechanism of action, storage requirements, application techniques, and integration into conventional technologies.

For a comprehensive understanding of the key principles for using inoculants effectively on leguminous crops, users would need to sift through a vast array of specialized literature. Consolidating all recommendations into a single publication would create an overwhelming long read. In our view, it is sufficient for the average user to focus on five fundamental principles for selecting and applying these products. These principles are based on global expertise and our own years of successful experience.

### Preparative Form

Currently, the most common inoculants are sterile or non-sterile powder-based products made from peat, organic residues, natural clays, or minerals. Peat is the primary carrier for creating loose products because it contains all the necessary nutrients for microbial development and has a structure that effectively protects bacteria from adverse factors.

However, it's important to note that the key requirement in producing highly effective inoculants is the absence of contaminating (foreign) microorganisms in the final product. These contaminants can outcompete the population of rhizobia in the substrate. Therefore, the carrier must be sterile, which is challenging and expensive when using peat or other organic substrates. Sterilizing mineral matrices is less complex but requires the addition of supplementary nutrients. Using non-sterile carriers renders the effectiveness of biofertilizers null, especially during long-term storage.



A significant breakthrough in improving the efficiency of liquid inoculants was the development of a "preservation" technology. This innovation allows for the production of sterile liquid microbial products with an extended shelf life. An additional advantage of this technology is its ability to enhance microorganism tolerance to seed treatments, adverse storage conditions, and application challenges (such as pre-treatment of seeds, drought, or exposure to extreme temperatures). This has expanded the conditions for transporting, applying, and storing liquid inoculants, making them technologically comparable to powder-based products. However, this technology remains inaccessible to many manufacturers as it requires specialized equipment. As a result, the effectiveness of liquid and powder-based products is currently equivalent in terms of key performance indicators, and the choice of form depends solely on the biofertilizer application method.

If the inoculant is applied directly to the soil or planter, the powder form is preferable. For pre-treatment of seeds in combination with other protective or nutritional agents, the liquid form is better suited. The main requirement is that the products must be manufactured on a sterile basis.

### **Mono- and Multicomponent Inoculants**

The question of the effectiveness of multicomponent inoculants remains unresolved. Analyzing specialized literature reveals hundreds of successful cases of using rhizobia in combination with other nitrogen-fixing, phosphate- and/or potassium-mobilizing, or biocontrol microorganisms. However, the negative experiences of combining multiple microorganisms with different properties currently outweigh the positive ones. This is one of the key reasons for the slow development of "all-in-one" products. Somewhat successful are attempts to combine several strains of the same species but with different properties. Such products are effective across a broader range of growing conditions but do not always guarantee higher yields compared to monostrain inoculants.

Using two or more species of microorganisms in one product is essentially equivalent to using non-sterile products. Under certain conditions, secondary microorganisms may dominate over the primary ones, nullifying the main function of the inoculant.

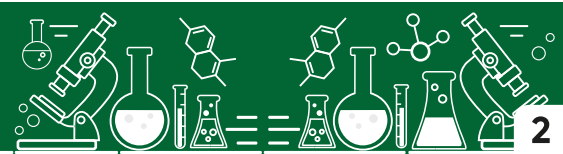
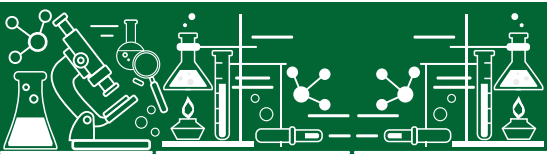
***Thus, the use of multistrain inoculants is fully justified when it comes to minimizing the impact of adverse conditions, even if it results in slight yield losses. However, combining two or more microorganisms of different species (even with complementary actions) in a single product jeopardizes the effectiveness of inoculation and may lead to significant economic losses.***

### **Simultaneous Use with Chemical Protection and Nutrition Products**

Seed treatment with inoculants and chemical protectants is one of the most extensively discussed topics in agricultural science. Numerous publications provide lists of pesticides compatible with inoculants and guidelines for their use. However, there are two critical aspects that require special attention.

**First**, it is essential to focus not on the active ingredient (AI) but on the specific product. Products containing the same AI can exhibit varying levels of toxicity toward plants or microorganisms due to differences in pesticide formulation. This discrepancy stems from imperfect formulations. As a result, agrochemical giants annually invest millions of dollars not only in discovering new active agents but also in enhancing existing products, improving their efficacy while reducing their toxicity to plants and the environment.

**Second**, avoid combining all nutritional and protective components into a single technological process. For instance, molybdenum is widely recognized for enhancing nitrogen fixation in symbiotic systems of legumes. However, little attention is paid to its toxicity (as well as that of other heavy metals) to microorganisms. While plants require micronutrient supplementation, it is better to apply these nutrients to the soil or as foliar treatments rather than incorporating them into seed dressing. When micronutrients are applied for fungicidal protection, the microorganisms forming the biological foundation of inoculants can protect plants from fungal or bacterial pathogens in the rhizosphere with similar efficiency.



The same caution applies to using various biologically active compounds such as phytohormones, polysaccharides, amino acids, organic acids, and polypeptides. While these compounds are unlikely to directly influence plants (as they are metabolized by soil microflora before seedling emergence), they may attract harmful soil microorganisms. These products are better applied foliar or through drip irrigation.

Finally, the choice between chemical and biological seed dressers, considering the safety of the latter, is not straightforward. Using a biological seed dresser can have effects similar to those observed with multicomponent or non-sterile inoculants.

***In such cases, combining a chemical seed dresser with an inoculant may provide higher efficiency, extended protection, and better productivity compared to the combined use of biofertilizers and biopesticides. If you opt for a biopesticide, ensure it is tested and recommended by the inoculant manufacturer. Successfully "harmonizing" multiple microorganisms is a complex task requiring highly qualified specialists and long-term research.***

## Methods of Inoculant Application

The timing and location of microbial product application are crucial factors that determine the effectiveness of inoculation. This can involve pre-treatment of seeds (up to 120 days before sowing), treating seeds directly before planting or in the planter, or applying inoculant to the soil before or during sowing. When choosing the form of inoculant and the technology for its application, two key aspects must be considered.

**First**, introducing microorganisms into the natural environment through seed treatment is 10–20 times more effective than applying them to the soil. This is because the microorganism is directly introduced into the rhizosphere of the target plant.

**Second**, the shorter the period between inoculating the seed and sowing, the more bacteria from the inoculant can survive and colonize the plant.

**However, seed inoculation limits the range of protection products available for disease and pest control. Inoculating the soil removes the restriction on seed treatment products, but it introduces risks for forming effective symbiotic relationships between the microorganism and the plant.**

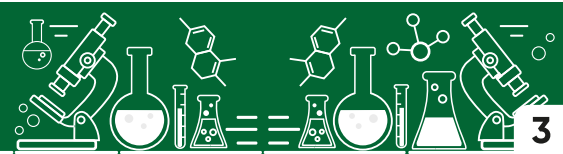
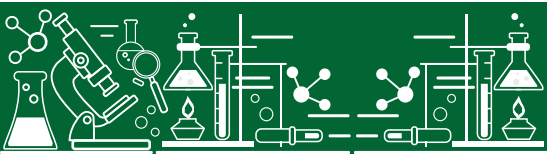
## Other Agro-technologies

A deficiency of any biogenic element, except nitrogen, reduces the effectiveness of legume–rhizobial symbiosis. Therefore, it is important to ensure that plants receive nutrients through soil fertilization. Foliar feeding is effective only for minor corrections of nutrient deficiencies.

Regarding nitrogen fertilizers, the following should be considered: Leguminous plants form nodules 14 days after emergence, and by the 21st day, these nodules are fully capable of supplying the plants with atmospheric nitrogen. During this period, the nitrogen consumption by the plant is minimal, and the soil contains enough of this element to nourish the crop until the symbiotic apparatus starts functioning. At the same time, the plant will not switch to symbiotrophic nutrition until nitrogen reserves in the soil decrease to a critical level. Therefore, by applying starter doses of nitrogen fertilizers, you extend the period for nodule formation and delay the transition to biologically fixed nitrogen. This can lead to two negative outcomes.

**Firstly**, nodule formation may coincide with periods of drought or temperature drops. Under stress conditions, the plant will slow down nodule formation, as this process is considered a biotic stress for the plant. If nitrogen reserves in the soil are depleted and biological nitrogen nutrition is unavailable, the plant may experience nitrogen starvation at a critical time.

**Secondly**, by applying nitrogen fertilizers, you shorten the effective functioning period of the symbiosis and, accordingly, reduce the potential reserves of biological nitrogen in the soil, which, according to scientists' calculations, could reach losses of 30 kg of active nitrogen per hectare. This, in turn, lowers the economic attractiveness of using inoculants.



An additional argument in favor of early nodule formation is that once the plant has "gone through" this process, it develops a nonspecific immune response, making it more resistant to pathogens, drought, and other stress factors in the future.

Moreover, legume-rhizobial systems are sensitive to a lack of moisture, aeration, and light. For example, to transform and assimilate 1 kg of nitrogen from the atmosphere, the plant uses eight times more photosynthesis products than when assimilating 1 kg of mineral nitrogen.

**Therefore, poor soil preparation or overcrowded sowing can bring the effectiveness curve of the symbiosis close to zero, and the calculated yield losses due to the low efficiency of photosynthetic and nitrogen-fixing apparatuses can reach up to 30%.**

Thus, the desire for plants to close the rows faster to conserve moisture and compete with weeds often has the opposite effect by increasing transpiration areas and reducing plant insulation.

## Summary

The intensification of agriculture has disrupted the water regime of soils, leading to widespread salinization and desertification. The extensive use of chemical plant protection products has not helped to combat diseases, but rather has depleted biodiversity and triggered the emergence of pesticide-tolerant pests. Mineral fertilizers deplete non-renewable natural resources used in their production and are also one of the causes of global warming, as up to 30% of greenhouse gas emissions come from agricultural lands due to humus degradation and the denitrification of nitrogen fertilizers. This points to the end of the "modern" model of the "green revolution."

Inoculants are the most effective and environmentally safe "local factories" for producing nitrogen fertilizers, with their nitrogen-fixing potential reaching 300 kg N/ha per season. However, their economic appeal is not limited to just cheap nitrogen or their multifaceted impact on plant development. Inoculated plants actively release various organic substances into the rhizosphere to communicate with soil microorganisms. These compounds can retain moisture, chelate microelements, or serve as nutrients for various microorganisms. Most importantly, the synthesis of these root exudates occurs from carbon dioxide during the photosynthesis process. Therefore, the use of inoculants is an extremely effective way to recycle greenhouse gases, making an important step toward environmentally safe, sustainable agricultural production.

