"Strategic partnership for competence based training in Biofertilizers"

BIOFERTILIZERS towards sustainable agricultural development

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Bio-FIT Book Summary

"BIOFERTILIZERS towards sustainable agricultural development" *provides in-depth coverage of all key issues concerning biofertilizers as a low-cost, renewable source of plant nutrients that supplement chemical fertilizers. This book provides general information about the benefits and impact of biofertilizers on organic farming practices. It describes the different types of biofertilizers, methods utilized for their production and different approaches of applications. Constraints in biofertilizer technology, as well as biofertilizers EU policy and potential market are highlighted.*

WHY BIOFERTILIZERS?

Contents

INTRODUCTION

"Despite the many accomplishments of mankind, we owe our existence to six-inch of top soil and fact that it rains." – Confucius

Sustainable agriculture *is the efficient production of safe, high quality agricultural products, in a way that protects and improves the natural* **environment**, *the* **social and economic conditions** *of farmers, their employees and local communities, and safeguards the* **health and welfare** *of all farmed species.*

For a sustainable agriculture system, it is essential to use renewable inputs (fertilizer, pesticides, water etc.) which benefit the plant and cause no or minimal damage to the environment. One possible way is to reduce the use of chemical fertilizers and pesticides. Chemical fertilizers are being used in increasing amounts in order to increase the output in high yielding varieties of crop plants. Chemical fertilizers are industrially manipulated substances composed of known quantities of nitrogen, phosphorus and potassium, and their exploitation causes air and groundwater pollution by eutrophication of water bodies.

However, chemical fertilizers cause pollution of water bodies as well as groundwater, besides getting stored in crop plants.

Modern agriculture is becoming more and more dependent upon the steady supply of synthetic inputs, mainly chemical fertilizers, which are products of fossil fuel (coal+ petroleum). Adverse effects are being observed due to the excessive and imbalanced use of these synthetic inputs. The soils have now become biologically dead. This situation has led to identifying harmless inputs like biofertilizers and biopesticides.

Environmentalists worldwide are pressing the market and society for a switch over **to *organic farming and biofertilizers***. ***Organic farming*** aims to be a more environmentally sustainable form of agricultural production, combining best environmental practices, and emphasizing biodiversity protection and the preservation of natural resources. It also emphasizes high animal welfare standards and the avoidance of synthetic chemical inputs such as fertilizers and pesticides and genetically modified organisms (GMOs).

Organic farming is one such strategy that not only ensures food safety, but also adds to the biodiversity of soil.

Organic farming is the raising of unpolluted crops through the use of manures, *biofertilizers* and *biopesticides* that provide optimum nutrients to crop plants, keeping pests and pathogens under control.

WHAT ARE BIOFERTILIZERS?

Generally, the term "fertilizer" is used for "fertilizing material or carrier", meaning any substance which contains one or more of the essential elements (nitrogen, phosphorus, potassium, sulphur, calcium, magnesium, iron, manganese, molybdenum, copper, boron, zinc, chlorine, sodium, cobalt, vanadium and silicon). Thus, fertilizers are used to improve the fertility of the land.

The term “biofertiliser” has been defined in different ways over the past 20 years, which derives from the improved understanding of the relationships occurring between the rhizosphere microorganisms and the plant. Biofertilizers may be defined as “substances which contain living microorganisms that colonize the rhizosphere or the interior of the plants and promote growth by increasing the supply or availability of primary nutrients to the target crops, when applied to soils, seeds or plant surfaces”. According to Vessey, the term biofertiliser is associated to “a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant". In 2005, biofertilizer was defined as "a product that contains living microorganisms, which exert direct or indirect beneficial effects on plant growth and crop yield through different mechanisms". The definition was extended as the bacteria were used to control plant pathogens. Nevertheless, microorganisms which promote plant growth by control of harmful organisms, such as biofungicides, bionematocides, bioinsecticides, or any other products with similar activity favoring plant health, are generally defined as biopesticides, not as biofertilizers.

Biofertilizers have an ability to mobilize nutritionally important elements from non-usable to usable form. These microorganisms require organic matter for their growth and activity in soil and provide valuable nutrients to the plant. The microorganisms in biofertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of biofertilizers, healthy plants can be grown while enhancing the sustainability and the health of soil. Thus, the term *biofertilizer means the product containing carrier based (solid or liquid) living microorganisms which are agriculturally useful in terms of nitrogen fixation, phosphorus solubilization or nutrient mobilization, to increase the productivity of the soil and/or crop.* Although at present biofertilizers are available for nitrogen and phosphorus only, efforts are on to identify the organisms which can solubilize or mobilize other minerals or nutrients. Recently, K-biofertilizer and Zn-biofertilizers have also been developed but these products are yet to be commercialized.

Biofertilizers are also living or biologically active products or microbial inoculants of bacteria, algae and fungi (separately or in combination) which are able to enrich the soil with nitrogen, phosphorus, organic matter etc. Biofertilizers act as a compound that enriches the nutrient quality of the soil by using microorganisms that establish symbiotic relationships with the plants.

Biofertilizers are low-cost renewable sources of plant nutrients which supplement chemical fertilizers. Biofertilizers generate plant nutrients like nitrogen and phosphorous through their activities in the soil or rhizosphere and make them available to the plants on the soil.

The use of biofertilizers is gaining importance because of the proper maintenance of soil health, the minimization of environmental pollutions and the cut-down in the use of chemicals.

Biofertilizers are one of the important components of integrated nutrient management, as they are a cost-effective and renewable source of plant nutrients to supplement and/or replace the chemical fertilizers for sustainable agriculture. These are preparations containing living cells or latent cells of efficient strains of microorganisms that help the uptake of nutrients in crop plants by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants.

WHAT ARE BIOPESTICIDES?

Biopesticedes are certain types of pesticides derived from such natural materials as animals, plants, bacteria and certain minerals. Biopesticides are pest management agents based on living microorganisms or natural products. They have proven potential for pest management and they are being used across the world. Biopesticides are living organisms (natural enemies) or their products (phytochemicals, microbial products) or byproducts (semiochemicals) which can be used for the management of pests that are injurious to plants. They are living organisms which are cultivated in the laboratory on a large scale and are used and exploited experimentally for the control of harmful organisms. Examples include insects, viruses, bacteria, fungi, protozoa and nematodes.

Biopesticides have an important role in crop protection, although most commonly in combination with other tools including chemical pesticides as part of Biointensive Integrated Pest Management. Biopesticides or biological pesticides pose less threat to the environment or to human health because they are specifically targeted to a single pathogenic pest.

The three main types of biopesticides are microbial pesticides, biochemical and plant-incorporated protectants.

Microbial Pesticides

Microbial pesticides contain active ingredients of specific types of microorganisms, such as a fungus, bacterium or protozoan. Each active ingredient can be utilized to target a specific type of pest. For example, some fungi can suppress certain weeds, while certain types of bacteria can control different species of insect larvae, such as mosquitoes, moths or flies. The most commonly utilized microbial pesticides come from strains of the bacteria *Bacillus thuringiensis* (Bt). The bacterial strains manufacture different protein mixes that can target specific insect larvae and will not affect other organisms.

Biochemical Pesticides

Biochemical pesticides use natural substances like insect sex pheromones, which can disrupt mating, thus controlling the insect population. Other types of biochemical pesticides can include the use of hormones, enzymes and scented plant extracts to attract and trap certain pests. These are good alternatives to conventional pesticides because the latter often contain synthetic toxic material to destroy insects.

Plant-Incorporated Protectants

By introducing genetic material into plants, scientists can make plants produce pesticide substances which can target and kill specific pests. In some cases, the addition of a gene with a particular Bt protein can produce these plant incorporated protectants, or plant pesticides.

There are considerable potential benefits to agriculture and public health programmes through the use of biopesticides. The interest in biopesticides is based on the advantages associated with such products, as follows:

1) They are less toxic and inherently less harmful and cause less environmental load;

2) Designed to affect only one specific pest or, in some cases, a few target organisms;

3) Often effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems.

4) When used as a component of Integrated Pest Management (IPM) programmes, biopesticides can contribute greatly.

5) They are safer for humans and the environment.

However, for the effective use of biological pesticides, it is important to have extensive knowledge of pest management.

WHY ARE BIOFERTILIZERS USED?

In recent years, a microbial green revolution is underway. Biofertilizers have their own advantages over chemical fertilizers and are economically and environmentally friendly as well. With the increasing demand in agriculture, it has become important for scientists and society to increase the productivity of the sector by using various fertilizers, insecticides and pesticides. However, with the tremendous use of these products, the soil has been badly affected because of the depletion of the essential minerals of the soil. Therefore, to overcome this problem, it has become important to use a different remedy for the production of various biofertilizers. They have the best economic value.

The following basic reasons to explore biofertilizers are outlined:

WHY BIOFERTILIZERS?

- The demand is much higher than the availability. It is estimated that by 2020, to achieve the targeted production of 321 million tonnes of food grain, the requirement of nutrients will be 28.8 million tonnes, while their availability will be only 21.6 million tonnes, leaving a deficit of about 7.2 million tonnes.

- Depleting feedstock/fossil fuels (energy crisis) and increasing cost of fertilizers. This is becoming unaffordable by small and marginal farmers.

- Depleting soil fertility due to widening the gap between nutrient uptake and supplies.

- Growing concerns about environmental hazards.

- Increasing threat to sustainable agriculture. Besides the above facts, the long-term use of biofertilizers is economical, eco-friendly, more efficient, productive and accessible to marginal and small farmers over chemical fertilizers.

Bio-fertilizers, also known as microbial inoculants, have great potential as a supplementary, renewable and environmentally friendly source of plant nutrients and are an important component of Integrated Plant Nutrient System (IPNS).

HOW DO BIOFERTILIZERS WORK?

1) Biofertilizers fix atmospheric nitrogen in the soil and root nodules of legume crops and make them available to the plants.

2) They solubilize the insoluble forms of phosphate, such as tricalcium, iron and aluminum phosphates, into available forms.

3) They scavenge phosphates from soil layers.

4) They produce hormones and anti-metabolites which promote root growth.

5) They decompose organic matter and help in the mineralization of soil.

6) When applied to the soils or seeds, these biofertilizers increase the availability of nutrients and improve the yield by 10% to 20% without adversely affecting the soil and the environment.

WHY BIOFERTILIZERS?

Biofertilizers are ready-to-use live formulates of such beneficial microorganisms, which upon application to seeds, roots or soil, mobilize the availability of nutrients by their biological activity in particular, and help build up the microflora and, in turn, the soil health in general, which consequently benefits crops. Biofertilizers are designed to improve the soil fertility in N and P. They provide growth promoting substances.

WHAT ARE THE BENEFITS OF USING BIOFERTILIZERS?

- *Increasing harvest yields*
  - ✓ An average increase in crop yields by 20 to 37 percent.
  - ✓ Algae-based fertilizers give improved yields in rice at rates ranging between 10 and 45 %.
- *Improving soil structure*
  - ✓ The use of microbial biofertilizers improves the soil structure by influencing the aggregation of the soil particles
- *Better water relation*

  Arbuscular mycorrhizal colonization induces drought tolerance in plants by:
  - ✓ Improving leaf water and turgor potential,
  - ✓ Maintaining stomatal functioning and transpiration,
  - ✓ Increasing root length and development.
- *Lowering production costs*
  - ✓ Made from easily obtained organic materials such as rice husks, soil, bamboo and vegetables etc.
  - ✓ Reduce the input expenses by replacing the cost of chemical fertilizers.
- *Providing protection against drought and some soil-borne diseases*
  - ✓ Aquatic cyanobacteria provide natural growth hormones, proteins, vitamins and minerals to the soil.
  - ✓ *Azotobacter* infuse the soil with antibiotic pesticide and inhibit the spread of soil-borne pathogens like *Pythium* and *Phytophthora*.
- *Suppressing the incidence of insect pests and plant diseases*

Biofertilizers strengthen the soil profile, leave water sources untainted and improve plant growth without detrimental side effects.

WHY BIOFERTILIZERS?

WHAT ARE THE ADVANTAGES AND DISADVANTAGES?

We can list the basic *advantages* of using biofertilizers:

They help to achieve high yields of crops by enriching the soil with nutrients and useful microorganisms necessary for plant growth.

They replace the chemical fertilizers, as the latter are not beneficial for plants. Chemical fertilizers decrease the plant growth and pollute the environment by releasing harmful chemicals.

Plant growth can be increased because biofertilizers contain natural components which do not harm the plants but do the opposite.

- *They are eco-friendly due to the fact that they protect the environment against pollutants.*
- If the soil is free of chemicals, it will retain its fertility, which will be beneficial for the plants as well as the environment, because the plants will be protected against diseases and the environment will be free of pollutants.
- Biofertilizers destroy those harmful components from the soil which cause diseases in plants. By using biofertilizers, plants can also be protected against drought and other restrictive conditions.
- Biofertilizers are cost effective. They are not costly and even low-income farmers can make use of them.

As disadvantages, using biofertilizers:

- Gives much lower nutrient density – it requires large amounts to get enough for most crops;
- Requires a different type of machinery to apply from that used for chemical fertilizers;
- Sometimes is hard to locate in certain areas; odour; difficult to store;
- Specific to the plants;
- Requires skills in production and application.
- There is inadequate awareness about the use and benefits of biofertilizers.

TYPES OF BIOFERTILIZERS

Biofertilizers add nutrients through the natural processes of fixing atmospheric nitrogen, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. They can be categorised in different ways based on their nature and function.

One simple broadly disseminated classification is as follows:

A. Nitrogen Biofertilizers

This group fixes nitrogen symbiotically. Nitrogen biofertilizers help to correct the nitrogen levels in the soil. Nitrogen is a limiting factor for plant growth because plants need a certain amount of nitrogen in the soil to thrive. Different biofertilizers have an optimum effect for different soils, so the choice of nitrogen biofertilizer to be used depends on the cultivated crop. Rhizobia are used for legume crops, *Azotobacter* or *Azospirillum* for non-legume crops, *Acetobacter* for sugarcane and blue-green algae and *Azolla* for lowland rice paddies.

B. Phosphorus Biofertilizers

Just like nitrogen, phosphorus is also a limiting factor for plant growth. Phosphorus biofertilizers help the soil to reach its optimum level of phosphorus and correct the phosphorus levels in the soil. Unlike nitrogen biofertilizers, the usage of phosphorus biofertilizers is not dependent on the crops cultivated on the soil. Phosphatika is used for all crops with *Rhizobium*, *Azotobacter*, *Azospirillum* and *Acetobacter*.

C. Compost Biofertilizers

Biofertilizers are also used for enrichment of your compost and for enhancement of the bacterial processes that break down the compost waste. Suitable biofertilizers for compost use are cellulolytic fungal cultures and Phosphotika and *Azotobacter* cultures. A 100% pure eco-friendly organic fertilizer is **Vermi Compost**: this organic fertilizer has nitrogen, phosphorus, potassium, organic carbon, sulphur, hormones, vitamins, enzymes and antibiotics, which helps to improve the quality and quantity of yield. It is observed that, due to continuous misuse of chemical fertilizers, the soil looses its fertility and becomes saline day by day. To overcome such problems, natural farming is the only remedy and Vermi compost is the best solution.

Another eco-friendly organic fertilizer which is prepared from sugar industry waste material that is decomposed and enriched with various plants and human-friendly bacteria and fungi is **Biocompost**. Biocompost consists of nitrogen, phosphate-solubilizing bacteria and various beneficial fungi like the decomposing fungus *Trichoderma viridae*, which protects plants from various soil-borne diseases and also helps to increase the soil fertility, resulting in a good quality product for farmers.

A more detailed classification of biofertilizers is as follows:

Classification of Biofertilizers

S.N	Groups		examples
A	**N2 fixing Biofertilizer**		
	1.	Free-living	Azotobacter, Clostridium, Anabaena, Nostoc,
		Symbiotic	Rhizobium, Anabaena azollae
	3.	Associative Symbiotic	Azospirillum
B	**P Solubilizing Biofertilizer**		
	1.	Bacteria	*Bacillus subtilis, Pseudomonas striata*
	2.	Fungi	*Penicillium sp, Aspergillus awamori*
C	**P Mobilizing Biofertilizers**		
	1.	Arbuscular Mycorrhiza	*Glomus sp. , Scutellospora sp..*
	2.	Ectomycorrhiza	*Laccaria sp., Pisolithus sp., Boletus sp., Amanita sp.*
	3.	Ericoid Mycorrhiza	*Pezizella ericae*
D	**Biofertilizer for Micro nutrients**		
	1.	Silicate and Zinc solubilizers	*Bacillus sp.*
E	**Plant Growth Promoting Rhizobacteria**		
	1.	Pseudomonas	*Pseudomonas fluorescence*

Just to remind, biofertilizers are defined as biologically active products or microbial inoculants of bacteria, algae and fungi (separately or in combination), which may facilitate the biological nitrogen fixation for the benefit of plants. Biofertilizers also include organic fertilizers (manure, etc.), which are rendered in an available form due to the interaction of microorganisms or due to their association with plants.

Biofertilizers thus include the following: (i) symbiotic nitrogen fixers, *Rhizobium* spp.; (ii) non-symbiotic, free-living nitrogen fixers (*Azotobacter*, *Azospirillum*, etc.); (iii) algal biofertilizers (blue-green algae or blue-green algae in association with *Azolla*); (iv) phosphate-solubilising bacteria; (v) mycorrhizae; (vi) organic fertilizers.

The various biofertilizers are as follows:

(i) *Nitrogen-fixing biofertilizers* Nitrogen-fixing bacteria function under two types of conditions, symbiotically and as free-living (non-symbiotic) as well as associative symbiotic bacteria.

Free-Living Nitrogen-Fixing Bacteria:

They live freely in the soil and perform nitrogen fixation. Some of them are saprotrophic, living on organic remains, e.g., *Azotobacter*, *Bacillus polymyxa*, *Clostridium*, *Beijerinckia*. They are further distinguished into aerobic and anaerobic forms.

The property of nitrogen fixation is also found in photoautotrophic bacteria, e.g., *Rhizobium, Rhodopseudomonas, Rhodospirillum, Chromatium*. Inoculation of soil with these bacteria helps in increasing the yield and cutting down on nitrogen fertilizers. For example, *Azotobacter* occurring in fields of cotton, maize, jowar and rice not only increases the yield, but also cuts down on nitrogen fertilizer to about 10–25 kg/ha. Its inoculant is available under the trade name of Azotobactrin.

Rhizobia are soil bacteria which are able to colonize the legume roots and fix the atmospheric nitrogen symbiotically. The morphology and physiology of rhizobia will vary from free-living conditions to the bacteroid of nodules. They are the most efficient biofertilizer as per the quantity of fixed nitrogen. There are seven genera that are highly specific in forming nodules in legumes, referred to as a cross-inoculation group.

Azotobacter is a genus of heterotrophic free-living nitrogen-fixing bacteria present in alkaline and neutral soils. It is aerobic in nature, recommended for non-leguminous crops like paddy, millets, cotton, tomato, cabbage and other monocotyledonous crops. *Azotobacter* also produces growth-promoting compounds. *Azotobacter* performs well if the soil organic matter content is high. Response to *Azotobacter* has been seen in rice, maize, cotton, sugarcane, pearl millet, vegetable and some plantation crops.

(ii) *Free-Living Nitrogen-Fixing Cyanobacteria:*

A number of free-living cyanobacteria, or blue-green algae, have the property of nitrogen fixation, e.g., *Anabaena, Nostoc, Aulosira, Totypothrix, Cylindrospermum, Stigonema*. Cyanobacteria are photosynthetic microorganisms. Therefore, they add organic matter as well as extra nitrogen to the soil. These chlorophyll-containing prokaryotic organisms fix atmospheric nitrogen.

Aulosira fertilissima is considered to be the most active nitrogen fixer of rice fields. *Cylindrospermum licheniforme* grows in sugarcane and maize fields. Cyanobacteria are extremely low-cost biofertilisers. Phosphate, molybdenum and potassium are supplied additionally.

(iii) *Loose Association of Nitrogen-Fixing Bacteria:*

This bacterial group live partly within the root and partly outside. There is a fair degree of symbiosis between the host and the bacteria. Hence, they are called associative symbiotic bacteria. *Azospirillum* is an important bacterium in this group, recommended for millets, grass, wheat, maize, sorghum, rice etc.

(iv) *Symbiotic Nitrogen-Fixing Bacteria:*

They form a mutually beneficial association with the plants. The bacteria obtain food and shelter from plants. In return, they give to the plants part of their fixed nitrogen. The most important group of symbiotic nitrogen-fixing bacteria are rhizobia (Sg. rhizobium). They form nodules on the roots of legume plants. There are about a dozen *Rhizobium* species which form associations with the roots of different legumes, e.g. *R. leguminosarum*, *R. lupini*, *R. trifolii*, *R. meliloti*, *R. phaseoli*.

These bacteria, also called rhizobia, can live freely in the soil but cannot fix nitrogen except for a strain of cowpea *Rhizobium*. They develop the ability to fix nitrogen only when they are present inside the root nodules. In the nodule cells, bacteria (bacteroids) lie in groups surrounded by the membrane of the host cells, which is lined by a pink-red pigment called leghemoglobin. Presently cultures of *Rhizobium* specific for different crops are raised in the laboratory.

Frankia, a nitrogen-fixing mycelial bacterium (actinomycete), is associated symbiotically with the root nodules of several non-legume plants like *Casuarina*, *Alnus* (*Alder*) *Myrica*, *Rubus* etc. The leaves of a few plants (e.g., *Ardisia*) develop special internal cavities for providing space to symbiotic nitrogen-fixing bacteria, *Xanthomonas* and *Mycobacterium*. Such leaves are a constant source of nitrogen fertilizer to the soil.

(v) *Symbiotic Nitrogen-Fixing Cyanobacteria*:

Nitrogen-fixing cyanobacteria (blue-green algae) form symbiotic associations with several plants, e.g. cycad roots, liverworts, *Azolla* (fern), and lichenized fungi. *Azolla* is an aquatic floating fern, found in temperate climate suitable for paddy cultivation. The fern appears as a green mat over water, which becomes reddish due to excess anthocyanin pigmentation. The blue-green algae, cyanobacteria (*Anabaena azollae*), present as a symbiont with this fern in the lower cavities actually fixes atmospheric nitrogen.

Azolla pinnata is a small free-floating fresh water fern which multiplies rapidly, doubling every 5–7 days. The fern can coexist with rice plants because it does not interfere with their growth.

Anabaena azollae resides in the leaf cavities of the fern. It fixes nitrogen. A part of the fixed nitrogen is excreted in the cavities and becomes available to the fern. The decaying fern plants release this nitrogen for utilization by the rice plants. When a field is dried up at the time of harvesting, the fern functions as green manure, decomposing and enriching the field for the next crop.

(vi) *Microphos Biofertilizers:*

They release phosphate from bound and insoluble states, e.g., *Bacillus polymyxa*, *Pseudomonas striata*, *Aspergillus* species.

(vii) *Mycorrhiza (Pl. Mycorrhizae, Frank, 1885):*

WHY BIOFERTILIZERS?

The mycorrhiza is a mutually beneficial or symbiotic association of a fungus with the root of a higher plant. The most common fungal partners of mycorrhiza are *Glomus* species. Mycorrhizal roots show a sparse or dense wooly growth of fungal hyphae on their surface. Root cap and root hairs are absent.

Mycorrhiza is a potential biofertilizer which mobilizes P, Fe, Zn, B and other trace elements. It supplies moisture from far-off inches and is ideal for long duration crops. It can be stored up to 2 years and is dry powder resistant.

Depending upon the residence of the fungus, mycorrhizae are of two types—ectomycorrhiza and endomycorrhiza.

(a) *Ectomycorrhiza (= Ectotrophic Mycorrhiza):*

The fungus forms a mantle on the surface of the root. Internally, it lies in the intercellular spaces of the cortex. The root cells secrete sugars and other food ingredients into the intercellular spaces that feed the fungal hyphae. The exposed fungal hyphae increase the surface of the root to several times. They perform several functions for the plant as follows:

(i) Absorption of water,

(ii) Solubilisation of organic matter of the soil humus, release of inorganic nutrients, absorption and their transfer to root,

(iii) Direct absorption of minerals from the soil over a large area and handing over the same to the root. Plants with ectomycorrhiza are known to absorb 2–3 times more of nitrogen, phosphorus, potassium and calcium,

(iv) The fungus secretes antimicrobial substances which protect the young roots from attack of pathogens. Ectomycorrhiza occurs in trees such as Eucalyptus, oak (*Quercus*), peach, pine, etc. The fungus partner is generally specific. It belongs to Basidiomycetes.

(b) *Endomycorrhiza (Endotrophic Mycorrhiza):*

Fewer fungal hyphae lie on the surface. The remaining live in the cortex of the root, mostly in the intercellular spaces with some hyphal tips passing inside the cortical cells, e.g., grasses, crop plants, orchids and some woody plants. At the seedling stage of orchids, the fungal hyphae also provide nourishment by forming nutrient-rich cells called pelotons. Intracellular growth occurs in order to obtain nourishment because, unlike ectomycorrhiza, the cortical cells do not secrete sugars in the intercellular spaces.

Vesicular Arbuscular Mycorrhizal (VAM) fungi possess special structures known as vesicles and arbusculars. VAM fungi are intercellular, obligate endosymbionts and, on establishment on the root system, act as an extended root system. Besides harvesting moisture from deeper and faraway nitches in the soil, they also harvest various micronutrients and provide them to the host plants. VAM facilitates the phosphorus nutrition by not only increasing its availability, but also increasing its mobility. VAM are obligate symbionts and improve the uptake of Zn, Co, P and H_2O. Its large-scale application is limited to perennial crops and transplanted crops. A single fungus may form a mycorrhizal association with a number of plants, e.g., *Glomus*.

The different types of biofertilizers are preparations made from natural beneficial microorganisms. They are safe for all plants, animals and human beings. Being beneficial to crops and natural nutrient cycles, they not only are environmentally friendly, but also help in saving of chemical inputs.

MAIN ROLES OF BIOFERTILIZERS

- ✓ Make nutrients available.
- ✓ Make the root rhizosphere livelier.
- ✓ Growth-promoting substances are produced.
- ✓ More root proliferation.
- ✓ Better germination.
- ✓ Improve the quality and quantity of produce.
- ✓ Improve the fertilizer use efficiency.
- ✓ Higher biotic and abiotic stress tolerance.
- ✓ Improve soil health.
- ✓ Residual effect.
- ✓ Make the system more sustainable.

Liquid Biofertilizers

At present, biofertilizers are supplied to the farmers as carrier-based inoculants. As an alternative, liquid formulation technology has been developed which has more advantages than the carrier inoculants.

Benefits:

The advantages of liquid biofertilizer over conventional carrier-based biofertilizers are listed below:

a. Longer shelf-life – 12–24 months;
b. No contamination;
c. No loss of properties due to storage up to 45º C;
d. Greater potential to fight with native population;

e. Easy identification by typical fermented smell;
f. Better survival on seeds and soil;
g. Very easy to use by the farmer;
h. High commercial revenues;
i. High export potential.

CHARACTERISTICS OF DIFFERENT LIQUID BIOFERTILIZERS

Rhizobium

Physical features of liquid *Rhizobium* biofertilizer:

a) Dull white in colour;
b) No bad smell;
c) No foam formation, pH 6.8–7.5

Azospirillum

Physical features of liquid *Azospirillum* biofertilizer:

a. The colour of the liquid may be blue or dull white.
b. Bad odour confirms improper liquid formulation and may be considered as mere broth.
c. Production of yellow gummy colour materials confirms the quality product.
d. Acidic pH always confirms that there are no *Azospirillum* bacteria in the liquid.
e. Role of liquid *Azospirillum* under field conditions:
f. Stimulates growth and imparts green colour which is a characteristic of a healthy plant.
g. Aids utilization of potash, phosphorous and other nutrients.
h. Enhances the plumpness and succulence of fruits and increases the protein content.

Azotobacter

Physical features of liquid *Azotobacter* biofertilizer:

The pigment that is produced by *Azotobacter* in aged culture is melanin, which is due to oxidation of tyrosine by a copper-containing enzyme, tyrosinase. The colour can be seen in liquid forms. Some of the pigmentations are described below:

a) Produces brown-black pigmentation in liquid inoculum;
b) Produces yellow-light brown pigmentation in liquid inoculum;
c) Produces green fluorescent pigmentation in liquid inoculum;
d) Produces green fluorescent pigmentation in liquid inoculum;
e) Produces, pink pigmentation in liquid inoculum;
f) Produces less, gum-less, greyish-blue pigmentation in liquid inoculum;
g) Produces green-fluorescent pigmentation in liquid inoculum.

Acetobacter

These are sacharophillic bacteria associated with sugarcane, sweet potato and sweet sorghum plants. *Acetobacter* fixes 30 kg N/ha/year. This bacterium is mainly commercialized for sugarcane crops. It is known to increase the yield by 10–20 t/acre and sugar content by about 10–15 percent.

ADVANTAGES OF THE PRODUCTION TECHNOLOGY OF BIOFERTILIZERS

Carrier-based	**Liquid-based**
Cheap	Longer shelf-life
Easier to produce	Easier to produce
Less investment	Temperature tolerant
	High cell counts
	Contamination-free
	More effective
	Product can be 100% sterile
Disadvantages	
Low shelf-life	High cost
Temperature sensitive	Higher investment for production unit
Contamination prone	
Low cell counts	
Less effective	
Automation difficult	

CONSTRAINS OF BIOFERTILIZERS

a) Hard to find in some areas;
b) Sensitive to humidity and temperature;
c) Slower effect on plant growth;
d) Some biofertilizers need special types of machines or sprayers to use;
e) Difficult to store.

There are three main ways of using biofertilizers (liquid and carrier).

APPLICATION OF BIOFERTILIZERS

1. Seed treatment or seed inoculation;
2. Seedling root dip;
3. Main field application.

Seed treatment

One package of the inoculant is mixed with 200 mL of rice kanji to make a slurry. The seeds required for an acre are mixed in the slurry so as to have a uniform coating of the inoculant over the seeds and then shade-dried for 30 minutes. The shade-dried seeds should be sown within 24 hours. One package of the inoculant (200 g) is sufficient to treat 10 kg of seeds.

Seedling root dip

Suspend 1 to 2 kg each of nitrogen-fixing (*Azotobacter*/*Azospirillum*) and phosphate-solubilizing biofertilizer into just sufficient quantity of water (5–10 L depending upon the quantity of seedlings to be planted in one acre). Dip the roots of seedlings in this suspension for 20–30 min before transplanting. In case of paddy, make a bed of sufficient size (2 m x 1.5 m x 0.15 m) in the field, fill it with 5 cm of water and suspend 2 kg each of *Azospirillum* and phosphate-solubilizing biofertilizer and mix thoroughly. Now dip the roots of seedlings in this bed for 8–12 hours (overnight) and then transplant.

CONSTRAINTS IN BIOFERTILIZER TECHNOLOGY

Although the biofertilizer technology is a low cost, eco-friendly technology, several constraints limit the application or implementation of the technology. The constraints may be environmental, technological, infrastructural, financial, human resources, unawareness, quality, marketing, etc. The different constraints, in one way or another, affect the technique at production or marketing or usage.

Technological constraints

- Use of improper, less efficient strains for production;
- Lack of qualified technical personnel in production units;
- Production of poor quality inoculants without understanding the basic microbiological techniques;
- Short shelf-life of inoculants.

Infrastructural constraints

- Non-availability of suitable facilities for production;
- Lack of essential equipment, power supply, etc.;
- Space availability for laboratory, production, storage, etc.;
- Lack of facilities for cold storage of inoculant packages.

Financial constraints

- Non-availability of sufficient funds and problems in getting bank loans;
- Less return by sale of products in smaller production units.

Environmental constraints

- Seasonal demand for biofertilizers;
- Simultaneous cropping operations and short span of sowing/planting in a particular locality;
- Soil characteristics like salinity, acidity, drought, water logging, etc.

Human resources and quality constraints

- Lack of technically qualified staff in the production units;
- Lack of suitable training on the production techniques;
- Ignorance on the quality of the manufactured product;
- Non-availability of quality specifications and quick quality control methods;
- No regulation or act on the quality of the products;
- Awareness on the technology;
- Unawareness on the benefits of the technology;

WHY BIOFERTILIZERS?

- Problems in the adoption of the technology by the farmers due to different methods of inoculation;
- No immediate visual difference in the crop growth like that of inorganic fertilizers.

Biofertilizers have a great role in increasing the crop production. They improve the soil health status and provide different growth-promoting hormones and phytohormones to the plant. Moreover, they do not leave residual effects like those of chemical fertilizers. Thus, the use of biofertilizers could be the proper option for sustainable agriculture.

WHAT PRECAUTIONS SHOULD ONE TAKE BEFORE USING BIOFERTILIZERS?

- Biofertilizer packages need to be stored in a cool and dry place away from direct sunlight and heat.
- The right combinations of biofertilizers have to be used.
- Other chemicals (fertilizers and pesticides) should not be mixed with the biofertilizers.
- Seed treatment chemicals like Bavistine etc. should be mixed 3 days prior to mixing with biofertilizer treatment.
- Sow the treated seeds (with biofertilizer) immediately, preferably in the morning or afternoon avoiding scorching sunlight.
- The package has to be used before its expiry, only for the specified crop and by the recommended method of application.

SPECIAL LEGISLATIVE ACTS ARRANGING FERTILIZERS ACTIVITIES IN BULGARIA AND EC

- LAW on plant protection (promulgated in State Gazette 91/10.10.1997, amended in State Gazette 18/5.3.2004); Art. 1 2a of the Plant Protection Law as well as Ordinance 22 regulate the strict rules for production of plants, plant products and foodstuffs of plant origin and indications referring thereto on them. Through these legislative acts, the EC Regulations on organic plant growing or production of organic plant food products are harmonized. Such plant products are organic only in case the requirements of the Ordinance are followed – for soil fertility preservation and improvement, for utilization of plant protection materials and for usage of organic seed material.

- Ordinance No 36/18 August 2004 for the conditions and order of bio-provision and control of fertilizers (State gazette No 87/2004);
- LAW on animal husbandry (promulgated in State Gazette 65/8.8.2000, amended in State Gazette 18/5.3.2004);
- LAW on foodstuff (promulgated in State Gazette 90/15.10.1999, amended in State Gazette 70/10.8.2004).
- ORDINANCE No 22 of 4 July 2001 on organic production of plants, plant products and foodstuffs of plant origin and indications referring thereto on them (promulgated in State Gazette 68/3.8.200 1);
- ORDINANCE No 35 of 30 August 2001 on organic production of livestock, livestock products and Food stuffs of animal origin and indications referring thereto on them (promulgated in State Gazette 80/18.9.2001).

The above-mentioned acts laid down the basis for development of organic farming compliant with the sustainable development requirements in the agricultural sector and its contribution to biodiversity conservation.

In the EU, microorganisms (bacteria, viruses and fungi) are included as possible inputs in the EU Commission Regulation n. 889/2008 on organic production, but only for the biological control of pests and diseases. As such, they are thus listed within the legal framework dealing with plant protection products, as biocontrol agents.

Another document is the **EU Landfill Directive,** which currently is the primary driver for initiatives on biodegradable waste. Its implementation at a national level often also includes separate collection of organic waste, and composting/AD as its primary destination. Anyway, no general provision is included for the destination of biodegradables; hence, the way that composting and anaerobic digestion shall be combined with incineration will be a matter of local strategies, and they factually vary widely from country to country.

REGULATIONS **Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91**

CONCLUSION

Biofertilizers increase the availability of plant nutrients and can help in maintenance of the soil fertility over a long period. As discussed earlier, some microorganisms have the beneficial role of biological nitrogen fixation to supply nitrogen to crops, solubilizing insoluble phosphates to plant-available (soluble) forms and synthesizing biomass for manuring of crops like rice. Biofertilizers are, therefore, economical, renewable and eco-friendly, but they cannot totally replace chemical fertilizers. Biofertilizer use is an important component of Integrated Nutrient Management and organic farming. These technologies are becoming vital in modern-day agricultural practices. The changing scenario of agricultural practices and environmental hazards associated with chemical fertilizers demand a more significant role of biofertilizers in coming years.

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TRENDS IN BIOFERTILIZERS PRODUCTION

Contents

INTRODUCTION

Biofertilizers hold a promising future in the development of the market, production, technologies, tools and instruments etc. They are promising in reducing soil quality problems with optimum crop yield. As it was highlighted in Part I of Module 1, biofertilizers are a complex product of live microbial inoculants which are able to fix atmospheric nitrogen, solubilize soil phosphorus, decompose organic material or oxidize sulphur in the soil. Biofertilizers are artificially multiplied cultures of beneficial soil microorganisms that can improve soil fertility and crop productivity. They add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus and stimulating plant growth through the synthesis of growth-promoting substances. They are made from biological wastes and do not contain any chemicals. The main sources of biofertilizers are bacteria, fungi and cynobacteria (blue-green algae).

TRENDS

Development of new eco-friendly technologies for production

The new eco-friendly technologies for production of biofertilizers will overcome the shortcomings of the conventional chemical-based farming which dominates at present. The implementation of technologies shows positive influence on both soil sustainability and plant growth. They support and gradually improve soil fertility by fixing atmospheric nitrogen. They increase the phosphorous content of the soil by solubilizing and releasing unavailable phosphorous. They participate in restoring depleted nutrients in the soil. Growth-promoting substances released by biofertilizers improve plant root proliferation. They also guard the plant against some soil-borne diseases. To popularize and implement more biofertilizers, there is a need of development of new technologies as follows:

Correct soil treatment

The role of plant nutrients in crop production is well-established and 16 essential plant nutrients have to be available to the crops in required quantities to achieve the yield target. Many studies have also emphasized the importance of N, P and K in enhancing the natural ability of plants to resist stress from drought and cold, pests and diseases. The essential plant nutrients such as N, P, K, Ca, Mg and S are called macronutrients, while Fe, Zn, Cu, Mo, Mn, B and Cl are called micronutrients.

It is necessary to assess the capacity of a soil to supply the lacking amounts of needed plant nutrients (total crop requirement–soil supply). This is also important to produce a good biofertilizer formulation and to supply nutrients that can improve soil health and plant fertility. Several authors have focused their attention on the potential usage of nitrogen from animal manures. Nonetheless, the effort to find a source alternative to animal manure needs further study. Granite powder has also been studied as a good source of slow-release K fertilizer.

Generally, the addition of nitrogen to high C:N ratio residues is capable of accelerating the microbial activity during the fermentation process.

The number of microorganisms and the level of macro- and micronutrients obviously affect the growth of plants. One of the benefits of fertilizers is that they contribute to the availability of the microorganism population. Having a higher initial count of appropriate microbes in a ready biofertilizer right after the fermentation is essential. One of the ways to increase the number of selected microorganisms is by using the concept of an effective microorganism (EM) as introduced by Higa and Wididana (1991). Field experiments are needed to determine the nutrient availability and efficacy of most organic fertilizers. Such an experiment is important because the nutrient content of organic fertilizers varies widely. The quality is directly determined by the number of selected microorganisms in an active form per gram and their capability to promote plant growth and soil fertility.

Water-in-oil emulsions appear to be a good, yet underutilized, method for storing and delivering microorganisms through liquid formulations. The oil traps the water around the organism and, therefore, slows down water evaporation once applied. This is particularly beneficial for organisms that are sensitive to desiccation or in the case of use for horticultural crops where irrigation systems are in place. Water-in-oil emulsions allow the addition of substances to the oil and/or aqueous phases which could improve both the cell viability and the kinetics of release. However, cell sedimentation during storage is a major issue to be considered. *Studies aimed at solving this problem with the help of nanomaterials are underway.* Thickening the oil phase using hydrophobic silica nanoparticles can significantly reduce cell sedimentation and improve cell viability during storage.

Preparation of bacterial inoculants is supported by implementation of a new process based on the application of supercritical fluid properties which has been tested to encapsulate virus formulations. The process, named PGSS (Particles from Gas Saturated Solutions), is carried out at low temperatures and uses carbon dioxide as a supercritical fluid. Therefore, there should be no negative effects on the microbial viability, and the cost of production would be relatively low. The final product of the process is almost spherical particles that form a free-flowing powder which can be suspended in water. The possibilities of the PGSS process have already successfully been demonstrated for several solids and liquids.

Another interesting new technology is the exploitation of the natural production of bacterial biofilms as a possible carrier, and not only for the production of the inoculum, of defined bacterial or fungal–bacterial consortia. Biofilm production is already obtained for different industrial applications (e.g., wastewater treatment, production of chemical compounds). Two types of biofilms are employed in that case: biofilms growing onto inert supports (charcoal, resin, concrete, clay brick, and sand particles) and biofilms that are formed as a result of aggregate formation. In the first case, biofilms grow all around the particles, and the size of the biofilm particles grows with time usually to several millimeters in diameter. Biofilms formed by aggregation are called granular biofilms; granule formation may take from several weeks to several months.

There are four stages to the development of a mature biofilm: initial attachment, irreversible attachment by the production of EPS, early development, and maturation of biofilm architecture. What is particularly critical is the production of EPS, which serves to bind the cell to the surface and to protect it from the surrounding environment. EPS can be composed of polysaccharides, proteins, nucleic acids or phospholipids. A common EPS produced by bacterial cells in biofilms is the exopolysaccharide alginate. Beneficial biofilms developed in *in vitro* cultures containing both fungal and bacterial strains have been used as biofertilizers for non-legume species with good efficacy. Application of a biofilmed inoculant containing a fungal-rhizobia consortium significantly increased N_2 fixation in soybean compared to a traditional rhizobium inoculant. Wheat seedlings inoculated with biofilm-producing bacteria exhibited an increased yield in moderate saline soils. Biofilms seem also to help the microorganisms to survive after inoculation even under stress conditions: this is a key aspect for the effectiveness of PGPM inoculation under agricultural conditions. Inoculants made with biofilms were shown to allow their rhizobia to survive at high salinity (400 mM NaCl) by 105-fold compared to rhizobial monocultures. Interestingly, beneficial endophytes were observed to produce higher acidity and plant growth-promoting hormones than their mono- or mixed cultures with no biofilm formation.

Technologies used for the production of living hybrid materials could be a new frontier in the development of carriers for PGPMs. Silica has appeared as a promising host for microorganism encapsulation: immobilization pathways are based on immobilization of a population of bacteria dispersed into a silica gel. Bacteria can be either entrapped into alginate microbeads coated with silica membranes or into macrocavities created inside the silica matrix. Such materials improve the mechanical properties of the alginate bead, the reduce cell leakage and enhance the cell viability.

The application of bio-nanotechnology could also provide new avenues for the development of carrier-based microbial inoculants. Nanotechnology employs nanoparticles which are made of inorganic or organic materials that are defined by having one or more dimensions in the order of 100 nm or less. The integration of whole cells with nanostructures leads to hybrid systems that have numerous applications in many fields, including agriculture. Indeed, even though nanoscale constructs are smaller than cells, macroscopic filters, made of radially aligned carbon nanotube walls, able to absorb *Escherichia coli*, were fabricated. The same technology could therefore be applied to collect bacterial cells from fermentation processes and deliver them to the plant. The physical stability and the high surface area of nanotubes, together with the ease and cost-effective fabrication of nanotube membranes may thus expand their use in the production of biofertilizer. The use of nanoformulations may enhance the stability of biofertilizers and biostimulators with respect to desiccation, heat and UV inactivation. The addition of hydrophobic silica nanoparticles of 7–14 nm to the water-in-oil emulsion formulation of the biopesticide fungus *Lagenidium giganteum* reduced the desiccation of the mycelium. The physical features of the formulation were improved and the microorganism was still effective after 12 weeks of storage at room temperature.

PRODUCT MODIFICATION AND INTRODUCTION OF INNOVATIVE PRODUCTS

The basic need of modern marketing is to regularly keep track of the consumers behaviour and adapt immediately to the requirements or the benefits sought by the consumers. As far as biofertilizers are concerned, it has been consistently argued for over a decade that there are tremendous product- and market-related constraints; however, the marketing organizations have not been able to adapt to the needs of the business environment.

The biofertilizers in a powder form have several constraints, as discussed above, which could be overcome to a great extent by product modification from a "powder form" to a "liquid form", which has tremendous superior benefits, as discussed below. The product innovation is another step forward towards tackling farmers' issues and some of them are the potash mobilizers like *Frateuria aurentia*, zinc and sulphur solubilizers like *Thiobacillus* species and manganese solubilizer fungal cultures like *Penicillium citrinum*, which have been identified for commercial operations and are highly useful and economical for enhancing agricultural productivity.

DEVELOPMENT OF BIOFERTILIZERS LEGISLATION

There are no specific regulations in the European Union that set parameters for biofertilizers. Each country locally regulates this matter. For example, the Polish Law on Fertilizers and Fertilization of July 10th 2007 includes "growth stimulators" in the category of plant conditioners. These are products which have "a positive impact on plant growth or other metabolic processes of plants in other ways than plant nutrients" and shall "pose no threat to [the] health of humans or animals or to the environment after their use according to use and storage instruction". This definition can be applied to biofertilizers, but no specific requirements are foreseen for such a category of products.

Spain, which is the second largest producer of conventional fruit and vegetables after Italy and among the leading countries in organic crops in Europe, does not include the term 'biofertiliser' in its legislation. The newest legal provision dealing with fertilizers (Real Decreto 506/2013) defines the number of microorganisms in organic amendments and compost but does not mention plant beneficial microorganisms. Fertilizers are defined as "Products used in agriculture or gardening, which, for their nutrient content, facilitate plant growth, increase performance and improve crop quality or which, by their specific action, amending, as appropriate, modify soil fertility or its physical, chemical or biological properties and that meet the requirements of Article 4.2 of this Royal Decree characteristics." Fertilizers, specialty products and amendments are also included in this definition. The Spanish administrative system allows local administrations to additionally regulate the matter (http://www.juntadeandalucia.es).

In Italy, only the mycorrhizal fungi inoculants are included within the group of "Products with action on the soil" and in the miscellaneous category of "Products with specific action" foreseen in the Decreto Legislativo of 29th April 2010, n. 75. The quality requirements established by the legal provision foresee that the inoculum is reproduced under sterile conditions on roots of sorghum in a substrate formed by an organic soil conditioner and rhizosphere bacteria. These conditions, particularly the "sterile conditions" requirement, are practically very difficult to achieve, considering the need of organic substrate. Besides, the presence of rhizosphere bacteria requires, from the point of view of the mycorrhizal fungus, unsterile conditions of the substrate. The label of such products shall indicate which organic matrix is used (presumably as a carrier), the name of the mycorrhizal fungal species included, and the name of rhizosphere bacteria and trichoderma species, even though the last two types of microorganisms are not AMF. No genetically modified organisms are allowed to be utilized for making this product; pathogens such as *Salmonella* spp., *Escherichia coli*, and other aerobic mesophilic microorganisms and nematode eggs shall not be present.

Proposals for an EU legislation on biofertilisers

The overall EU policy for the development of the agricultural sector in the next programming period (EU COM (2020)) underlines the need of reducing the impact on the environment of agricultural practices and the possibility of an increased use of alternatives to chemical inputs. The achievement of the objectives of rural development, which contribute to the Europe 2020 strategy for smart, sustainable and inclusive growth shall be pursued, among others, through the improvement of soil management, the preservation of biodiversity, the fostering of knowledge transfer and innovation and the promotion of resource efficiency. Furthermore, there is a strong emphasis on a wider application of agricultural practices based on low input (e.g. EU Directive 2009/128 on the sustainable use of pesticides) and on organic farming practices. Based on these policies, the support to research dedicated to biotechnological processes and products has a strong focus through the Horizon 2020 Programme (EU COM (2011) 808). In such a context, it is thus feasible to expect an increased interest among producers to develop products based on biological compounds and microorganisms.

DEVELOPMENTS OF THE BIOFERTILIZERS MARKET

There is a nascent but aggressively growing biofertilizers market. Among the major concerns in today's world are the pollution and contamination of soil by excessive and injudicious use of agrochemicals, as well as their detrimental effects to humans, in particular, by agricultural workers and rural communities. The concerns on both the health and environmental front have compelled governments to look for environmentally friendly options and switching from 'risk reduction' and 'safe use' procedures, in sustainable agricultural production. The use of biofertilizers and biopesticides offers a better option to augment the ‘Fertilizer Use Efficiency’ and maintain soil health. Biofertilizers are seen as an important component in Integrated Nutrient Management, with a supplementary role for the largest consumers of fertilizers.

TRENDS IN BIOFERTILIZERS PRODUCTION

Challenges and options in the biofertilizer business

In spite of being a cost-effective input, biofertilizers have not been completely accepted by the farmers till now. Some of the reasons/constraints for this low acceptance of biofertilizers are narrated below. However, the product modification as a "liquid form" has overcome some limitations and has provided opportunities for marketers.

Marketing challenges:

a) Biofertilizers are live microorganisms which die in case of high temperature.

b) The shelf-life of biofertilizers is limited to 6–12 months in powder form.

c) Biofertilizers are used before sowing and delay in dispatches leads to inventory carry-over and expiry of product.

d) Some biofertilizers are crop specific as well as location specific and, therefore, their efficacy does not remain the same at different locations due to differences in agro-climatic conditions and soil edaphic factors.

e) Soil characteristics like high nitrate, low organic matter, less available phosphate, high soil acidity or alkalinity, high temperature as well as presence of high levels of agro-chemicals or low levels of micro-nutrients contribute to failure of inoculants or adversely affect their efficacy.

f) The changes in the cropping patterns by farmers also adversely affect the sales.

g) Supply of sub-standard or spurious material by some manufacturers also adversely affects the credibility of biofertilizers, as they are a new product.

h) Some firms are selling organic manures as biofertilizers. Some organizations state a shelf-life of two/one year despite the norm of maximum 3–6 months.

i) Naturally occurring soil microflora and fauna also often inhibit the growth of introduced inoculums due to competition.

j) Lack of awareness among farmers regarding the benefits of biofertilizers.

k) There is no magic effect of biofertilizers and their impact is not visible in standing crop and, therefore, farmers are not convinced with the benefits of biofertilizer use.

Trend option – switching over to liquid biofertilizers, as they are superior than powder-based ones

1. Longer shelf-life to as long as 12 to 24 months.

2. No contamination.

3. No effect of high temperature, as tolerant up to 45 degrees Celsius with no activity loss.

4. Greater potential to fight with native population.

5. High population density of more than 10^9 cells/mL can be maintained up to 12 to 24 months.

6. Easy identification by typical fermented smell.

7. Cost saving on carrier material, pulverization, neutralization, sterilization, packing and transport.

8. Quality control protocols are easy and quick.

9. Better survival on seeds and soil.

10. No need of running biofertilizer production units throughout the year.

11. Very easy to use by the farmer.

12. Dosage is 10 times less than that of carrier-based powder based biofertilizers.

13. High commercial revenues.

14. High export potential.

15. Very high enzymatic activity, since contamination is nil.

Some examples of marketing strategies, as suggested below, may work strongly in the marketing of biofertilizers:

1. Field demonstration.

The farmers do what they see because "seeing is believing" and, therefore, result as well as method demonstration is a very effective tool in promoting the use of biofertilizers. The producers may synergize their efforts on this front, as biofertilizers are new and it is very crucial to show the impact of biofertilizer use to farmers and educate them about the economics/returns. Therefore, a demonstration farm may be developed jointly, at different locations, defining a catchment area, which could be shown to farmers at different crop stages.

2. Market segmentation and product positioning.

The segmentation is primarily dividing the market into various groups of buyers. The biofertilizer market can be segmented by "specific crop grower (Fruits/ Vegetables/ Oilseed/ Pulses/ Sugarcane/ Cereals), institutional buyers (Cane / Tea / Coffee / cotton/ oilseeds/ pulses federations and research-farms, SFCI, Agro-industries, etc) and customer size (major/minor), geographical location (high/low-consuming area and accessibility), and product application (supplementary/exclusive)". Once the market is segmented, it is important to target the market and concentrate on the most profitable one. Positioning starts with a product, but positioning is not what one does to a product; rather, it is what one does to the mind of a prospective customer. Thus, the product is being positioned in the mind of the customer, i.e. how he/she perceives the product. In an "over-communicated society", the marketer must create distinctiveness. The appropriate

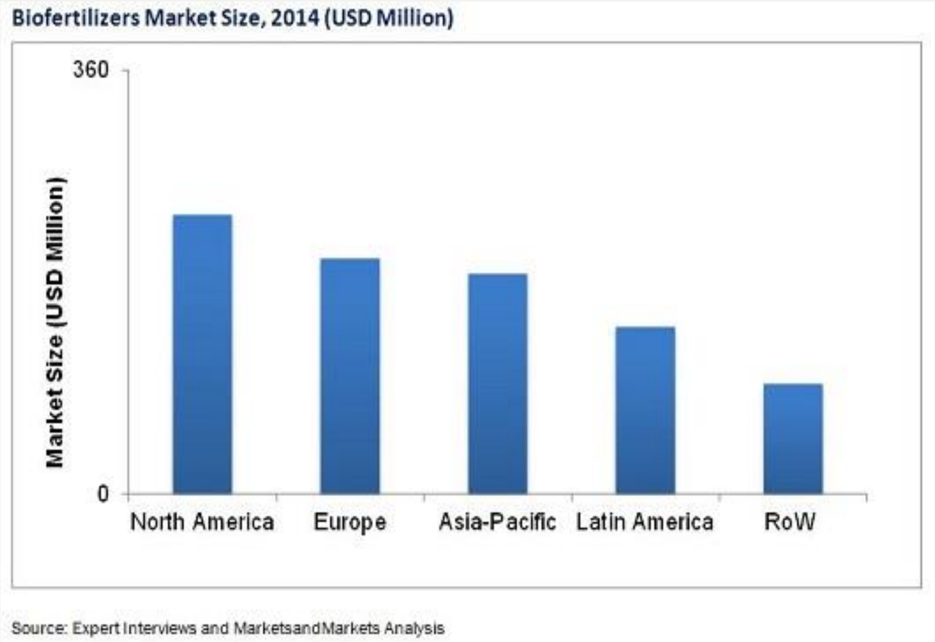
"USP" (Unique Selling Proposition) needs to be identified and propagated widely, for example: (a) Save cost through reduced dosage of chemical fertilizer; (b) Improves resistance power against disease; (c) Enhance sugar recovery percent in sugarcane.

Trends in pricing and sales promotion of right biofertilizers

Rural markets are quite "price sensitive" and particularly biofertilizers, being technical and new to farmers with a lot of constraints, do not fall under the category of "zero elasticity of demand" and need more push in view of lack of pull. The company generally determines the price of a product on the basis of its marketing objectives. Here, it is important to understand how biofertilizers are perceived in terms of value offered for money spent by customers. Biofertilizers have derived demand and so far, they have not really been perceived by farmers as giving those economic returns by reduction in the quantity of chemical fertilizers used. Unless farmers are convinced about substantial savings in cost of production through reduced usage of chemical fertilizers and getting similar yield, biofertilizer manufacturers will probably not be able to apply "pricing strategies".

The global biofertilizers market

The global biofertilizers market is expected to reach USD 1.88 billion by 2020 at a CAGR of 14.0% from 2015 to 2020. In 2012, the overall market was worth US $440.0 mln.



The biofertilizers market is projected to grow at a CAGR of 14.0% from 2015 to 2020. The increasing demand for organic products from emerging economies due to increased spending power and awareness level regarding health and wellness are expected to accelerate the growth of the biofertilizers market.

Sales and usage promotion

There is a great need to promote the product, from the point of view of both sales and usage. The channel members, i.e. dealer/distributors, need to be motivated by offering tangible benefits/incentives linking sales targets, such as “free family tour, gifts etc.” Similarly, the consumer also needs to be attracted by offers of coupons, premiums, contests, buying allowances etc. based on customer characteristics/buying behaviour. The progressive farmer village leaders, besides dealers, may also be identified for the purpose of conducting demonstrations and should be appropriately compensated.

Publicity and training

The POS (Point of Sales) material must be made available to all dealer/distributors and it also needs to be ensured that the product is displayed visibly. Wider publicity through Radio and educational films screening also needs to be taken up vigorously. Free distribution of biofertilizer during farmer meetings must be avoided. The orientation and training programmes for field sales force and dealers/distributors also need to be chalked out. There is a need of an exclusive team of Extension Executives for promoting biofertilizers with constant visits and developing a close connection with farmers and undertaking demonstrations with replication in nearby villages.

The major research focus is and should be on the production of efficient and sustainable biofertilizers for crop plants, wherein inorganic fertilizer application can be reduced significantly to avoid further pollution problems.

The most important and specific research needs, according to Swapna Latha Aggani from Kakatiya University, should highlight the following points:

1. Selection of effective and competitive multi-functional biofertilizers for a variety of crops;

2. Quality control system for the production of inoculants and their application in the field, to ensure and explore the benefits of plant microorganism symbiosis;

3. Study of microbial persistence of biofertilizers in soil environments under stressful conditions;

4. Agronomic, soil and economic evaluation of biofertilizers for diverse agricultural production systems;

5. Transferring technological know-how on biofertilizer production to the industrial level and for optimum formulation;

6. Establishment of legislation and strict regulation for quality control in markets and application.

TRENDS IN INNOVATIVE PRODUCTION OF BIOFERTILIZERS AS KEY PLAYERS IN SUSTAINABLE AGRICULTURE BY IMPROVING SOIL FERTILITY, PLANT TOLERANCE AND CROP PRODUCTIVITY

The microbiome: potential significance of beneficial microbes in sustainable agriculture

The rhizosphere, which is the narrow zone of soil surrounding plant roots, can comprise up to 10^{11} microbial cells per gram of root and above 30,000 prokaryotic species that, in general, improve plant productivity. The collective genome of the rhizosphere microbial community enveloping the plant roots is larger compared to that of plants and is referred to as microbiome, whose interactions determine the crop health in natural agro-ecosystems by providing numerous services to crop plants *viz.*, organic matter decomposition, nutrient acquisition, water absorption, nutrient recycling, weed control and biocontrol.

The metagenomic study provides the individual, the core rhizosphere and endophytic microbiomes activity in *Arabidopsis thaliana* using 454 sequencing (Roche) of 16S rRNA gene amplicons. It has been proposed that exploiting tailor-made core microbiome transfer therapy in agriculture can be a potential approach in managing plant diseases for different crops. Rhizosphere microbial communities as an alternative to chemical fertilizers has become a subject of great interest in sustainable agriculture and biosafety programmes.

A major focus in the coming decades would be on safe and eco-friendly methods by exploiting the beneficial microorganisms in sustainable crop production. Such microorganisms, in general, consist of diverse naturally occurring microbes whose inoculation into the soil ecosystem advances soil physicochemical properties, soil microbial biodiversity, soil health, plant growth and development and crop productivity. The agriculturally useful microbial populations cover plant growth-promoting rhizobacteria, N_2-fixing cyanobacteria, mycorrhiza, plant disease suppressive beneficial bacteria, stress-tolerant endophytes and biodegrading microbes. Biofertilizers are a supplementary component to soil and crop management traditions, *viz.* crop rotation, organic adjustments, tillage maintenance, recycling of crop residue, soil fertility renovation and the biocontrol of pathogens and insect pests, whose operation can be significantly useful in maintaining the sustainability of various crop productions. *Azotobacter*, *Azospirillum*, *Rhizobium*, cyanobacteria, phosphorus- and potassium-solubilizing microorganisms and mycorrhizae are some of the PGPRs that have been found to increase in the soil under no tillage or minimum tillage treatment. Efficient strains of *Azotobacter*, *Azospirillum, Phosphobacter* and *Rhizobacter* can provide significant amount of nitrogen to *Helianthus annus* and to increase the plant height, number of leaves, stem diameter percentage of seed filling and seed dry weight. Similarly, in rice, addition of *Azotobacter*, *Azospirillum* and *Rhizobium* promotes the physiology and improves the root morphology.

Azotobacter plays an important role in the nitrogen cycle in nature, as it possesses a variety of metabolic functions. Besides playing a role in nitrogen fixation, *Azotobacter* has the capacity to produce vitamins, such as thiamine and riboflavin, and plant hormones, *viz.* indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK). *A. chroococcum* improves the plant growth by enhancing seed germination and advancing the root architecture by inhibiting the pathogenic microorganisms around the root systems of crop plants. This genus includes diverse species, namely, *A. chroococcum, A. vinelandii, A. beijerinckii, A. nigricans, A. armeniacus* and *A. paspali*.

It is used as a biofertilizer for different crops, *viz.* wheat, oat, barley mustard, sesame, rice, linseeds, sunflower, castor, maize, sorghum, cotton, jute, sugar beets, tobacco, tea, coffee, rubber and coconuts. *Azospirillum* is another free-living, motile, Gram-variable, aerobic bacterium that can thrive in flooded conditions and promotes various aspects of plant growth and development. *Azospirillum* has been shown to exert beneficial effects on plant growth and crop yields both in greenhouse and in field trials. Diverse species of the *Azospirillum* genus, including *A. lipoferum*, *A. brasilense, A. amazonense, A. halopraeferens* and *A. irakense* have been reported to improve the productivity of various crops. Interestingly, it was observed that *Azospirillum* inoculation can change the root morphology via producing plant growth-regulating substances via siderophore production. It also increases the number of lateral roots and enhances the formation of root hairs to provide more root surface area to absorb sufficient nutrients. This improves the water status of the plant and aids the nutrient profile in the advancement of plant growth and development. Co-inoculation of *Azospirillum brasilense* and *Rhizobium meliloti* plus 2,4-D had a positive effect on the grain yield and N, P, K content of *Triticum aestivum*. *Rhizobium* has been used as an efficient nitrogen fixer for many years. It plays an important role in increasing yields by converting atmospheric nitrogen into usable forms. Being resistant to different temperature ranges, *Rhizobium* normally enters the root hairs, multiplies there and forms nodules. *Rhizobium* inoculants in different locations and soil types have been reported to significantly increase the grain yields of Bengal gram and lentil and enhance the rhizosphere of pea, alfalfa and sugar beet, berseem, ground nut and soybean. *Rhizobium* isolates obtained from wild rice have been reported to supply nitrogen to the rice plant to promote growth and development. A Rhizobiaceae species, *Sinorhizobium meliloti* 1021, infects plants other than legumes, e.g. rice, to promote growth by enhancing the endogenous level of plant hormone and photosynthesis performance to confer plant tolerance to stress. In groundnut, the IRC-6 rhizobium strain has resulted in the enhancement of several useful traits such as increased number of pink coloured nodules, nitrate reductase activity and leghaemoglobin content in 50 DAI (days after inoculation). Rhizobial symbiosis provides defence to plants against pathogens and herbivores, such as, Mexican bean beetle and the greenhouse whitefly *Trialeurodes vaporariorum*.

TRENDS IN BIOFERTILIZERS PRODUCTION

Potential use of soil microbes in sustainable crop production

The beneficial soil microorganisms sustain crop production either as biofertilizers or as symbionts. They perform nutrient solubilization, which facilitates the nutrient availability and thereby uptake. This improves the plant growth by advancing the root architecture. Their activity provides several useful traits to plants such as increased root hairs, nodules and nitrate reductase activity, and efficient strains of *Azotobacter*, *Azospirillum*, *Phosphobacter* and *Rhizobacter* can provide a significant amount of available nitrogen through nitrogen cycling. Biofertilizers produce plant hormones, which include indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK). Biofertilizers improve photosynthesis performance to confer plant tolerance to stress and increase the resistance to pathogens, thereby resulting in crop improvement.

Biofertlizers exploitation and nutrient profile of crops

A key advantage of beneficial microorganisms is to assimilate phosphorus for their own requirements, which in turn, becomes available in its soluble form in sufficient quantities in the soil. *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Fusarium, Sclerotium, Aspergillus* and *Penicillium* have been reported to be active in the solubilization process. A phosphate-solubilizing bacterial strain NII-0909 of *Micrococcus* sp. has polyvalent properties, including phosphate solubilization and siderophore production. Similarly, two fungi, *Aspergillus fumigatus* and *A. niger*, isolated from decaying cassava peels have been found to convert cassava wastes by the semi-solid fermentation technique to phosphate biofertilizers. *Burkholderia vietnamiensis,* a species of stress tolerant bacteria, produces gluconic and 2-ketogluconic acids, which are involved in phosphate solubilization. *Enterobacter* and *Burkholderia* isolated from the rhizosphere of sunflower produce siderophores and indolic compounds (ICs) which can solubilize phosphate. Potassium-solubilizing microorganisms (KSM), such as the genera *Aspergillus*, *Bacillus* and *Clostridium*, are efficient in potassium solubilization in the soil and mobilization in different crops. Mycorrhizal mutualistic symbiosis with plant roots satisfies the plant nutrients demand, which leads to enhanced plant growth and development, and protects plants from pathogen attacks and environmental stress. It leads to the absorption of phosphate by the hyphae from outside to the internal cortical mycelia, which finally transfer phosphate to the cortical root cells. Nitrogen-fixing cyanobacteria, such as *Aulosira, Tolypothrix, Scytonema*, *Nostoc, Anabaena* and *Plectonema*, are commonly used as biofertilizers. Besides the contribution of nitrogen, growth-promoting substances and vitamins liberated by these algae, *Cylindrospermum musicola* increases the root growth and yield of rice plants. Interestingly, genetic engineering was used to improve the nitrogen-fixing potential of *Anabaena* sp. strain PCC7120. Constitutive expression of the *hetR* gene driven by a light-inducible promoter enhanced HetR protein expression, leading to higher nitrogenase activity in *Anabaena* sp. strain PCC7120 as compared with the wild-type strain. This, in turn, caused better growth of paddy when applied to the fields.

Biofertilizers relevance and plant tolerance to environmental stress

Abiotic and biotic stresses are the major constraints that affect the productivity of crops. Many tools of modern science have been extensively applied for crop improvement under stress, of which the role of PGPRs as bioprotectants has become of paramount importance in this regard. *Trifolium alexandrinum* inoculated with *Rhizobium trifolii* showed higher biomass and increased nodulation under salinity stress conditions. *Pseudomonas aeruginosa* has been shown to withstand biotic and abiotic stresses. Paul and Nair found that *P. fluorescens* MSP-393 produces osmolytes and salt-stress induced proteins that overcome the negative effects of salt. *P. putida* Rs-198 enhanced the germination rate and several growth parameters, *viz,* plant height, fresh weight and dry weight, of cotton under alkaline and high-salt conditions via increasing the rate of uptake of K^+, Mg^{2+} and Ca^{2+}, and by decreasing the absorption of Na^+. A few strains of *Pseudomonas* reportedly confer plant tolerance via 2,4-diacetylphloroglucinol (DAPG). Interestingly, systemic response was found to be induced against *P. syringae* in *Arabidopsis thaliana* by *P. fluorescens* DAPG. Calcisol produced by PGPRs, *viz. P. alcaligenes* PsA15, *Bacillus polymyxa* BcP26 and *Mycobacterium phlei* MbP18, provides tolerance to high temperatures and salinity stress. It has been demonstrated that inoculation of plants with AM fungi also improves plant growth under salt stress. *Achromobacter piechaudii* was also shown to increase the biomass of tomato and pepper plants under 172 mM NaCl and water stress. Interestingly, a root endophytic fungus *Piriformospora indica* was found to defend its host plants against salt stress. It has been found that inoculation of PGPR alone or along with AM like *Glomus intraradices* or *G. mosseae* resulted in better nutrient uptake and improvement in the normal physiological processes in *Lactuca sativa* under stress conditions. The same plant treated with *P. mendocina* increased its shoot biomass under salt stress. Studies on the mechanisms involved in osmotic stress tolerance employing transcriptomic and microscopic strategies have revealed a considerable change in the transcriptome of *Stenotrophomonas rhizophila* DSM14405^T in response to salt stress. A combination of AM fungi and N_2-fixing bacteria helped the legume plants in overcoming drought stress. The effect of *A. brasilense* along with AM can be seen in other crops such as tomato, maize and cassava. *A. brasilense* and AM in combination improved the plant tolerance to various abiotic stresses. The additive effect of *Pseudomonas putida* or *Bacillus megaterium* and AM fungi was effective in alleviating drought stress. Application of *Pseudomonades* sp. under water stress improved the synthesis of antioxidant and photosynthetic pigments in basil plants. Interestingly, a combination of three bacterial species caused the highest CAT, GPX and APX activity and chlorophyll content in leaves under water stress. *Pseudomonas* spp. was found to have a positive effect on the seedling growth and seed germination of *A. officinalis* L. under water stress. The photosynthetic efficiency and the antioxidant response of rice plants subjected to drought stress have been found to increase after inoculation of arbuscular mycorrhiza. The beneficial effects of mycorrhizae have also been reported under both drought and saline conditions. Heavy metals such as cadmium, lead and mercury from hospital and factory waste accumulate in the soil and enter plants through the roots. *Azospirillium* spp., *Phosphobacteria* spp. and *Glucanacetobacter* spp. isolated from the rhizosphere of rice fields and mangroves have been found to be more tolerant to heavy metals, especially iron. *P. potida* strain 11 (P.p.11), *P. potida* strain 4 (P.p.4) and *P.*

fluorescens strain 169 (P.f.169) can protect canola and barley plants from the inhibitory effects of cadmium via IAA, siderophore and 1-aminocyclopropane-1-carboxylate deaminase (ACCD). It has been reported that rhizoremediation of petroleum contaminated soil can be expedited by adding microorganisms in the form of effective microbial agent (EMA) to different plant species such as cotton, ryegrass, tall fescue and alfalfa.

PGPRs as biological agents proved to be one of the alternatives of chemical agents to provide resistance to various pathogen attacks. Apart from acting as growth-promoting agents, they can provide resistance against pathogens by producing metabolites. *Bacillus subtilis* GBO3 can induce defense-related pathways, *viz.* salicylic acid (SA) and jasmonic acid (JA). Application of PGPR isolates, *viz. B. amyloliquefaciens* 937b and *B. pumilus* SE-34, provides immunity against tomato mottle virus. *B. megaterium* IISRBP 17 characterized from black pepper stem acts against *Phytophthor capsici*. *Bacillus subtilis* N11 along with mature composts was found to control *Fusarium* infestation on banana roots. Similarly, *B. subtilis* (UFLA285) was found to provide resistance against *R. solani* and also to induce foliar and root growth in cotton plants. In another interesting study, *Paenibacillus polymyxa* SQR-21 was identified as a potential agent for the biocontrol of *Fusarium* wilt in watermelon. Further, the exploitation of PGPRs was found to be effective to manage the spotted wilt viruses in tomato, cucumber mosaic virus of tomato and pepper, and banana bunchy top virus in banana. In some cases, along with bacteria, mycorrhizae can also confer resistance to fungal pathogens and inhibit the growth of many root pathogens, such as *R. solani*, *Pythium* spp., *F. oxysporum*, *A. obscura* and *H. annosum*, by improving the plant nutrient profile and thereby the productivity. For instance, *Glomus mosseae* is effective against *Fusarium oxysporum* f. sp. *basilica*, which causes root-rot disease of basil plants. *Medicago tranculata* also showed induction of various defense-related genes with mycorrhizal colonization. It was shown that addition of arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* to the soil can reduce the development of root-rot disease and enhance the yield of *Phaseolus vulgaris* L.

Mechanism of action of various biofertilizers

Mycorrhiza is the association of fungi with the roots of higher plants. While it remains an enigma, it serves as a model system to understand the mechanism behind stimulation of growth in the root cells as a result of mycorrhizal inhabitation. The genome sequencing of two EM fungi (ectomycorrhizae), *L. bicolor* 13 and *T. melanosporum* (black truffle) 14, has helped in the identification of factors that regulate the development of mycorrhiza and its function in the plant cell. Fifteen genes up-regulated during symbiosis have been identified as putative hexose transporters in *L. bicolor*. Its genome lacks genes encoding invertases, making it dependent on plants for glucose. However, *T. melanosporum* possesses one invertase gene, and unlike *L. bicolor*, it can directly use the sucrose of the host. The up-regulation of transporter genes during symbiosis indicated the role of transportation of useful compounds like amino acids, oligopeptides and polyamines through the symbiotic interface from one organism to the other. Free-living mycelium can take nitrate and ammonium from the soil. Subsequently, these compounds reach the mantle and Hartig net and are then transferred to the plants. Cysteine-rich proteins (MISSP7) of the fungus play an important role as effectors and facilitators in the formation of symbiotic interfaces. Many genes related to auxin biosynthesis and root morphogenesis showed up-regulation during mycorrhizal colonization. Further, *G. versiforme* possesses inorganic phosphate (Pi) transporters on its hyphae, which help in the direct absorption of phosphate from the soil, and a glutamine synthase gene was found in *G. intraradice*, which strengthens the possibility that nitrogen metabolized in the fungal hyphae can be transported later to the plant. Bioactive compounds called Myc factors similar to the Nod factors of *Rhizobium* are suggested to be secreted by mycorrhiza and *Rhizobium* and to be perceived by host roots for the activation of signal transduction pathways or the common symbiosis (SYM) pathway. The pathways that prepare the plant for both AM and *Rhizobium* infection have some common points. The common SYM pathway prepares the host plant to bring about changes at the molecular and anatomical level with the first contact of fungal hyphae. So far, calcium is supposed to be the hub of secondary messengers via Ca^{2+} spiking in the nuclear region of root hairs. *Rhizobium leguminosarum* biovar *viciae* can induce various genes in plants like pea, alfalfa and sugar beet, as evident from the microarray studies. PGPRs produce IAA which, in turn, induces the production of nitric oxide (NO), which acts as a second messenger to trigger a complex signaling network leading to improved root growth and developmental processes.

Expression of ENOD11 and many defense-related genes and root-remodelling genes get up-regulated during entry. Subsequently, this allows the formation of a pre-penetration apparatus (PPA). Although the biology behind the development of arbuscules is unknown, a gene called *Vapyrin*, when knocked down, causes a decline in the growth of arbuscules. Many other genes, including those encoding subtilisin protease, phosphate transporter or two ABC transporters, are known to be involved in arbuscule formation. Nitrogen-fixation genes are popularly used by scientists today to create engineered plants that can fix atmospheric nitrogen. The induction of *nif* genes in case of nitrogen-fixing bacteria takes place under low concentration of nitrogen and oxygen in the rhizosphere. Interestingly, sugarcane plantlets inoculated with a wild strain of *G. diazotrophicus*, have demonstrated fixation of radioactive N_2 when compared with the *G. diazotrophicus* mutant that has a mutant *nifD* gene, which proved the significance of *nif* genes. The efficiency of nitrogen fixation is dependent on the utilization of carbon. Bacteria like *Bacillus subtilis* (UFLA285) can differentially induce 247 genes in cotton plants as compared to controls where no PGPR was supplied to the cotton plant. Many disease-resistance genes that work via jasmonate/ethylene signaling as well as osmotic regulation via proline synthesis genes were differentially expressed with UFLA285 induction. Various differentially expressed genes were identified, including ones encoding metallothionein-like protein type 1, a NOD26-like membrane integral protein, ZmNIP2-1, a thionin family protein, an oryzain gamma chain precursor, stress-associated protein 1 (OsISAP1), probenazole-inducible protein PBZ1, as well as auxin- and ethylene-responsive genes. The expression of the defense-related proteins PBZ1 and thionins have been found to get repressed in the rice–*H. seropedicae* association, suggesting the modulation of plant defense responses during colonization.

Among the PGPR species, *Azospirillum* has been suggested to secrete gibberellins, ethylene and auxins. Some plant-associated bacteria can also induce phytohormone synthesis. For example, lodgepole pine, when inoculated with *Paenibacillus polymyxa*, had elevated levels of IAA in the roots. *Rhizobium* and *Bacillus* were found to synthesize IAA at different cultural conditions such as pH, temperature and in the presence of agro-waste as a substrate. Ethylene, unlike other phytohormones, is responsible for the inhibition of growth of dicot plants. It was found by Glick *et al*. that PGPR could enhance the growth of the plant by suppressing the expression of ethylene. Interestingly, a model has been suggested in which ethylene synthesis from 1-aminocyclopropane-1-carboxylate (ACC), an immediate precursor of ethylene, which is hydrolyzed by bacterial ACC-deaminase enzyme in the need of nitrogen and carbon source is also one of the mechanisms of induction of conditions suitable for growth. ACC-deaminase activity has also been found in bacteria such as *Alcaligenes* sp., *Bacillus pumilus*, *Pseudomonas* sp. and *Variovorax paradoxus*. The involvement of ACC deaminase in the indirect influence on the growth of plants was proved in canola, where mutations in the ACC deaminase gene caused the loss of effect of growth-promoting *Pseudomonas putida*. Interestingly, the potential of PGPRs was further enhanced by introducing genes involved in the direct oxidation (DO) pathway and mineral phosphate solubilisation (MPS) into some useful strains of PGPRs. The gene encoding glucose dehydrogenase (*gcd*) involved in the DO pathway was cloned and characterized from *Acinetobacter calcoaceticus* and *E. coli* and *Enterobacter asburiae*. Moreover, a gene encoding a soluble form of GCD has been cloned from *Acinetobacter calcoaceticus* and *G. oxydans*. Furthermore, there are reports of site-directed mutagenesis of glucose dehydrogenase (GDH) and gluconate dehydrogenase (GADH) that has improved the activity of this enzyme. Mere substitution of S771M provided thermal stability to *E. coli*, whereas mutation of glutamate 742 to lysine improved the EDTA tolerance of *E. coli* PQQGDH. The application of this technology was achieved by transferring genes involved in the DO pathway, *viz. GDH, GADH* and pyrroloquinoline quinine (*PQQ*), to rhizobacteria and phosphoenolpyruvate carboxylase (*PPC*) to *P. fluorescens*, providing the MPS trait.

To recapitulate briefly, excess nutrients are accumulated in soils, particularly phosphorus, as a result of over-application of chemical fertilizers by farmers during intensive agricultural practices. The major research focus is and should be on the production of efficient and sustainable biofertilizers for crop plants, wherein inorganic fertilizer application can be reduced significantly to avoid further pollution problems.

Finally, let us reiterate the most important and specific points, as defined by Swapna Latha Aggani from Kakatiya University, on which the research on biofertilizers should focus:

1. Selection of effective and competitive multi-functional biofertilizers for a variety of crops;

2. Quality control systems for the production of inoculants and their application in the field, to ensure and explore the benefits of plant microorganism symbiosis;

3. Studies on microbial persistence of biofertilizers in soil environments under stressful conditions;

4. Agronomic, soil and economic evaluation of biofertilizers for diverse agricultural production systems;

5. Transferring technological know-how on biofertilizer production to the industrial level and for optimum formulation;

6. Establishment of legislation and strict regulation for quality control in markets and application.

CONCLUSIONS

Environmental stresses are becoming a major problem and productivity is declining at an unprecedented rate. Our dependence on chemical fertilizers and pesticides has encouraged the thriving of industries that are producing life-threatening chemicals and which are not only hazardous for human consumption, but can also disturb the ecological balance. Biofertilizers can help solve the problem of feeding an increasing global population at a time when agriculture is facing various environmental stresses. It is important to realise the useful aspects of biofertilizers and implement their application to modern agricultural practices. The new technology developed using the powerful tool of molecular biotechnology can enhance the biological pathways of production of phytohormones. If identified and transferred to the useful PGPRs, these technologies can help provide relief from environmental stresses. However, the lack of awareness regarding improved protocols of biofertilizer applications to the field is one of the few reasons why many useful PGPRs are still beyond the knowledge of ecologists and agriculturists. Nevertheless, the recent progresses in technologies related to microbial science, plant–pathogen interactions and genomics will help to optimize the required protocols. The success of the science related to biofertilizers depends on invention of innovative strategies related to the functions of PGPRs and their proper application to the field of agriculture. The major challenge in this area of research lies in the fact that, along with the identification of various strains of PGPRs and their properties, it is essential to dissect the actual mechanism of functioning of PGPRs for their efficacy towards exploitation in sustainable agriculture.

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COMMON-USED BIOFERTILIZERS

Contents

BIOFERTILIZERS: DEFINITION AND GENERAL ASPECTS

The increasing demand for safe and healthy food and the concerns on environmental pollution have led to the emergence and development of organic farming. It is globally an important priority area in the crop and livestock production, which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. Organic farming is based on the development and application of biofertilizers and plant strengtheners. The use of chemical fertilizers in large amounts has resulted in a manifold increase in the productivity of farm commodities but they also have an adverse effect on the soil. Continuous and excess use of chemical fertilizers and other agrochemicals to increase yield may lead to ground water contamination and depletion of soil nutrients, eventually resulting in reduction of crop yield. This problem could be overcome using a different technology to produce various biofertilizers. Biofertilizers from microorganisms can replace chemical fertilizers; they are less expensive and are more environmentally friendly than chemical fertilizers. The current global market for organically raised agricultural products is valued at around US$ 30 billion with a growth rate of around 8 percent. Nearly 22 million hectares of land are now cultivated organically. Organic cultivation represents less than 1 percent of the world's conventional agricultural production and about 9 percent of the total agricultural area. Biofertilizers, or more appropriately "microbial inoculants" in the strict sense, are not fertilizers, which directly give nutrition to crop plants. They represent natural and organic formulations that contain living or latent cells of beneficial soil microorganisms which, after being added to the seeds, plant surfaces or soil, colonize the rhizosphere or the interior of the plant and promote its growth by increasing the supply or availability of primary nutrients to the host plant. The inoculation with beneficial soil microorganisms is a promising method for raising soil fertility because, in this way, the accessibility of plants to a number of important elements, such as nitrogen, phosphorus and potassium, increases. As a result, the use of synthetic fertilizers can be significantly reduced. In the world literature, there is evidence of promotion of vegetable yields by inoculation with microorganisms. Microorganisms (bacteria, mycorrhizal fungi and algae) are the living components of the soil. Their activities related to soil fertility and plant nutrition are diverse. They affect the soil structure, the dynamics of nutrients in it, participate in plant nutrition and increase plant resistance to soil-borne pathogens.

These microorganisms are responsible for the process of nitrogen fixation, solubilization of insoluble soil phosphates, convertion of complex organic biomass into mineral compounds which are utilized by plants, and synthesis of growth-promoting substances such as amino acids, vitamins, etc. There are 17 essential non-mineral and mineral elements required for proper plant growth. The lack of any of these nutrients can result in severe damage to crop health. Three essential nutrients are carbon (C), hydrogen (H) and oxygen, which are taken up from atmospheric carbon dioxide and water. Of the mineral elements, the primary macronutrients (nitrogen, phosphorous, and potassium) are needed in largest quantities and are most likely to be in short supply in agricultural soils. Secondary macronutrients, such as Mg, S, Zn, Mn, Fe and Cu, are needed in smaller quantities and are typically found in sufficient quantities in agricultural soil, and therefore do not often limit crop growth. Micronutrients, or trace nutrients (B, Mo, Cl, and Ni) are needed in very small amounts and can be toxic to plants in excess. Silicon (Si) and sodium (Na) are sometimes considered essential plant nutrients, but due to their ubiquitous presence in soils, they are never in short supply. Microorganisms encourage plants to absorb a greater quantity of nutrients on their own which, even if naturally present in the soil, on occasion, cannot be assimilated by plants because of being in an insoluble form.

At present, biofertilizers are supplied to the farmers as carrier-based inoculants or as liquid formulations as an alternative technology, which has more advantages than the carrier-based inoculants.

COMMON-USED BIOFERTILIZERS

TYPES OF BIOFERTILIZERS ON THE BASIS OF BENEFICIAL MICROORGANISMS AND THEIR FUNCTIONS

Biofertilizers contain microorganisms that are able to activate a biological process which stimulates the development of plants and ensures healthy growth. These microorganisms do not function only as a fertilizer. They transform the inaccessible forms of soil elements into ones accessible to plants. Although they are called fertilizers, they do not contain all nutrients that may be added directly into the soil to increase soil fertility. On the contrary, microorganisms slowly and reliably improve the soil stability and phytosanitation. The difference between biofertilizes and composts lies in the amount of microorganisms contained in them. Biofertilizes can comprise only a specific strain of microorganism, which is intended for a specific activity in the soil. These microorganisms are classified into three main groups: nitrogen-fixing, phosphate-transforming and cellulose-degrading microorganisms. They help to fix atmospheric nitrogen and to convert the phosphorus into a form usable to plants.

Microorganisms also help plants to produce hormones, vitamins and amino acids that are of substantial importance for building resistance to pathogens. Almost all crops need different types of biofertilizers depending on their needs. The various types of biofertilizers which help plants grow at different phases of growth can be grouped into four categories:

- N-fixing biofertilizers: These include the bacteria *Rhizobium*, *Azotobacter*, *Azospirillum*, *Clostridium* and *Acetobacter* among others; blue-green algae (BGA), or cyanobacteria, and the fern *Azolla* (which works in symbiosis with BGA).
- P-solubilizing/mobilizing biofertilizers: These include phosphate-solubilizing bacteria (PSB) and phosphate-solubilizing microorganisms (PSMs) like *Bacillus*, *Pseudomonas* and *Aspergillus*. *Mycorrhizae* are nutrient-mobilizing fungi, also known as vesicular-arbuscular mycorrhizae, or VA-mycorrhizae or VAM.
- Plant-growth-promoting rhizobacteria (PGPR): Mainly represented by species of *Pseudomonas*. These bacteria do not provide plant nutrients but they enhance plant growth and performance.
- Composting accelerators: cellulolytic (*Trichoderma*) and lignolytic (*Humicola*) fungal species and different Gram-positive and Gram-negative bacteria.

Nitrogen-fixing biofertilizers

Nitrogen is the most limiting nutritional factor for plant growth. Suitable nitrogen application to growing plants has a favourable enhancing effect on growth, yield and quality. Since nitrogen is the main element in the composition of amino acids, which are required for the synthesis of proteins and other related compounds, it plays a role in almost all plant metabolic processes. Nitrogen is also an integral part of the chlorophyll molecule responsible for plant photosynthesis. Symptoms of nitrogen deficiency generally appear on the bottom leaves first; the lower leaves on the tips turn brown, usually disintegrate, and fall off. However, the excessive use of nitrogen fertilizers increases the total costs of crop production, pollutes the agro-ecosystem and enhances the deterioration of soil fertility. Therefore, it became essential for researchers to develop and adopt a strategy of supplementing or substituting inorganic nitrogen with organic sources, especially ones of microbial origin. Nitrogen-fixing biofertilizers were the ones majorly utilized in the industry in 2012, accounting for over 78% of the global demand. These biofertilizers are mainly used for crop yield improvement and involve several potential benefits in environmental application, in addition to their agricultural usefulness. Furthermore, increasing consumption of leguminous and non-leguminous plant products is also expected to augment the demand for nitrogen fixing biofertilizers over the forecast period.

Nitrogen biofertilizers help agriculturists to determine the nitrogen level in the soil. The type of crops also determines the level of nitrogen. Some crops need more nitrogen for their growth, while others need fewer amounts. The type of soil is an important factor which determines which type of biofertilizers is needed for a crop.

Though the atmospheres contain 79% N_2, eukaryotes cannot utilize it directly. Atmospheric N_2 must be first reduced to nitrogen compounds that can be assimilated by plants (either NH_4^+ or NO_3^-). This process is called biological nitrogen fixation (BNF) and is exclusively carried out by prokaryotes (bacteria and cyanobacteria) (Fig.1).

1. Uptake of NH_4 or NO_3 by organisms
2. Release of NH_4 by decomposition
3, 4. Microbial oxidation of NH_4 (yields energy in aerobic conditions)
5. Denitrification (NO_3 respiration) by microbes in anaerobic conditions (NO_3 is used as a terminal electron acceptor during decomposition of organic matter)
6. Nitrogen fixation

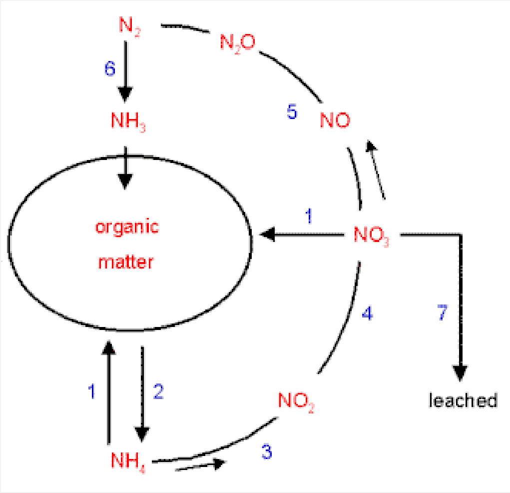

Fig. 1. Nitrogen cycle in nature

The diagram above shows an overview of the nitrogen cycle in soil or aquatic environments. At any time, a large proportion of the total fixed nitrogen will be locked up in the biomass or in the dead remains of organisms (shown collectively as "organic matter"). So, the only nitrogen available to support new growth will be that supplied by nitrogen fixation from the atmosphere (pathway 6 in the diagram) or by the release of ammonium or simple organic nitrogen compounds through the decomposition of organic matter (pathway 2).

Biological nitrogen fixation was discovered by the Dutch microbiologist Martinus Beijerinck. It accounts for 60% of the total nitrogen fixation. The microorganisms that fix nitrogen are called diazotrophs.

In this way, they increase the soil nitrogen level and, respectively, the soil fertility. Biological nitrogen fixation is catalyzed by a microbial multimeric enzyme complex, nitrogenase. The nitrogenase complex exists in all diazotrophs. It consists of two conserved proteins: an iron (Fe)-containing dinitrogenase reductase (Fe protein) encoded by the *nifH* gene and a molybdenum iron (Mo Fe) dinitrogenase (or Mo Fe protein), which is encoded by the *nifDK* genes (Matthew et al., 2008). The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin), HN=NH is reduced to $H_2N\text{-}NH_2$, and this in turn is reduced to $2NH_3$. Depending on the type of microorganism, reduced ferredoxin, which supplies electrons for this process, is generated by photosynthesis, respiration or fermentation. There is a remarkable degree of functional conservation between the nitrogenase proteins of all nitrogen-fixing bacteria. The Fe protein and the Mo-Fe protein have been isolated from many of these bacteria, and nitrogen fixation can be shown to occur in cell-free systems in the laboratory when the Fe protein of one species is mixed with the Mo-Fe protein of another bacterium, even if the species are very distantly related. The nitrogenase is irreversibly inhibited by molecular oxygen and reactive oxygen species, because the oxygen reacts with the iron component of the proteins. Although this is not a problem for anaerobic bacteria, it could be a major problem for the aerobic species such as cyanobacteria (which generate oxygen during photosynthesis) and the free-living aerobic bacteria of soils, such as *Azotobacter* and *Beijerinckia*. These microorganisms have various defense mechanisms to overcome the problem. For example, *Azotobacter* species have the highest known rate of respiratory metabolism of any organism, so they might protect the enzyme by maintaining a very low level of oxygen in their cells. These species also produce extracellular polysaccharide, which retains water and in this way limits the diffusion rate of oxygen to the cells.

Plant growth-promoting bacteria (PGPB) have been used as biofertilizers worldwide, due to their ability to promote plant growth and therefore crop yields and soil fertility and hence, the potential to contribute to more sustainable agriculture and forestry.

Generally, PGPB facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development, in the form of biocontrol agents. They suppress the activity of pathogens by producing numerous metabolites like siderophores, hydrolytic enzymes, and antibiotics. PGPB live freely in soil, colonize plant roots aggressively and establish symbiotic association with plants. The existence of PGPB with the plant roots is generally classified by two environments;

rhizosphere and endosphere. The rhizosphere is the soil volume under the direct influence of roots, while the endosphere is the internal root tissue. The strains inhabiting the rhizosphere and endosphere are called rhizobacteria and endophytes, respectively.

Only N-fixing microorganisms bring additional supplies of a nutrient (N) into the soil/plant system. All other biofertilizers simply solubilize or mobilize the nutrients that are already present in soils. Microorganisms that have the capacity to fix atmospheric N_2 can be used as efficient biofertilizers. Their application in soil improves the soil biota and reduces the need of chemical fertilizers. Among all PGPB, the diazotrophic (N_2-fixing) bacteria, which are involved in the transformation or fixation of N_2 from the unavailable gaseous form in the atmosphere, are divided into:

- Free-living heterotrophic or autotrophic bacteria;
- Bacteria in associative symbiotic relationships;
- Bacteria in symbiotic relationships with plants.

Free-living nitrogen fixers

The free-living, or non-symbiotic, nitrogen-fixing bacteria live outside plant cells and are associated with the rhizosphere, the part of soil under the influence of plant roots and their exudates. They are of four types:

- Free-living non-photosynthetic aerobic nitrogen-fixing bacteria such as *Azotobacter*, *Beijerinckia* and *Derxia*;
- Free-living non-photosynthetic anaerobic nitrogen-fixing bacteria such as *Clostridium*;
- Free-living photosynthetic nitrogen-fixing bacteria such as *Chromatium*, *Rhodopseudomonas*, *Rhodospirillum*, cyanobacteria;
- Free-living chemosynthetic nitrogen-fixing bacteria such as *Desulfovibrio*.

Free-living non-photosynthetic nitrogen-fixing bacteria

Although many genera and species of N_2-fixing bacteria are isolated from the rhizosphere of various cereals, mainly members of the *Azotobacter* and *Azospirillum* genera have been widely tested to increase the yield of cereals and legumes under field conditions. *Azotobacter* is an obligate aerobe, although it can grow under limited O_2 concentration. Its six species are: *Azotobacter armeniacus*, *A. beijerinckii*, *A. chroococcum*, *A. nigricans*, *A. paspali* and *A. vinelandi*. These species play an important role in nitrogen fixation in rice crops and are used as a biofertilizer for wheat, barley, oat, rice, sunflower, maize, line, beetroot, tobacco, tea, coffee and coconuts. *Azotobacter* species are different in terms of morphological and physiological characteristics. Some of them have higher nitrogen-fixing ability than others. Inoculation of soil with *Azotobacter* species lead to increase in crop yields due to the increase in the concentration not only of nitrogen, but also of other substances, such as vitamins, gibberellins, naphthalene and acetic acid, which improve plant growth. *Azotobacter* also synthesizes growth-promoting substances, produces group B vitamins such as nicotinic acid and pantothenic acid, biotin and heteroauxins, gibberellins and cytokinin-like substances, and improves the seed germination in several crops. Both carrier-based and liquid-based *Azotobacter* biofertilizers are available.

Free-living photosynthetic nitrogen-fixing bacteria

Free-living nitrogen-fixing photosynthetic cyanobacteria (blue-green algae) belong to 15 genera, which are found freely in the soil where they fix free N_2 into nitrogenous and ammonium compounds. Mostly they are heterocysts, e.g *Nostoc*, *Anabaena*, *Aulosira*, *Cylindrospernum*, *Calothrix, Totypothrix* and *Stigonema.* Cyanobacteria are photosynthetic and hence add organic matter and extra nitrogen into the soil. Amongst these, *Aulosira* is the most active nitrogen fixer in the rice fields of India. Nitrogen fixation occurs in special thick walled cells called heterocysts, or heterocytes (H), which occur at intervals along the cyanobacterial filaments. This separation of cellular functions is necessary because cyanobacteria have oxygen-evolving photosynthesis but the nitrogen-fixing enzyme, nitrogenase, is unstable in the presence of oxygen. This problem is overcome because the heterocysts contain only part of the photosynthetic apparatus, photosystem I, which can be used to generate energy (as ATP). But the heterocysts do not contain photosystem II, which is used to split water into hydrogen (for combination with CO_2 to produce organic products) and oxygen. There are fewer non-heterocystous nitrogen-fixing blue-green algae, e.g. *Oscillatoria, Phormidium* and *Gleocapsa*.

Associative symbiotic nitrogen fixers

This group comprises bacteria from the family Spirillaceae with two main genera, *Azospirillum* and *Herbaspirillum*. Bacteria of the genus *Azospirillum* are widespread in the soils of tropical, subtropical and temperate regions where they live in symbiotic mutualism around the root of various wild and agricultural plants, which is also known as a risosphere association. They are a good example of the so-called associative nitrogen fixers. *Azospirillum* belong to the facultative endophytic diazotrophs groups, which colonize the surface and the interior of non-legume plants. They are able to fix a considerable quantity of nitrogen in the range of 20– 40 kg N/ha in the rhizosphere in non-leguminous plants such as cereals, millets, oilseeds, cotton, rice, sugar cane etc. Nitrogen fixers such as *Azospirillum* benefits plant by improving shoot and root development and increasing the rate of water and mineral uptake by roots (Gonzales et al., 2005). The yield increases can be substantial, up to 30 percent, but generally range from 5 to 30 percent. These yield increases by *Azospirillum* are possibly a result of the production of growth-promoting substances rather than N_2 fixation (Okon, 1985). The main problem that limits the use of *Azospirillum* on a large scale is the great uncertainty and unpredictability of the results. Regardless of these uncertainties, *Azospirillum* bears great promise as a growth-promoting N_2-fixing biofertilizer. The species *A. lipoferum*, *A. brasilense* and *A. amazonense* have been commercially used as nitrogen-supplying biofertilizers.

COMMON-USED BIOFERTILIZERS

Symbiotic nitrogen fixers

The best known and most exploited symbiotic nitrogen fixers comprise mutualistic (symbiotic) bacteria belonging to the group of Alphaproteobacteria, family Rhizobiaceae, which include the following genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium* and *Mesorhizobium* and *Allorhizobium*, collectively called rhizobia. Rhizobia participate in mutually useful associations with the roots of leguminous plants where they form noodles and carry out the nitrogen fixation process. Within the nodules, the bacteria convert free nitrogen to ammonia, which the host plant utilizes for its development. To ensure sufficient nodule formation and optimum growth of legumes (e.g. alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium. *Rhizobium* can fix 15–20 kg N/ha and increase crop yields up to 20% in pulses. It has been estimated that 40–250 kg N/ha/year is fixed by different legume crops by the microbial activities of *Rhizobium*. The N_2-fixing capability of rhizobia varies significantly among host plant species and bacterial strains.

Therefore, for the production of biofertilizers not only the bacterial strain, but also the rhizobia-host compatibility must be taken into account.

The N_2-fixers from the genus *Frankia* also participate in symbiotic relationships with certain dicotyledonous species (actinorhizal plants). *Frankia* are a free-living gram-positive filamentous actinobacteria found in root nodules or soil. Inoculation of actinorhizal plants with *Frankia* significantly improves plant growth, biomass, shoot and root N content, as well as the survival rate after transplanting in fields. However, the success of establishment of an actinorhizal plantation in degraded sites depends upon the choice of effective *Frankia* strains. Species from this genus are capable of infecting and nodulating eight families of actinorhizal plants (mainly woody plants), which are used for wood production, land reclamation, for timber and fuel wood production, in mixed plantations, for windbreaks, as well as for shelterbelts along deserts and coastlines. *Frankia* inoculation can be advantageous in arid environments, disturbed sites, and areas where native actinorhizal plants are absent. The symbiosis between actinorhizal plants and *Frankia* induces the formation of a perennial root organ called nodule, wherein bacteria are hosted and nitrogen is fixed. In the field, actinorhizal nodules can have variable forms and colours. Comparison of actinorhizal and leguminous nodules shows that the morphology, anatomy, origin, and functioning of the nodules are different for these two nitrogen-fixing plants. Two types of nodule formation occur in actinorhizal symbiosis: intercellular and extracellular infection.

Cyanobacteria are ecologically important because they contribute significantly to the global N_2-fixation. Their capability to fix molecular nitrogen is essential in rice cultivation and in the remediation of arid soils. Nevertheless, the production and application of cyanobacteria is still fairly poorly developed. However, cyanobacteria should be seriously considered as a biofertilizer supporting sustainable agricultural practices in various environments.

Besides cyanobacteria (blue-green algae), which are an important biological factor in rice cultivation, *Azolla* forms another inexpensive, economical, and ecologically friendly biofertilizer. The important factor in using *Azolla* as biofertilizer for rice crops is its quick decomposition in the soil, efficient availability of its nitrogen to rice plants, requirement of a shallow freshwater habitat, rapid growth, and growth along with rice without competition for light and space. Increase in grain yields of rice from 14% to40 % have been reported with *Azolla* being used as a dual crop. It improves the height of rice plants, the number of tillers, grains and the straw yield. It is supplemented with 8–20 kg phosphate per hectare.

Besides N-fixation, these biofertilizers or biomanures also contribute significant amounts of P, K, S, Zn, Fe, Mb and other micronutrients. Widely cultivated in the Asian regions, *Azolla* is either incorporated into the soil before rice transplanting or is grown as a dual crop along with rice. The Asians have recognized the benefits of growing *Azolla* as a biofertilizer, human food and medicine. It also improves water quality by removal of excess quantities of nitrate and phosphorous and is also used as fodder, feed for fish, ducks and rabbits. *Azolla* is a small floating pteridophyte which has symbiotic associations with cyanobacteria and eubacteria that remain associated throughout its life cycle. It is unique in the sense that it acts as a host to the N-fixing cyanobacteria, after which it is used virtually as a green manure. In this process, it adds not only the biologically fixed N, but also the other nutrients absorbed from the soil and present in its biomass. There are seven species of the Azzolaceae family: *Azolla caroliniana*, *A. filiculoides, A. maxicana*, *A. microphylla*, *A. pinnata*, *A. rubra* and *A. nilotica*. In India, *A. pinnata* is commonly observed. The algal symbiont belongs to family Nostocaceae and is generally referred to as *Anabaena azollae*. In the associations between *Azolla* and the cyanobacteria *Anabaena azollae*, the eukaryotic partner *Azolla* houses the prokaryotic endosymbiont *Anabaena azollae* in its leaf cavities and provides carbon sources and, in turn, gets its nitrogen requirements satisfied. The atmospheric nitrogen is harvested by the algal symbiont. The sites of nitrogen fixation are heterocysts. The heterocyst counts increase along the stem from the apex towards the base in the successive leaves. This symbiosis helps in the quick growth and multiplication of the fern and in the creation of a huge amount of biomass on the water surface. It is then harvested, dried and used as biofertilizer to supplement the needs of nitrogen in coffee farms as well.

Phosphorus biofertilizers

Phosphorous (P) is the next essential macroelement after nitrogen. Phosphorus is required in a soluble form for maximizing crop growth and production. It plays a significant role in plant metabolism and is important for the functioning of key enzymes that regulate the metabolic pathways. The phosphate available in soil occurs in three forms: soil solution phosphate, insoluble organic phosphate and insoluble inorganic phosphate. The greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates. This means that soils contain a high amount of total phosphorus, but its availability to plants is very low and it is often a limiting factor for plant growth.

A major characteristic of phosphorus biogeochemistry is that only 1% of the total soil phosphorus (400–4,000 kg P/ha in the top 30 cm) is incorporated into living plant biomass during each growing season (10–30 kg P/ha), reflecting its low availability for plant uptake. Phosphorus deficiency in plants leads to chlorosis, weak stem and slow growth. Therefore, it is considered to be the most important chemical factor that restricts plant growth because of its vital role in the physiological and biochemical functions of plants. The application of chemical phosphorous fertilizers to circumvent the phosphorus deficiency in soil is not a very efficient method due to the high reactivity of phosphate anions through precipitation with cations such as Fe^{3+} and Al^{3+} in acidic soils or Ca^{2+} in calcareous soils. The application of microbial inoculants with phosphate-solubilizing activity will be a promising approach to increase the phosphorus availability in agricultural soil and is an environmentally-friendly alternative to the use of chemical fertilizers. Organic phosphate solubilization is also called mineralization of organic phosphorus, and it occurs in soil at the expense of plant and animal remains, which contain a large amount of organic phosphorus-containing compounds. The decomposition of organic matter in soil is carried out by the action of numerous saprophytes, which release orthophosphate from the carbon structure of molecules. Various bacterial species are able to solubilize inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphates. It is important to determine the actual mechanism of phosphorus solubilisation by PSM for optimal utilization of these microorganisms in various field conditions. Microorganisms must assimilate phosphorus via membrane transport, so dissolution of calcium phosphate [$Ca(H_2PO_4)_2$] to dihydrogen phosphate anion ($H_2PO_4^-$) is considered essential to the global phosphorus cycle.

The solubilization of phosphorus in nature is due to the activity of phosphate-solubilizing microorganisms (PSM) which belong to several genera: *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aereobacter*, *Flavobacterium* and *Erwinia.* The symbiotic nitrogenous rhizobia, which fix atmospheric nitrogen into ammonia and export the fixed nitrogen to the host plants, also show phosphate-solubilizing activity. For instance, *Rhizobium leguminosarum* bv. *trifolii*, and *Rhizobium* species nodulating *Crotalaria* species improved plant phosphorus nutrition by mobilizing inorganic and organic phosphorus. Various phosphate-solubilizing bacteria have also been isolated from stressed environments; for example, the halophilic bacteria *Kushneria sinocarni* isolated from the sediment of Daqiao saltern on the eastern coast of China, which may be useful in salt-affected agricultural soils.

Two types of phosphate biofertilizers have been developed based on the application of phosphate-solubilizing bacteria and phosphate-mobilizing microorganisms.

Phosphate-solubilizing biofertilizers

The members of this group are bacterial and fungal species which solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate. The most efficient ones belong to *Bacillus* and *Pseudomonas* among Bacteria and *Aspergillus* and *Penicillium* among Fungi. They could be isolated in higher concentrations from rhizosphere soil rather than non-rhizosphere soil. Their application in biofertilizers aims to increase the yields of legume, cereals, vegetables and fruit crops. The phosphate-solubilizing fungi produce more acids than bacteria and consequently exhibit greater phosphate-solubilizing activity. Among the filamentous fungi that solubilize phosphate, the genera *Aspergillus* and *Penicillium* are the most representative ones, although strains of *Trichoderma* and

Rhizoctonia solani have also been reported as phosphate solubilizers. A number of theories have been proposed to explain the mechanisms of phosphate solubilization. The most important theories are the acid production theory and the proton and enzyme theory.

- *Acid production theory*

The major mechanism involved in the solubilization of phosphate by phosphate-solubilizing microorganisms is the production of organic acids which either directly dissolve rock phosphate as a result of anion exchange of phosphate by acid anion or chelate Fe, Al, Ca ions to bring the phosphate into solution. Due to the ability of PSM to secrete and release organic acids (citric, oxalic, succinic, tartaric, malic, alpha keto butyric, 2-ketogluconic, gluconic and fumaric acids) in the soil environments, these bacteria lower the pH in their vicinity, which is a prerequisite for solubilization of bound phosphates in soil and consequently dissociate the bound form of phosphates like $Ca_3(PO_4)_2$ in calcareous soil. The microbial organic acids are produced as a result of oxidative respiration or by fermentation of organic carbon sources. Gluconic and fumaric acids have the highest ability to solubilize phosphate from inorganic phosphate compounds. The amount of soluble phosphate released depends on the strength and type of acid. Aliphatic acids are found to be more effective in phosphate solubilization than phenolic acids and citric acids. *Pseudomonas* sp., *Erwinia herbicola*, *Pseudomonas cepacia* and *Burkholderia cepacia* are phosphorus-solubilizing bacteria, which produce a higher amount of gluconic acid. Besides organic acids, inorganic acids such as nitric and sulphuric acids are also produced by the nitrifying *Nitrosomonas* and sulphur-oxidizing *Thiobacillus* bacteria during the oxidation of nitrogenous or inorganic compounds of sulphur which react with calcium phosphate and convert them into soluble forms. The introduction of efficient phosphate solubilizers in the rhizosphere of crops increases the availability of phosphorus and thus increases the crop yield up to 200–500 kg/ha. In this way, microorganisms play a major role in the solubilization and uptake of native and applied phosphorus.

- *Enzyme and proton theory*

Phosphate-solubilizing microorganisms are also known to produce phosphatase enzyme along with acids which cause the solubilization of phosphate in aquatic environment. Esterases are involved in liberating phosphorous from organic compounds. Solubilization without acid production is due to the release of protons accompanying respiration or ammonium assimilation. Besides these mechanisms, some bacterial species synthesize syderophores – iron-chelating compounds which bind the iron present in the root area and, thus, make it unavailable for harmful microorganisms so that crop plants are protected from them. The production of other chelating substances, mineral acids and biologically active substances like indole, acetic acids, gibberellins and cytokinins, is also correlated with phosphate solubilization.

Phosphorus mobilizing biofertilizers: Mycorrhiza

This type of biofertilizers contain mycorrhizal fungi also known as phosphate absorbers. They are a heterogeneous taxonomic group which inhabits the plant root system and establishes a symbiotic association with them. Mycorrhizal fungi live in symbiosis with over 90 % of all vascular plant species, including many important crop species, such as maize, wheat, rice and potato. Mycorrhizal fungi form a bridge between the roots and the soil, gathering nutrients from the soil and giving them to the roots. There are two major types of mycorrhizae: ectomycorrhizal fungi (EM) and endomycorrhizal fungi (AM). Endomycorrhizae are the most common type, and are found in grasses, shrubs, some trees and many other plants. Ectomycorrhizal fungi are usually specific to a certain host species, but most species of endomycorrhizae will form relationships with almost any AM-fungi host plant, and are therefore much easier to specify. The arbuscule-forming mycorrhiza (AMF) are a widespread type of endomycorrhiza associated with crop and horticultural plants, where fungal hyphae of *Glomeromycota* species penetrate root cortical cells and form branched structures called arbuscules. The host plant is benefited by obtaining needed nutrients, especially phosphorus, calcium, copper, zinc etc., which are otherwise inaccessible to it, with the help of the fine absorbing hyphae of the fungus. Phosphorus is a highly immobile element because it is easily absorbed by soil particles and a phosphate-free zone rapidly occurs around plant roots. Some of the external hyphae of mycorrhizal fungi may extend more than 10 cm from the root surface, which allows them to have access to a greater volume of non-depleted soil than the root alone. The small diameter of hyphae (20 to 50 µm) permits access to soil pores that cannot be explored by roots as well. They also produce extracellular alkaline phosphatases which can mobilize phophate from organic sources. Through the excretion of protons, hydroxyls and organic acids, mycorrhizae modify the redox potential around the root and the mycelium, which also enhances the transformation of insoluble phosphate from the soil into a soluble form in the soil solution. Therefore, a root system forming a mycorrhizal network will have a greater effective surface area for absorbing nutrients and exploring a greater volume of soil than nonmycorrhizal roots. AM hyphae also excrete gluey, sugar-based compounds called glomalin, which helps to bind soil particles, and make stable soil aggregates. There is an increasing interest in the use of mycorrhiza to promote sustainable agriculture, considering the widely accepted benefits of the symbioses to nutrition efficiency (for both macronutrients, especially P, and micronutrients), water balance and biotic and abiotic stress protection of plants. Vesicular Arbuscular Mycorrhiza Root Inoculant (VAMRI) is a biofertilizer based on chopped dried corn roots infected with *Glomus* species (*G. mosseae* or *G. fasciculatum*). Besides a microbial inoculant, this product also serves as a biocontrol agent of soil-borne diseases of different crops under various conditions. VAMRI can be applied for pepper, tomato, papaya, onion, corn, peanut, sugarcane, eggplant, banana, fruit crops, watermelon, etc.

Potassium (K)-solubilizing biofertilizers

Potassium (K) is the third essential nutrient necessary for plant growth. Some rhizobacteria are able to solubilize insoluble potassium forms. *Bacillus edaphicus* has been reported to increase potassium uptake in wheat and *Paenibacillus glucanolyticus* has been found to increase the dry weight of black pepper. Sudan grass inoculated with the potassium-solubilizing bacterium *Bacillus mucilaginosus* had higher biomass yields. Moreover, *Bacillus mucilaginosus* in co-inoculation with the phosphate-solubilizing *Bacillus megaterium* promoted the growth of eggplant, pepper and cucumber.

Biofertilizers for secondary macronutrients: zinc and iron solubilizers

Zinc is of utmost importance. It is found in the earth's crust at a concentration of 0.008%, but there are soils which exhibit zinc deficiency with content far below the critical level of 1.5 ppm of available zinc. The plant deficiencies in absorbing zinc from the soil are overcome by external application of soluble zinc sulphate ($ZnSO_4$). Microorganisms found in the soil can be used as biofertilizers to provide micronutrients like Zn, Fe, Cu, etc. Zinc can be solubilized by *B. subtilis*, *Thiobacillus thioxidans* and *Saccharomyces* sp. These species are responsible for Zn extraction in soils where native zinc is higher or in conjunction with insoluble cheaper zinc compounds like zinc oxide (ZnO), zinc carbonate ($ZnCO_3$) and zinc sulphide (ZnS) instead of costly zinc sulphate. The zinc fixation occurs through two main mechanisms: the first one operates in acidic soils and is based on cation exchange; the second mechanism operates in alkaline soils where fixation takes place by sorption of Zn on $CaCO_3$ and, as a result, a solid-solution of $Zn_{\chi}Ca_{\chi-1}CO_3$ is formed.

Plant-growth-promoting rhizobacteria (PGPR)

A group of rhizosphere bacteria (rhizobacteria) that exerts a beneficial effect on plant growth is referred to as plant-growth-promoting rhizobacteria or PGPR. PGPR is a generic acronym that indicates bacteria which, in some often unknown way, can stimulate plant growth. They belong to several genera, e.g. *Agrobacterium*, *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Actinoplanes*, *Azotobacter*, *Bacillus*, *Pseudomonas* sp., *Rhizobium*, *Bradyrhizobium*, *Erwinia*, *Enterobacter*, *Amorphosporangium*, *Cellulomonas*, *Flavobacterium*, *Streptomyces* and *Xanthomonas*. These bacteria vary in their mechanism of plant growth promotion but generally influence growth via phosphate solubilization, nutrient uptake enhancement, plant growth hormone production or production of a variety of antimicrobial compounds that act in different ways. Bertrand et al. (2000) showed that a rhizobacterium belonging to the genus *Achromobacter* could enhance the root hair number and length in oilseed rape (*Brassica napus*). *Achromobacter* increased the NO_3 and K uptake and, consequently, the shoot and root dry weights by 22 to 33 percent and 6 to 21 percent, respectively. One of the plant-growth-promoting mechanisms of rhizobacteria is the antagonism against phytopathogenic microorganisms due to the production of antimicrobial metabolites like siderophores, antibiotics, cyanides, fungal cell-wall-degrading enzymes and gaseous products including ammonia (Idris et al., 2007; Lugtenberg and Kamilova, 2009). The mechanism of antifungal effects lies in the production of a variety of antimicrobial compounds that act in different ways. The antagonistic effects are caused by cytolysis, leakage of potassium ions, disruption of the structural integrity of membranes, inhibition of mycelial growth and protein biosynthesis. Most of the identified *Pseudomonas* biocontrol strains produce antifungal metabolites such phenazines, pyrrolnitrin, pyoluteorin and cyclic lipopeptides like viscosinamide. It was demonstrated that viscosinamide prevents the infection of sugar beet by *Pythium ultimum*. These bacterial strains, besides having an antagonistic effect, also influence the defense system of plants. The siderophore-mediated competition for iron is one of the mechanisms responsible for the antagonistic activity of *Pseudomonas* spp. The secreted iron-chelating compounds bind ferric ions (Fe^{3+}), and are taken up by microbial cells through specific recognition by membrane proteins (Srivastava and Shalini, 2008). The presence of iron-chelating compounds makes the bacteria better competitors for iron, in this way preventing the growth of pathogenic microorganisms. *Pseudomonas* species produce two different types of siderophores: pseudobactin and pyoverdin (Oldal et al., 2002). Siderophores produced by biocontrol bacteria have a higher affinity for iron than those produced by some fungal pathogens, allowing the former microbes to scavenge most of the available iron, preventing the proliferation of fungal pathogens (Hillel, 2005). Some authors have reported that *Pseudomonas fluorescens* belonging to the PGPR class produces siderophores and has a biocontrol effect against *P. ultimum*, *R. batatticola* and *Fusarium oxysporum*. Other *Pseudomonas* species like *Pythium stutzeri* produce extracellular enzymes like chitinase and laminase capable of lysing the mycelia of *Fusarium solani*. *Pseudomonas aeruginosa* produces three types of siderophores under iron-limiting conditions: pyoverdine, pyochelin and its precursor salicylic acid, and induces resistance to plant diseases caused by *Botrytis cinerea* on bean and tomato, *Colletotrichum lindemuthianum* on bean. *F. oxysporum* causes vascular wilt and foot-, root- and bulbrot diseases in a wide variety of economically important crops. *Alternaria* spp., *Sclerotium* spp. cause leaf spots, root rot and stem rot, which also leads to serious yield losses. The antifungal effect of PGPRs is influenced by a lot of environmental and genetic factors. Biotic and abiotic environmental signals may have an important input on the regulation of biocontrol genes in pseudomonads, e.g. on the repression of siderophore biosynthesis. Together with low oxygen concentrations, the available carbon and nitrogen sources that influence the molecular mechanisms are involved in biocontrol activity.

Compost as fertilizer

What is compost?

Composting is a controlled microbial bio-oxidative process in which organic biodegradable wastes are converted into a hygienic, humus-rich product (compost) for use as a soil conditioner and an organic fertilizer. It is an inexpensive, efficient, and sustainable treatment for solid wastes. The process is dependent on a number of factors, including temperature, moisture (typically 40–60% by weight), sufficient oxygen to support an aerobic environment (typically 5% or more), particle size, the C/N ratio and the degree of turning involved. The effective management of these factors will accelerate the composting process. Compost can be defined as organic manure or fertilizer produced as a result of aerobic, anaerobic or partially aerobic decomposition of a wide variety of crop, animal, human and industrial wastes. Composting has a long tradition almost everywhere in the world. It was a central concept of early Chinese agriculture, but it has also been practiced in India and Europe for centuries. Compost is a dark, crumbly, earthy material, which usually contains less than 2% (*w*/*w*) of nitrogen, phosphorous and potassium (N:P:K). It also has microscopic fungi, bacteria, earthworms and dung beetles. This mixture creates a symbiotic food web within the soil. The decomposing material feeds the organisms and helps to aerate the soil while also keeping it moist. The nutrient value of composts varies widely, depending upon the nature of feedstock composted.

Composts are generally classified as:

- Rural compost: This is produced from materials available on the farm and in other rural areas. The raw materials used can be straw, leaves, cattle-shed bedding, fruit and vegetable wastes, and biogas plant slurry. On average, it contains 0.5% N, 0.2% P_2O_5 and 0.5% K_2O. Rural compost primarily finds use on farms as bulky organic manure.
- Urban or town compost: This refers to compost prepared from urban and industrial wastes, city garbage, sewage sludge, factory waste, etc. Its typical composition is 1.5–2.0% N, 1.0% P_2O_5 and 1.5% K_2O. Commercially prepared urban compost has been reported to contain 1% Fe, about 375 mg/kg Cu, 705 mg/kg Zn, 740 mg/kg Mn and small amounts of other micronutrients.
- Vermicompost: This is an important type of compost that contains earthworm cocoons, excreta, beneficial microorganisms, actinomycetes, plant nutrients, organic matter, enzymes, hormones, etc. It is an organic fertilizer produced by earthworms and contains on average 0.6% N, 1.5% P_2O_5 and 0.4% K_2O. In addition to NPK, it is also a source of micronutrients, containing an average of 22 mg/kg Fe, 13 mg/kg Zn, 19 mg/kg Mn and 6 mg/kg Cu. It helps in cost-effective and efficient recycling of animal wastes (poultry, horse, piggery excreta and cattle dung), agricultural residues and industrial wastes using low energy.

Various parameters are commonly used to evaluate compost quality. In general, these parameters include germination index (GI), water-soluble organic carbon (WSOC), water-soluble organic nitrogen (WSON), pH, electrical conductivity (EC), moisture and total organic matter (TOM) content. It is accepted that any sole parameter cannot determine the compost maturity, which must be assessed by a combination of different physical (odour, colour, temperature and particle size), chemical (C/N ratio, mineral N, pollutants content (heavy metals and organics), pH, organic matter quality and humification) and biological properties (microbial activity indicators such as respiration, ATP content, enzyme activity, microbial biomass, nitrogen mineralization). The pH of the mature compost is usually around 7.5 and it has a C:N ratio ranging from 10:1 to 20:1. The temperature in the pile is equal to that of the surrounding air. Compost smells earthy, no longer heats up after turned or watered, looks like dark soil, and does not have identifiable food items, leaves or grass. The application of immature compost to soil results in seed germination inhibition, root destruction, and a decrease in the O_2 concentration and redox potential, which imposes the need to assess the compost maturity.

Compost benefits and use

When applied to soil, composts (organic manure) or compost extracts have beneficial effects on plant growth and are considered as a valuable soil amendment. Compost application is very popular as a means of improving the soil physical properties and supplying plant nutrition. It also provides nutrients rich in organic carbon for the microbial biomass, which converts the unavailable nutrients in plant residues to ones available for crops, and it enhances the biodiversity of soil microorganisms. Organic fertilizers (animal/plant based) also activate the natural microflora in the soil and rhizosphere of the plant and are excellent means of enhancing the natural microbial population. Composts contain macro and micronutrients that are often absent in synthetic fertilizers and release nutrients slowly—over months or years, unlike synthetic fertilizers. Composts buffer the soil, neutralizing both acid and alkaline soils, bringing the pH levels to the optimum range for nutrient availability to plants. Composts help bind clusters of soil particles, called aggregates, which provide good soil structure. Such soil is full of tiny air channels and pores that hold air, moisture and nutrients. This makes any soil easier to work and is also useful for erosion control. Erosion is often the end result of low soil fertility. Compost and the humus it contains can actually bind to soil, building a good structure that encourages optimum fertility and erosion resistance. A comparatively new application for compost is bioremediation. Many things can contaminate surface waters, soils and reservoirs. The microorganisms in compost degrade contaminants in water or soil. Contaminants are digested, metabolized and transformed into humus and inert byproducts such as carbon dioxide, water and salts. Compost bioremediation is effective in degrading or altering chlorinated and non-chlorinated hydrocarbons, wood-preserving chemicals, solvents, heavy metals, pesticides, petroleum products and explosives.

Microbial community in compost

During composting, different animal/plant wastes like dead plants, farm yard waste and cattle waste are degraded by various decomposing microorganisms with cellulolytic/ lignolytic activity such as *Trichoderma viridae*, *Aspergillus niger*, *Aspergillus terreus*, *Bacillus* sp., etc. Composts support high population levels of bacteria with higher percent of Gram-negative cultures. Some isolates show proteolytic activity, which is considered a potential mechanism of suppression or competition with other microorganisms. The major Gram-negative genera identified in mature compost are *Pseudomonas*, *Serratia*, *Klebsiella*, and *Enterobacter*. All Gram-positives are identified as *Bacillus* spp. The essential elements required by the composting microorganisms are carbon, nitrogen and oxygen, as well as moisture. If there is a lack of any of these elements, or if they are not provided in the proper proportion, the microorganisms will not flourish and will not provide adequate heat. A composting process that operates at optimum performance will convert organic matter into stable compost that is odour and pathogen free, and a poor breeding substrate for flies and other insects. In addition, it will significantly reduce the volume and weight of organic waste, as the composting process converts much of the biodegradable component to gaseous carbon dioxide.

The composting period is governed by a number of factors including, temperature, moisture, oxygen, particle size, the carbon-to-nitrogen ratio and the degree of turning involved. Generally, effective management of these factors will accelerate the composting process.

Compost preparation

The composting process is carried out by three classes of microbes:

• Psychrophiles - low temperature microbes;

• Mesophiles -medium temperature microbes;

• Thermophiles - high temperature microbes.

Generally, composting begins at mesophilic temperatures and progresses into the thermophilic range. This is due to the oxidative metabolism of microorganisms, which is exothermic and the heat produced is sufficient to increase the temperature of organic matter to 65–75 °C over a period of up to 10 days. The thermophilic stage of composting appears as a self-sanitizing mechanism by which pathogens, thermolabile microbial and plant toxins are destroyed. Temperature is directly proportional to the biological activity within the composting system. As the metabolic rate of the microbes accelerates, the temperature within the system increases. Conversely, as the metabolic rate of the microbes decreases, the system temperature decreases. Not all organic matter is degraded completely. Lignin, lignocellulosic and other plant components are modified slowly and become part of the final stable compost. Soluble plant exudates and sap are bio-degraded more rapidly. After the most readily decomposable organic matter in the compost is consumed, the biological activity decreases in intensity, and the temperatures and oxygen consumption decline. The compost then enters the curing phase, during which decomposition proceeds more slowly and organic matter is converted to stable humic substances—the finished or mature compost. Crops residues are compostable matter but, although high in carbon, they are deficient in nitrogen. On the contrary, animal wastes are rich in nitrogen and very often low in carbon content.

Compost as a plant protectant

Compost can be transformed into suppressive compost after inoculation of biological control agents specifically active against a plant disease. In practice, composts are not consistently or naturally colonized by a broad spectrum of biocontrol agents because the latter are destroyed by high temperatures during active composting. To be effective, biocontrol agents must recolonize composts during the curing process and this does not always occur. For example, composts produced near a forest are much more likely to become colonized by effective biocontrol agents and more consistent in suppressing rhizoctonia diseases than those produced in an enclosed system. Microbes that show a preference for colonizing and lysing plant pathogens might be classified as biocontrol agents.

The microorganisms stimulated by compost amendments contribute to the suppressive activity of the amended soil through four control mechanisms: antibiosis, competition, parasitism and induced systemic resistance.

Antibiosis is the inhibition of one organism's growth by a metabolic product such as antibiotic produced by another organism. Agrobacterium radiobacter 84 produces bacteriocin, called agrocin, which is a widely accepted commercial product for controlling of crown gall – a serious disease of stone-fruit trees in nurseries and of many other woody plants. Lysobacter and Myxobacteria are known to produce copious amounts of lytic enzymes, and some isolates have been shown to be effective at suppressing fungal plant pathogens. Expression and secretion of these enzymes by different microbes can sometimes directly result in the suppression of plant pathogen activities. For example, control of Sclerotium rolfsii by Serratia marcescens appeared to be mediated by chitinase expression. Some products of lytic enzyme activity may contribute to indirect disease suppression. For example, oligosaccharides derived from fungal cell walls are known to be potent inducers of plant host defenses. The enzyme β-1,3-glucanase contributes significantly to biocontrol activities of Lysobacter enzymogenes strain C3.

Competition is when microorganisms compete for nutrients such as high-energy carbohydrates, nitrogen and iron, as well as for infection sites, oxygen and space.

An example of parasitism are parasitic fungi which invade plant pathogens resulting in lysis and death. Effective control of Rhizoctonia solani can be achieved by applying isolates of Trichoderma species combined with any of several bacterial biocontrol agents. The representatives of the Trichoderma genera are the main microorganism isolated from compost prepared from lignocellulosic wastes and capable of parasitizing Rhizoctonia solani.

The mechanism of induced systemic resistance is based on activation of the production of plant metabolites such as salicylic acid, defense-related proteins or other compounds which lead to systemic plant resistance to pathogens. Some biocontrol strains of Pseudomonas sp. and Trichoderma sp. are known to strongly induce plant host defenses. In several instances, inoculations with plant-growth-promoting rhizobacteria (PGPR) were effective in controlling multiple diseases caused by different pathogens, including anthracnose (Colletotrichum lagenarium), angular leaf spot (Pseudomonas syringae pv. lachrymans and bacterial wilt (Erwinia tracheiphila).

The quantitative contribution of biologically active compounds to disease suppression is likely to be dependent on the composition and carbon-to-nitrogen ratio of the soil organic matter that serves as a food source for microbial populations in the soil and rhizosphere. However, such activities can be manipulated so as to result in greater disease suppression. When suitable antagonists are already presented in the soil or substrate but do not provide a satisfactory level of disease control, their activity must be intensified. For example, in post-harvest disease control, addition of chitosan can stimulate microbial degradation of pathogens similar to that of an applied hyperparasite. Chitosan is a non-toxic and biodegradable polymer of beta-1,4-glucosamine produced from chitin by alkaline deacylation. Amendment of the plant growth substratum with chitosan suppressed root rot caused by Fusarium oxysporum f. sp. radicis-lycopersici in tomato. Although the exact mechanism of action of chitosan is not fully understood, it has been observed that treatment with chitosan increases the resistance to pathogens. The extent to which composts suppress this disease depends on the chemical-physical nature of the composted materials and increases with the compost maturity.

COMMON-USED BIOFERTILIZERS

TYPES OF BIOFERTILIZERS ON THE BASIS OF THE PHYSICAL NATURE AND CARRIER MATERIALS USED

Based on the physical nature and carrier materials used, various types of biofertilizers are manufactured by different producers. These are carrier-based inoculants, agar-based inoculants, broth cultures and dried cultures. New developments in biofertilizer production like (i) freeze-dried inoculants (e.g. BAIF, IARI, India), (ii) Rhizobium-paste (e.g. KALO Inc. USA), (iii) granular inoculant (e.g. Soil implant of Nitragin, USA), (iv) pelleting (e.g. Pelinoc of Nitragin), (v) polyacrylamide-entrapped rhizobia (e.g. Agrosoke) and (vi) pre-coated seeds (e.g. Prillcote of New Zealand), appear to be more promising for inoculation success in tropical legumes.

Carrier-based biofertilizers

At present, biofertilizers are supplied as carrier-based microbial inoculants which are added to the soil to enrich the soil fertility. The carrier is a medium that can carry the microorganisms in sufficient quantities and keep them viable under specified conditions, easy to supply to the farmers. The use of ideal carrier material is necessary in the production of good quality biofertilizer.

A good carrier should have the following qualities:

- Highly absorptive (water-holding capacity) and easy to process;
- Non-toxic to microorganisms;
- Easy to sterilize effectively;
- Available in adequate amounts and low-cost;
- Provide good adhesion to seeds;
- Has good buffering capacity;
- High organic matter content and water-holding capacity of more than 50%.

Other essential criteria for carrier selection relating to the survival of the inoculant bacteria should be considered.

- Survival of the inoculant bacteria on seeds. Seeds are not always sown immediately after seed coating with the inoculant bacteria. The bacteria have to survive on seed surface against drying condition until placed into soil.
- Survival of the inoculant bacteria during the storage period.
- Survival of the inoculant bacteria in soil. After being introduced into the soil, the inoculant bacteria have to compete with native soil microorganisms for the nutrient and habitable niche, and have to survive against grazing protozoa. Such carrier materials that offer the available nutrient and/or habitable micro-pores to the inoculant bacteria will be desirable. In this sense, materials with micro-porous structure, such as soil aggregate and charcoal, will be good carriers for soil inoculants.

COMMON-USED BIOFERTILIZERS

Biofertilizers are supplied to the soil either by “seed inoculation”, in which the inoculant (bacteria-carrier mixture) is mixed with water to make slurry-form and then mixed with seeds, or by “soil inoculation”, i.e. by spreading over the field during cultivation. In the case of seed inoculation, the carrier must be a form of fine powder. To achieve a tight coating of inoculant on the seed surface, use of an adhesive, such as gum arabic, methylethylcellulose, sucrose solutions and vegetable oils, is recommended. Seed inoculations may not always be successful due to the low nodule occupancy of the inoculated rhizobia strain as a result of the inoculation or low establishment of the inoculated rhizobacterial strain. This might be due to low population and/or low survival of the inoculated bacterial strain on the seed surface and in the soil. In such instance, “soil inoculation” will be adopted, whereby a large population of a bacterial strain can be introduced into the soil. For soil inoculation in general, granular inoculant is placed into the furrow under or alongside the seed. This enhances the chance for the inoculated strain to be in contact with plant roots. Various types of material are used as carriers for seed or soil inoculation. Peat soil, lignite, vermiculite, charcoal, press mud, farmyard manure and soil mixture can be used as carrier materials. Neutralized peat soil/lignite are found to be better carrier materials for biofertilizer production. For preparation of seed inoculant, the carrier material is milled to fine powder with a particle size of 10–40 µm. For soil inoculation, carrier material with granular form (0.5–1.5 mm) is generally used. Granular forms of peat, perlite, charcoal or soil aggregates are suitable for soil inoculation.

Liquid biofertilizers

The strength of biofertilizers is determined by two basic parameters: number of cells and efficiency of the microorganisms to fix nitrogen or solubilize phosphates.

Liquid biofertilizers are liquid formulations containing the dormant form of desired microorganisms and their nutrients along with the substances that encourage formation of resting spores or cysts for longer shelf-life and tolerance to adverse conditions. The dormant forms, on reaching the soil, germinate to produce a fresh batch of active cells. These cells grow and multiply by utilizing the carbon source in the soil or from root exudates.

As an alternative to conventional carrier–based biofertilizers, liquid formulation technology, which has more advantages than the carrier-based inoculants, has been developed in the Department of Agricultural Microbiology, TNAU, Coimbatore. The advantages of liquid biofertilizers over conventional carrier-based biofertilizers are listed below:

- Longer shelf life, 12-24 months;
- No contamination;
- No loss of properties due to storage up to 45º C;
- Greater potential to fight with native populations;
- High populations can be maintained at more than 10^9 cells/ml up to 12 to 24 months;
- Easy identification by typical fermented smell;
- Cost saving on carrier material, pulverization, neutralization, sterilization, packing and transport;

- Quality control protocols are easy and quick;
- Better survival on seeds and soil;
- No need of running biofertilizer production units throughout the year;
- Very much easy to use by the farmer;
- Dosages are 10 times less than those of carrier-based powder biofertilizers;
- High commercial revenues;
- High export potential;
- Very high enzymatic activity, since contamination is nil.

Among different techniques to produce biofertilizer, the concept of effective microorganisms (EM), which are available in liquid form, was introduced in 1991 by Dr. Teruo Higa of Japan. The major groups of microorganisms contained in the EM include filamentous fungi, yeast, lactic acid bacteria and other soil bacteria. The application of EM aims to function as inoculum of microorganisms to the soil in which it will help to establish or re-establish soil ecosystems. EM is commercially available in concentrated form that needs to be processed before the application. According to the procedure suggested by the EM manufacturer, the concentrated EM (EM Bokashi) can be used directly by mixing with molasses and water. However, the common method is to use EM Bokashi as a starter to ferment the raw materials and produce either liquid or solid biofertilizer. The common raw materials include left-over plant or animal materials in the farms. The fermentation period was suggested to be at least seven days and the product is recommended to be used within three months. Today, the production of ready-to-use liquid biofertilizer from EM is becoming available in the market due to the convenience for small-scale farming or domestic application in which the users do not have space and raw materials available for fermentation.

There are three ways of using liquid biofertilizers.

- Seed treatment

  Seed treatment is the most common method adopted for all types of inoculants. The seed treatment is effective and economic. For a small quantity of seeds (up to 5 kg), the coating can be done in a plastic bag. For this purpose, a plastic bag sized 21” x 10” or larger can be used. The bag should be filled with 2 kg of seeds or more. The bag should be closed in such a way so as to trap the air as much as possible. The bag should be squeezed for 2 minutes or more until all the seeds are uniformly wetted. Then the bag is opened, inflated again and shaken gently. The shaking should stop after each seed gets a uniform layer of culture coating. The bag is opened and the seeds are shade-dried for 20–30 minutes. For large amounts of seeds, coating can be done in a bucket and the inoculant can be mixed directly by hand. Seed treatment with *Rhizobium, Azotobacter, Azospirillum*, along with PSM can be done. The seed treatment can be done with any of two or more bacteria. There is no side (antagonistic) effect. The important things that have to be kept in mind are that the seeds must be coated first with *Rhizobium, Azotobacter* or *Azospirillum*. When each seed gets a layer of these bacteria, then the PSM inoculant has to be coated as an outer layer. This method will provide maximum cell counts of all bacteria required for better results.

Treatments of seeds with any two bacteria will not provide a maximum number of bacteria on individual seeds.

- Root dipping

This method is used for application of *Azospirillum*/ /PSM on paddy transplating/ vegetable crops. The required quantity of *Azospirillum*/ /PSM has to be mixed with 5–10 litres of water at one corner of the field and the roots of seedlings has to be dipped for a minimum of half an hour before transplantation.

- Soil application

Use 200 ml of PSM per acre. Mix PSM with 400 to 600 kg of cow dung farmyard manure along with ½ bag of rock phosphate, if available. The mixture of PSM, cow dung and rock phosphate has to be kept under any tree or in the shade overnight and 50% moisture should be maintained. The mixture is used for soil application in rows or during leveling of soil.

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NANO-FERTILIZERS

Contents

NANO-FERTILIZERS

Strategic role of nanotechnology for fertilizers: potential and limitations

The ability of people to construct and manipulate materials at nano-scale has increased tremendously during the last decade building the fundamentals of the interdisciplinary science nanotechnology. Nanomaterials behave differently than the same material at non-nano scale; they have high surface area to volume ratio, high solubility, and specific targeting due to small size, high mobility, and low toxicity. They can be engineered for surface reactivity or other desired characteristics - unique behavior that can be both useful and profitable. As of March 2011, over 1300 commercially available products contain nanomaterials. Nanotechnology was a $1 trillion industry in 2015.

According to the National Nanotechnology Initiative (NNI) (https://www.nano.gov/about-nni), *"Nanotechnology research and development is directed towards understanding and creating improved materials, devices and systems that exploit nanoscale properties"*. Following the definition of Royal Society, *"Nanotechnologies are the design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scale"*.

NANO-FERTILIZERS

Recently nanotechnology has emerged as the sixth revolutionary technology after the green revolution of the 1960s and the biotechnology revolution of the 1990s. Nanotechnology is a novel scientific approach that involves the use of materials and equipment capable of manipulating physical and chemical properties of a substance at molecular levels. It merges science and technology leading to revolutionary breakthrough in electronics, energy, remediation, automobile, space technology, and life sciences. The potential uses and benefits of nanotechnology are enormous. Nowadays, nanotechnology is progressively moved away from the experimental into the practical areas. Among others, it promises significant contribution to agricultural research in solving important agricultural problems, such as detection of pollutants, plant diseases, pests, and pathogens; controlled delivery of pesticide, fertilizers, nutrients, and genetic material; formation and binding of soil structure. Today, when agricultural scientists are facing major challenges such as reduced crop production, nutrient deficiency and climate change, nanotechnology has offered promising applications for precision farming. This innovative technology embraces wide applications such as plant disease control, enhanced nutrient uptake, improved plant growth and sustained release of agrochemicals. Interestingly, a nanoparticle (NP)-based strategy has gained momentum and become increasingly popular in the agricultural sector as a result of its unique properties compared with that of the biopesticides. The application of nanotechnology to agriculture (the so called agri-nanotechnology, Fig. 1) is getting significant attention, primary in the following several categories:

- Increase production rates and yield;
- Increase efficiency of resource utilization;
- Minimize waste production;
- Specific applications that include nano-fertilizers and nano-pesticides;
- Nano-based treatment of agricultural waste;
- Nano-sensors.

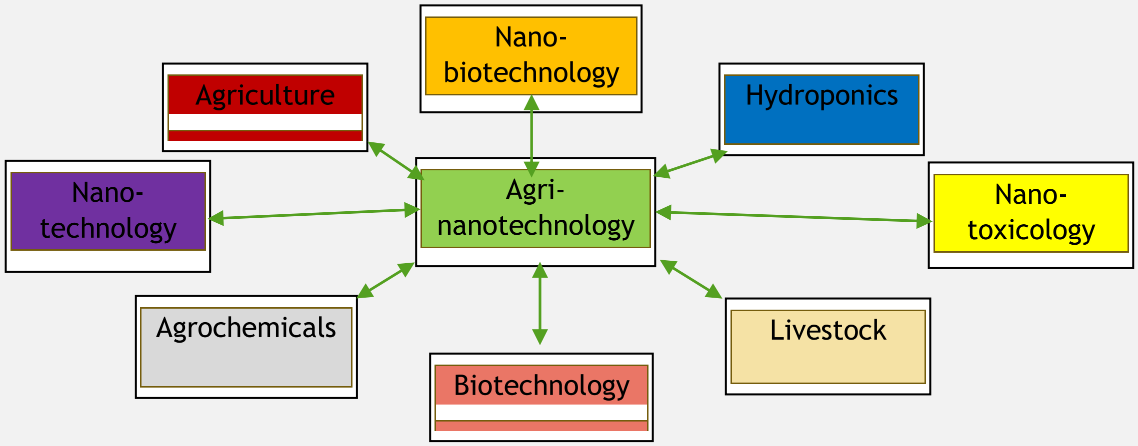

Fig. 1. Multidisciplinary nature of agri-nanotechnology.

Currently, nanotechnology potential in sustainable agriculture management is clearly recognized. It occupies a prominent position in transforming agriculture and food production. The development of nano-devices and nanomaterials could forward novel applications in plant biotechnology and agriculture. Thus, the development of slow/controlled release fertilizers on the basis of nanotechnology has now become crucial for promoting the development of environment friendly and sustainable agriculture. Applying nanoscale or nanostructured materials as fertilizer carriers leads to the development of the so-called “smart fertilizer” - new facilities that enhance nutrient use efficiency and reduce costs of environmental protection.

Nano-fertilizers *vs.* conventional fertilizers - formulation and delivery of nano-fertilizers

Outburst of world population in the last 10 – 15 years has imposed the necessity for higher agriculture productivity to satisfy the food needs of billions of people. The increasing nutrient deficiency in soils causes significant economic losses for farmers on the one hand and considerable decreases in nutritional quality of grain for food and feed. The crop productivity can be enhanced through application of fertilizers, although they have an additional role in enhancing the food production especially after the introduction of high yielding and fertilizer responsive crop varieties. Conventional fertilizers are generally applied on the crops by either spraying or broadcasting. An important factor, on which the mode of application depends, is the real final concentration of the fertilizers in the plants. Conventional fertilizers offer nutrients in chemical forms that are not fully accessible to plants. Additionally, the inversion of these chemicals to insoluble form in soil is the reason for the very low utilization of most of the macronutrients. A concentration much below the minimal desired one reaches to the targeted site due to leaching of chemicals, drift, runoff, evaporation, hydrolysis by soil moisture, and photolytic and microbial degradation. It has been estimated that around 40–70 % of nitrogen, 80–90 % of phosphorus, and 50–90 % of potassium content of applied fertilizers are lost in the environment and never reach the plant. These problems superimpose repeated use of fertilizers. According to the International Fertilizer Industry Association, world fertilizer consumption sharply picked up in 2009–2010 and 2010–2011 with growth rates of 5–6 %. World demand is estimated to reach 192.8 Mt by 2016–2017. The repeated use on its turn adversely affects the inherent nutrient balance of the soil and results in environmental pollution affecting normal flora and fauna. It is reported that excess use of fertilizers increases pathogen and pest resistance, reduces soil microflora, diminishes nitrogen fixation, contributes to bioaccumulation of pesticides, and destroys habitats for birds. This vicious circle causes sustainable and economic losses.

It is well known that yields of many crops have begun to drop down as a result of imbalanced fertilization and decrease in soil organic matter. Moreover, excessive applications of nitrogen and phosphorus fertilizers affect the groundwater and also lead to eutrophication in aquatic ecosystems. The remaining minerals may either leach down and/or leak and become fixed in soil or contribute to air pollution. Considering these facts, the large-scale application of chemical fertilizers to increase the crop productivity is not an acceptable option for sustainability. Especially in a long term perspective, although the conventional fertilizers increase the crop production they disturb the soil mineral balance and decrease soil fertility. In addition to the irreparable damage that the excess use of chemical fertilizers causes to the soil structure and mineral cycles, it spoils the soil microflora, plants, and consequently - the food chains across ecosystems leading to heritable mutations in future generations of consumers. Thus, there is an urgent need to optimize the use of chemical fertilization to fulfill the crop nutrient requirements and to minimize the risk of environmental pollution. Accordingly, it is very important to develop smart materials that can systematically release chemicals to specific targeted sites in plants which could be beneficial in controlling nutrition deficiency in agriculture, while keeping the natural soil structure and contributing to clean environment. The nano-fertilizers are promising alternative in this context.

A nano-fertilizer refers to a product in nanometer scale that delivers nutrients to crops. Nano-fertilizer technology is recent innovation. Substituting traditional methods of fertilizer application by nano-fertilizers is an approach to release nutrients into the soil both gradually and in a controlled way. Nano-fertilizers show controlled release of agrochemicals through site targeted delivery, reduction in toxicity, and enhanced nutrient utilization of delivered fertilizers. They possess unique features that enhance plants' performance in terms of ultrahigh absorption, increase in production, rise in photosynthesis, and significant expansion in the leaves' surface area. Besides, the controlled release of nutrients contributes to preventing eutrophication and pollution of water resources.

In nano-fertilizers, nutrients can be encapsulated by nanomaterials, coated with a thin protective film, or delivered as emulsions or nanoparticles. There are many throughput examples of nano-fertilizers application. Thus, treatment with TiO_2 nanoparticles on maize had a considerable effect on growth, whereas the effect of TiO_2 bulk treatment was negligible. Titanium nanoparticles increased light absorption and photo energy transmission. In another experiment, a compound of SiO_2 and TiO_2 nanoparticles increased the activity of nitrate reductase in soybeans and intensified plant absorption capacity, making its use of water and fertilizer more efficient. Nano-organic iron-chelated fertilizer is proved to be environmentally sustainable. The positive effect from the uptake and penetration of ZnO_2 nanoparticles on tomato plants leaves supports its potential use as a future nano-fertilizer. Nano-fertilizers that ensure slow, targeted, efficient release have the potential to increase the efficiency of nutrient uptake. Engineered nano-particles are useful for mitigating the chronic problem of moisture retention in arid soils and enhancing crop production by increasing the availability of nutrients in the rhizosphere. Coating and binding of nano-particles help to regulate the release of nutrients from the fertilizer capsule. Application of a nano-composite consisting of nitrogen, phosphorus, potassium, micronutrients, mannose, and amino acids enhanced the uptake and use of nutrients by grain crops. Zn–Al layered double-hydroxide nano-composites have been employed for the controlled release of chemical compounds that act as plant growth regulators. Nano-porous zeolite based on nitrogen fertilizer can be used as alternate strategy to improve the efficiency of nitrogen use in crop production systems. As super-fertilizer, carbon nanotubes were found to penetrate tomato seeds and affect their germination and growth rates. Analytical methods indicated that the carbon nanotubes penetrated the thick seed coat and supported water uptake inside seeds.

These facts support the statement that fertilizers based on nanotechnology have the potential to surpass conventional fertilizers following several important indices (as showed in Table 1).

Table 1. Conventional fertilizers vs. nano-fertilizers

Index	Nano-fertilizer	Conventional fertilizer
Solubility	High	Low
Dispersion of mineral micronutrients	Improved dispersion of insoluble nutrients	Lower solubility due to large particle size
Soil adsorption and fixation	Reduced	High
Bioavailability	High	Low
Efficiency of nutrients' uptake	Increased uptake ratio; saves fertilizer resource	Conventional fertilizer is not available to roots and nutrients' uptake efficiency is low
Controlled release	Release rate and pattern precisely controlled	Excess release leading to toxicity and soil imbalance
Effective duration of release	Extended effective duration	Used by the plant at the site and time of application; the rest is converted in insoluble form
Loss rate	Reduced loss of fertilizer nutrients	High loss rate due to leachi8ng, drifting, run off

The nano-fertilizers should be formulated in a way that they retain important properties such as high solubility, stability, effectiveness, time-controlled release, enhanced targeted activity with effective concentration, and less eco-toxicity due to the safe, easy mode of delivery and disposal.

A great potential in targeted delivery of nutrients to living systems possess the nanoparticles. They can be loaded by nutrients most commonly through one of the following ways:

- absorption on the nanoparticles;
- attachment on the nanoparticles mediated by ligands;
- encapsulation in nanoparticulate polymeric shell;
- entrapment in nanoparticles.

Thus, it has been shown that chitosan nanoparticles suspensions containing N, P, and K fertilizers can be useful for agricultural applications. Similarly, urea-modified hydroxyapatite (HA) nanoparticles are exploited for slow and sustained release of nitrogen over time with the crop growth. The large surface area of HA facilitates the large amount of urea attachment on the HA surface and the strong interaction between HA nanoparticles and urea contributes to the slow and controlled release of urea. Polymer-based mesoporous nanoparticles can also provide efficient carrier system to agrochemical compounds. Mesoporous silica nanoparticles (150 nm) have been reported to entrap urea and to release it in a controlled manner in soil and water.

The efficiency of the nano-fertilizers and their impact on plant systems is influenced by the method of their application. The nano-fertilizers' delivery to plants can be realized through the listed below methods. The approaches include either *in vitro* or *in vivo* application, as shown in Table 2.

Table 2. Modes of nano-fertilizers' application

***In vitro* methods**	***In vivo* methods**
Aeroponics: ➢ Principle: the technique, first reported in 1992, consists of continuously spraying of a nutrient solution on suspended in air roots; ➢ Advantages: the technique allows strict control of the gaseous environment around the roots; ➢ Disadvantages: the techniques requires a high level of nutrients to sustain rapid plant growth, thus its application is restricted.	**Soil Application:** ➢ Principle: direct delivery to soli; ➢ Requirements: careful choose of the persistent time of the fertilizer in the soil; special attention to the soil texture, salinity, plant sensitivities to salts, and pH of the amendment. Negative soil particles affect the adsorption of mineral nutrients. The anion exchange capacity of most agricultural soils is small compared to cation one. Among anions, NO_3^- remains mobile in the soil solution and is susceptible to leaching by water, PO_4^{3-} binds to soil particles containing Al or Fe because the positively charged $Fe^{2+}/^{3+}$ and Al^{3+} exchanges OH^- group with phosphates, resulting in tightly bounding of the latter, which mobility and availability in soil can limit plant growth. ➢ Advantages: the most common method of nutrient supplement using chemical and organic fertilizers.
Hydroponics: ➢ Principle: the plants are grown with their roots immersed in a liquid nutrient solution (without soil), introduced in 1937 for dissolved inorganic salts, known as well as the so called "solution culture"; ➢ Requirements: careful choose of the volumes of nutrient solution, maintenance of oxygen demands and pH. ➢ Advantages: application of supporting materials (e.g. sand) that allow nutrient solution to be flushed from one end and old solution to be removed from the other end. ➢ Disadvantages: frequent pathogen attack and high moisture rates which may cause over wilting of soil-based plants.	**Foliar Application** ➢ Principle: liquid fertilizers are directly sprayed onto leaves, generally used for the supply of trace elements; ➢ Advantages: reduces the time lag between application and uptake by plant during the rapid growth phase; circumvent the problem of restricted uptake of a nutrient from soil; agronomic advantage of foliar application since stomata and leaf epidermal cells are majorly involved in nutrient uptake ➢ Disadvantages: further needs for standardization of application protocol to avoid damage to the leaves; need of specific time (morning and evening) of spraying because the stomata open during these time periods only; possibility of plant damage if incorrect concentration of fertilizer is applied.

NANO-FERTILIZERS

Technology expansion has improved ways for large-scale production of nanoparticles of physiologically important metals, which are now used as **“smart delivery systems”** in order to improve fertilizer formulation by minimizing nutrient loss and increasing the uptake in plant cell. “Smart delivery system” means combination of specifically targeted, highly controlled, remotely regulated, and multifunctional characteristic to avoid biological barriers for successful targeting. The specific properties of nano-fertilizers, i.e. their high surface area, sorption capacity, and controlled-release kinetics to targeted sites, attribute them as smart delivery system.

Smart fertilizers are becoming reality through transformed formulation of conventional products using nanotechnology. The nanostructured formulation allows a fertilizer to intelligently control the release speed of nutrients in order to match the uptake pattern of a specific crop. It improves solubility and dispersion of insoluble nutrients in soil, reduces soil absorption and fixation and increases the bioavailability, hence the nutrient uptake efficiency.

Biosynthesis of nanoparticles my microorganisms

Mediated synthesis of metal nanoparticles by microorganisms

Recently**,** the use of biological entities has emerged as a novel method for the synthesis of nanoparticles. Biotechnological way for the synthesis of nanoparticles possess many advantages, such as use of known microbial technologies and processes for scale up the obtaining of biomass. This is leading to economic viability, possibility of readily covering large surface areas by suitable growth of the microbes, which is of major advantage in the field of agriculture for easier production of bio-fertilizers.

The disadvantages of the convention methods for obtaining of metal nanoparticles like high energy and cost fabrication demands, as well as toxic by-products production makes the implementation of such approaches at large scale very complicated. Using of microbial cell factories like bacteria, fungi, algae, viruses and actinomycetes provide a smart alternative way of synthesising metallic nanoparticles. The biosynthesis of metallic nanoparticles in these microorganisms is a costly and eco friendly technology. The use of broad number of microorganisms belonging to prokaryotic as well as eukaryotic types takes part in the synthesis of long range of metal nanoparticles as gold (Au), silver (Ag), lead (Pb), platinum (Pt), copper (Cu), iron (Fe), cadmium (Cd) and metal oxides such as titanium oxide (TiO), zinc oxide (ZnO), etc. These microorganisms represent a varied ambience for the nanoparticles production. The nanoparticles produced are highly useful, safe and environmental friendly in nature with a lot of applications ((Syed, PhD Thesis). In agriculture, the most used nanoparticles as bioeffectors are coper (Cu), iron (Fe), silver (Ag), gold (Au). The future challenges in this respect comprise optimal biosynthesis of nanoparticles with defined size and shape as well as optimal duration of the fermentation process in order to enhance their stability.

Microbiological synthesis is a new approach for manufacture of nanoparticles and realization of the so called bio-nanofactories. The major characteristics of nanoparticles are revealed by the researchers, who prepared nanoparticles of desirable shape and size.

The principal flow chart for microbiological synthesis of metallic nanoparticles is presented in Fig. 2.

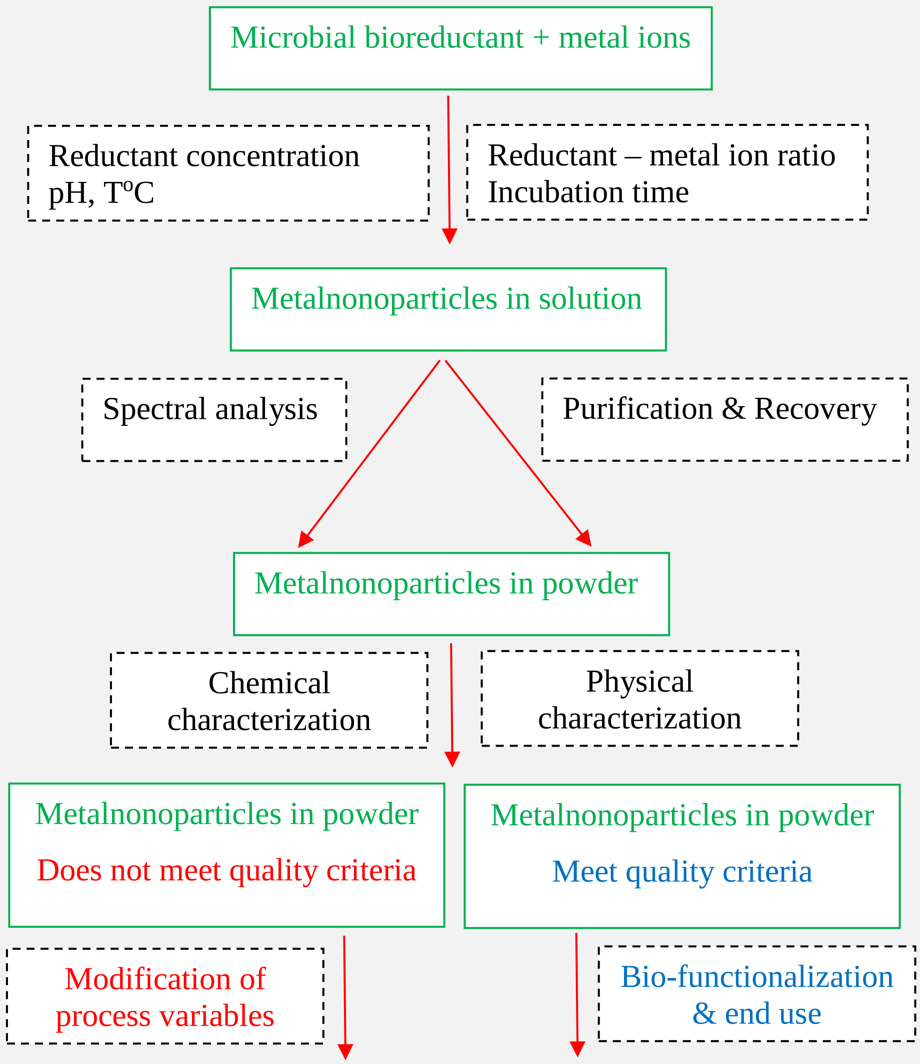

Fig.2. Principal flow chart for microbiological synthesis of metallic nanoparticles

The following important parameters play a significant role in biosynthesis of nanoparticles.

1 Bioresources used for nanoparticles biosynthesis: The synthesis of nanoparticles is characterized by choice of the most convenient microorganism in respect to: growth rate, enzyme production and the respective metabolic pathways. Some of the microorganisms like bacteria, viruses, fungi, yeasts and algae are used for the biosynthesis of metallic nanoparticles and are an object of specific research.

2. Cellular metabolites involved in biosynthesis: molecules like enzymes, proteins, polysaccharides etc. are acting as a reducing and stabilizing agents in the biosynthesis of nanoparticles. They can be utilized in the process as a whole cells of microorganisms, crude cell preparations, and crude or purified enzymes obtained from the microorganisms. The obtained nanoparticles are resulted mainly from bioreduction, which is realized by co-enzymes such as NADH, NADPH, FAD, etc. It is found that nanoparticles synthesis with the help of whole cell of fungi is much cheaper as compare to purified enzymes from the same fungus strain (Syed, PhD Thesis).

3. Reactions facilitating nanoparticles biosynthesis: the process of this biosynthesis is initiated with harvesting of microbial biomass, which is related with residual nutrients and metabolites to avoid wrong by product reactions. During the processes of scalling up the production rate and product yield are of special interest and optimization is necessary (e.g. production time, pH, temperature etc.). The process of optimization of these factors can influence the particles morphology and their properties. Thus, currently researchers have directed their investigations on arranging the optimal reaction conditions as well as the equipment used in the bioreduction process (Syed, PhD Thesis).

4 Growth of inoculum for biosynthesis of nanoparticles: biosynthesis of nanoparticles depends on growth conditions of microorganisms-producers like: nutrients, pH, temperature, etc. These factors need to be optimized. They are also important in case of using whole cells and crude enzymes. Another important parameter for optimization of the inoculums is the harvesting time, so that it is necessary to monitor the enzyme activities during the time course of growth (Syed, PhD Thesis).

Microbial nanoformulations: exploring potential for nano-farming

Nanoparticles, synthesized by microbes are highly stable and could offer a non-toxic, cost-effective and eco-friendly approach for synthesis over chemical ones. This green synthesis has a great advantage over the chemical methods, causing toxic effect on environment. Thus, the use of agriculturally important microorganisms for nanoparticles biosynthesis and their further role in agriculture is of substantial significance. The use of nanoformulations may enhance the stability of bio-fertilizers and bio-stimulators with respect to desiccation, heat, and UV inactivation.

Nano-fertilizers uptake, translocation, and fate in plants

The uptake and fate of nano-fertilizers in plant is an emerging field of research interest. The uptake, translocation, and accumulation of nanoparticles depend on the plant itself, more specifically on the plant species, age, and growth environment. Also these processes are linked to the physicochemical properties, functionalization, stability, and mode of delivery of the nanoparticles. A schematic representation of the uptake, translocation, and biotransformation pathway of various nanoparticles is proposed by Rico et al. (2011) along with possible modes of cellular uptake in the plant system. According to this presentation the root system uptakes and translocates to the foliar part of a plant, regardless it species appurtenance, ZnO^{2+}, Cu^{2+}, Al^{3+}, Ag^{2+} and Fe_3O_4 Nano-Particle (NP). In addition, indicatives for species dependence are available for translocation of Cu NP, ZnO NP, Al NP, Ag NP, (all in leaves), $Ni(OH)_2$ NP in the stem, and CeO_2 NP in both stem and leaves. A translocation of the Fe_3O_4 NP in the stem is also speculated.

The probable differential nanoparticle interaction on exposure in the root absorption zone can be summarized in Table 3.

Table 3. Localization and interaction of different nano-particles in the root absorption zone.

Nano-particle	Localization / interaction
Fe_3O_4 NP	Cambium
ZnO NP	Endodermis, metaxylem; Zn^{2+} - in the metaxylem
CeO_2 NP	Cortex
Al NP	Cortex Al^{3+} - in the metaxylem
Ag NP	Cortex; Ag^{2+} - in the metaxylem
Cu NP	Cortex; Cu^{2+} - in the cambium and metaxylem
TiO_2 NP	Cortex
Ni $(OH)_2$ NP	Metaxylem

The entry of the nanoparticles through the cell wall depends on the cell wall pore diameter (5–20 nm). Because of this, nanoparticles or nanoparticle aggregates with diameter less than the pore size of plant cell wall can easily enter through the cell wall and reach up to the plasma membrane. Functionalized nanoparticles can facilitate the enlargement of the pore size or the induction of new cell wall pore formation to enhance the nanoparticles uptake. Research discussions are going on about the uptake of nanoparticles into plant cell mediated by binding to carrier proteins through aquaporin, ion channels or endocytosis. Additionally, nanoparticles can also be transported into the plant by forming complexes with membrane transporter proteins or root exudates. Other studies reported that nanoparticles could enter through stomata or the base of trichome in leaf. Studies on the uptake and translocation of TiO_2-alizarin red S complex in *Arabidopsis thaliana* seedling have revealed that mucilage released by the roots develops pectin hydrogel complex around the root which is most probably responsible for the entry of the nanoparticle-dye complex.

Recent studies on the mechanism of nanoparticle uptake and translocation have exploited fluorescently labeled monodispersed mesoporous silica nanoparticles which were shown to penetrate the roots via symplastic and apoplastic pathways and translocate via xylem tissue to the aerial parts of the plants including the stem and leaves. However, the exact mechanism of nanoparticle uptake by plants is still not fully elucidated.

In the cytoplasm, nanoparticles are targeted to different cytoplasmic organelles and interfere with different metabolic processes of the cell (Table 3). It is shown that the uptake of TiO_2 nanoparticles in wheat include localization in parenchyma and vascular tissues of the root. The cell internalization and upward translocation of ZnO nanoparticles in *Lolium perenne* (ryegrasses) is realized through the root cells and then - move up to the vascular tissues.

The uptake and accumulation of ZnO nanoparticles when applied at higher concentration is straitened since the nanoparticles get agglomerated which inhibits their entry through the cell wall pores. Moreover, X-ray absorption spectroscopy of ZnO-treated seedlings revealed presence of Zn^{2+} ions instead of ZnO suggesting the role of the roots in ZnO ionization on its surface.

Another class of nanoparticles – the magnetite NP, behave in a way that their presence in root, stem and leaves is reported, and the extent of the nanoparticles uptake is proven to be affected by the type of the growth medium. A higher uptake was achieved in hydroponic medium as compared to the plant grown in sand, whereas no uptake was observed in plants grown in soil which might be due to the adherence of magnetite nanoparticles to soil and sand grains.

Finally, it should be mentioned that besides some conclusive studies on TiO_2 and ZnO nanoparticles, most of the uptake, translocation, and accumulation studies in plants are reported only up to the germination stage. Hence, the fate of nanoparticles in the plant system is still largely unknown.

Nano-fertilizers effect on plant physiology and metabolism

The majority of recent studies support the idea that nanoparticles exercise some adverse effects on plants. However, there are few studies that have suggested that nanoparticles, when delivered in controlled safe dose, may contribute to promotion of plant growth and yield. In this respect, multi-walled carbon nanoparticles (MWCNP) have been shown to promote seed germination and growth of tomato and enhance the growth of tobacco cells. The same phenomenon was observed in MWCNTs in mustard plant. Using the so called germination index and relative time of root elongation as etalon parameters it was shown that oxidized MWCNPs exercise better effect at lower concentration than the non-oxidized ones.

Comparative studies for evaluation of the seed yield and prevention of leaf abscission in borage plant, made with nanosilver and silver nitrate, have shown that the former was performing better. It is known that the plant hormone ethylene plays a key role in leaf abscission, and silver ions inhibit ethylene by replacing copper ions from the receptors. When the both compounds were applied on the plants through the foliar spray method it was observed that nanosilver was effective at a lower concentration than silver nitrate. Similar promoting effect of biosynthesized silver nanoparticles on emergence of seedling and various plant growth parameters of many economically important plant species were reported.

Various studies have been performed to clarify the effect of ZnO nanoparticles on the growth of different plants. Thus, it was shown a stimulatory effect on the growth of *Vigna radiata* and *Cicer arietinum*; ZnO nanoparticles adsorption on the root surface was observed through correlative light and scanning electron microscopy and such by the seedlings through inductively coupled plasma/atomic emission spectroscopy. The effect of ZnO nanoparticles on plant cell physiology was investigated using cellular antioxidant system as a model. Applying the foliar spray method on chickpea seedlings it was shown that low concentrations of ZnO nanoparticles has positive effect on the plant growth and the seedlings biomass accumulation has improved which may be due to lower reactive oxygen species (ROS) levels (evidenced by the lower malondialdehyde content). Field experiments confirmed that usage of 15 times lower dose of ZnO nanoparticles compared to the recommended dose of ZnSO4 led to 29.5 % higher pod yield.

Comparable positive effects of ZnO and CeO2 nanoparticles on *Cucumis sativus* fruit quality were observed. The application of both nanoparticles resulted in increased starch content and possibly – in altered carbohydrate pattern.

Stimulation of the antioxidant activity and nitrate reductase by a mixture of SiO2 and TiO2 nanoparticles in G. max was found, in addition to the better productive effect and increase in water and fertilizer uptake capacity of the model plant. The application of TiO2 nanoparticles was demonstrated to promote photosynthesis under both visible and ultraviolet light and growth in spinach. An increase of 73 % in dry weight, threefold higher photosynthetic rate, and 45 % increment in chlorophyll after seed treatment in spinach were observed. The authors speculate that the reason of increment in photosynthetic rate may be due to the increase in absorption of inorganic nutrients which enhanced the utilization of organic substance and quenching of oxygen-free radicals.

Unlike most of the nanoparticles, for which application at high concentration are not recommended due to the observed negative impact, TiO2 nanoparticles applied at concentrations as high as 2,000 ppm increased seed germination and seedling vigor in *Brassica napus*.

Hence, it is clear that different metal nanoparticles showed positive influence at various concentration range, e.g. Pd and Au at lower concentration, Si and Cu at higher concentration, and Au and Cu in combined mixture. This behavioral patter was confirmed by field studies with *G. max* and *Brassica juncea*: nanocrystalline powder of iron, cobalt, and copper at an extra low concentration promoted seed germination rate, and a marked increase in the chlorophyll index, number of nodules, and crop yield was observed. Similarly, foliar spray of gold on plant in field experiments showed positive effect resulting in increased plant height, stem diameter, number of branches, number of pods, seed yield, and – interestingly, improved the redox status of treated plants.

Ethical and safety issues of nano-fertilizers application

Undoubtedly nanotechnology has incredible potential to revolutionize many aspects of human life. However, the advancement of this multidisciplinary branch of science, especially the benefits from their practical application have to be considered with some precautions.

The major concern at world scale is whether the unknown risks of nanoparticles involving their environmental and health impact prevail over their potential benefits. Thus, the risks associated with the application of nanoparticles are yet to be evaluated before nanoparticles application is fully accepted and implemented. Hence, "nanotoxicology," has been developed which is responsible for assessing toxicological potential and promoting safe design and use of nanoparticles. Due to the thorough quantitative analysis of the potential health impacts, environmental clearance, and safe disposal of nanoparticles improvements in designing further applications of nanotechnology can be anticipated.

No direct human disease has been linked to nanoparticles so far. Nanoparticles which constitute a part of ultrafine particulate matter can enter in the human/animal system through oral, respiratory, or intradermal routes. Currently, there is a common assumption that the small size of nanoparticles allows them to easily enter tissues, cells, and organelles and interact with functional biomolecular structures (i.e., DNA, ribosomes) since the actual physical size of an engineered nanostructure is similar to many biological molecules (e.g., antibodies, proteins) and structures (e.g., viruses).

Of course there is still a need for proper physicochemical characterization and determination of appropriate exposure protocols and reliable methods for assessing nanoparticles outcome in the environment, their internalization, and their kinetics in living organisms. These are the prerequisites for establishment of optimal experimental conditions that will allow precise determination if a particular nanoparticle poses a threat to human health. However, the interdisciplinary research of materials scientists, environmentalists, and life scientists is contributing to identification of the true, if any, hazards of nanotechnology. The heterogeneous and developmental nature of nanotechnology is making risk assessment quite subjective. The absence of standardized methodologies and guidelines makes it difficult to compare the safety/toxicity assessments from different research groups. It is most likely that different types of nanoparticles vary as to their toxicological properties. To interpret correctly any toxicological data, it is essential to calculate and determine the expected concentrations of nanoparticles that may be exposed to the biological system or present in the ecosystem. The risk assessment of nanoparticles has to be performed on a case-by case basis. Thus the ethical issues must be specific for a specified product at a given time, and alternative assessments are needed to take into consideration ethical, social, and political values that relate policies such as those involving nanotechnology.

The use of nanotechnology in agriculture is very important as it directly affects humans. Nano-fertilizers enable nanoparticles to enter in the food chain allowing their distribution in every organism related to the food chain. Literally all substances can be toxic to plants, animals, or humans at some exposure level. However, this does not limit their use in various applications which are formulated minding the critical exposure concentration. As mentioned above the promoting effect of the nanoparticles on plant growth and physiology is expressed at very low concentrations, hence is hardly to believe that these concentrations will pose significant health and environmental damage.

Many countries have identified the potential of nanotechnology in the food and agriculture sectors. Meanwhile they recognize the need for assessment of the food safety implications of nanotechnology. As suggested by the scientific committee of the European Food Security Authority (EFSA), *"the risk assessment paradigm (hazard identification, hazard characterization*, *exposure assessment and risk characterization) is applicable for nanoparticles (EFSA Scientific Committee 2011). However, risk assessment of these nanoparticles in the food and feed area should consider the specific properties of the subject nanoparticles in addition to those common to the equivalent non-nanoforms."*

Deciding the risk associated with the use of a particular nanoparticle in food and feed means taking into consideration various parameters, among which physicochemical characterization of nanoparticles, their stability in the food and feed, toxicokinetics (absorption, distribution, metabolism/biotransformation, excretion/elimination) within the human and animal systems.

GENETICALLY ENGINEERED MICROBES

Genetically modified bacteria for agricultural purposes

There are numerous bacterial genera which representatives can influence plant growth and production. Among these representatives there are plant pathogens that can suppress plant diseases and they are used as biocontrol strains. Another group or bacterial species can contribute to increased plant growth by enhancing the availability of nutrients. These bacteria constitute the bio-fertilizers and are known as well as growth-promoting rhizobacteria (PGPR). The name of PGPR is associated with their ability to grow well at the interface between soil and plant root (the rhizosphere). PGPR can be applied either as seed coating or directly to soil. However, to exert their growth-promoting effect sufficient numbers of the introduced PGPR have to survive in soil and rhizosphere, which not always happens. Consequently, the efficacy of PGPR is not always sufficient for commercial applications and there is a need to improve their performance. One of the possible decisions is to apply genetic modifications to facilitate their survival efficiency.

Survival of genetically modified bacteria in soil

Any microbial cell introduced into the environment will encounter a large number of biotic and abiotic factors affecting its survival. Both biotic and abiotic factors are equally important. Thus, high clay content, high pH, and relatively high moisture content can have a positive effect on bacterial survival. On the contrary, dry periods, presence of competing microorganisms, predation by protozoa, and lysis by bacteriophages negatively affect the number of introduced bacteria. Speaking about biotic factors affecting the activity and survival of introduced bacteria, the presence of plant roots that provide nutrients to the microorganisms living in their vicinity is very important. Among the microorganisms that are well adapted to the rhizosphere are members of the genera *Agrobacterium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Erwinia*. *Pseudomonas*, *Rhizobium*, and *Xanthomonas*.

Microbial survival depends on the interrelation between the environmental conditions and the physiological state of the bacteria. As a result of this interactions bacterial cells can switch their metabolism to different physiological states. For instance, cells can become more stress resistant or form dwarf cells, they can produce exopolysaccharides for protection, they can enter a viable but non-culturable state, and some are able to form spores or associations with plants.

One can speculate that the survival pattern of the GM bacteria will follow the one of their wild-type parents. In fact, this extrapolation should be applied with some precautions. Firstly, the expression of the inserted genes requires an extra amount of energy, which could reduce their environmental fitness. In addition, the insertion could have disrupted unknown functions weakening the competitiveness of the strains. Secondly, it is possible the GMMs to evolve and adapt to the prevailing environmental conditions via natural selection. This last statement is supported by evidence for evolutionary adaptation of bacteria to degrade the herbicide 2,4-dichlorophenoxyacetic acid resulting in increased competitive fitness to use succinate as a substrate. Similarly, it is reported that environmental stresses could alleviate the debilitating effects of mutations - organisms may become more tolerant to genetic perturbations under certain environmental stresses.

GMMs have been shown to survive even better than the wild-type strain in studies with artificial growth conditions. However, enhanced survival of GMMs has rarely been observed under field conditions. Often, the population of introduced bacterial cells declines rapidly in soil, and the GM species survive in a mode similar to that of non-modified bacteria. There are a lot of experimental studies in which no difference in survival between GMM and parent strain could be detected (for *Pseudomonas chlororaphis, P. fiuorescens, Sinorhizobium meliloti).* Furthermore, some GMMs were reported to be out-competed by the parent strains. It is speculated that the presence of a number of constitutively expressed marker genes in a GMM had a negative effect on its survival in competition with the wild- type strain. Most probably it is the metabolic load that is responsible for the decreased fitness, since this effect does not occur under nutrient-rich conditions.

To correctly interpret bacterial survival data of crucial importance is to use a reliable method for detection, since cells that enter a non-culturable state cannot be detected with standard cultivation-based techniques. And various studies have shown that GMMs introduced into soil become non-cultuable. The presence of viable but non-culturable cells, dead cells, or naked DNA, detected with molecular techniques contributes to the complexity and the ecological significance of GMMs and their fitness in the context of the effect of the genetic modification introduced. The reliable way in which the effect of small differences in fitness will be measurable is to co-inoculate GMM and its parental strain placing them in direct competition. However, results from such direct competition experiments have to be interpreted with care as well, since commercial application of GMMs does not include direct competition between GMM and wild-type strain.

All these data, contradictory to some extend show that conclusion regarding survival of GMMs as compared to their parental strains cannot be definitely drawn. In each case where colonizing ability and survival of the GMM are of importance, these parameters will have to be determined.

Environmental impact of GMMs inoculated into soil

Possible effects of the release of GMMs in natural microbial ecosystems are quite diverse. The range encompasses events such as input of organic substrate, displacement of species, changes in population structure, and possible loss of certain functions; production of toxic metabolites, which might lead to disturbance of key ecological processes. It should be taken into consideration that small changes in community composition are difficult or even impossible to determine, and the relationship between microbial diversity and ecosystem functioning is not quite clear. Undoubtedly, soil microbial diversity is enormous with a high redundancy of functions. Disappearance of a few species with certain functions will be difficult to detect, since many functions can be performed by a large number of different microbes. In this sense, only extreme disturbances might affect soil microbial communities to the extent that certain functions will be negatively influenced.

The limited culturability of the indigenous soil microflora is one of the major problems in microbial ecology. DNA- and RNA-based techniques, which do not involve cultivation of the microorganisms, are currently used to detect the impact of GMMs on the indigenous microbial community. Methods that are suitable to analyze shifts in community structures are denaturing gradient gel electrophoresis (DGGE), amplified ribosomal DNA restriction analysis (ARDRA), terminal restriction fragment length polymorphisms (T-RFLP), and single-strand conformation polymorphism (SSCP).

Fate and effect of bio-fertilizer strains – field release

GM derivatives of bacteria that contribute to an enhanced nutrient availability for plants, and thereby increase plant growth.

The most important bio-fertilizers are bacteria, such as *Azospirillum* and *Rhizobium* that can fix nitrogen. *Rhizobium*, *Bradyrhizobium*, and *Sinorhizobium* are plant symbionts, which form root nodules in leguminous plants and fix atmospheric nitrogen. These bacteria have been used widely as plant inoculants to increase yield of leguminous crops. There is a long history of safe use of non-modified rhizobia as inoculants to increase yields of crops. However, yield increase is variable, and the success of inoculants seems to be dependent on competition with indigenous strains that are usually less effective. *Rhizobium*, *Bradyrhizobium*, and *Sinorhizobium* have been reported to survive in soil for years, in some cases even without the presence of their specific host. *Rhizobium* was shown to be able to form nodules when its host plant was planted again after several years. This shows that presence of the host plant is not strictly necessary for their survival, but also characteristics of the strain not related to symbiosis play a role in its survival in bulk soil for years. Fast-growing *Rhizobium* species were found to be more susceptible to desiccation than the slower-growing *Bradyrhizobium*.

Genetically modified *Azospirillum* and *Rhizobium* strains

Except for carbon dioxide (CO2) which plants obtain from the atmosphere, plants get all their nutrients from soil. Nature has developed various mechanisms to supply plant nutrients by means of renewable resources, and the best example of this principle is biological nitrogen fixation in leguminous plants. Nitrogen-fixing bacteria can be regarded as a self-propagating source of nitrogen for plants. Unfortunately, not all plants are able to perform such interaction with N_2-fixing bacteria. That is why at present plant production yields still largely depend on input of chemical fertilizers. Most of these fertilizers are very mobile in the soil and are supplied in greater quantities than required for optimal plant growth. The loss of valuable compounds is not only of economic importance; this also causes serious problems for the environment, through leakage in surface and ground water and accumulation of in the atmosphere.

Different strategies have been developed that aim at better uptake of fertilizers by plant roots. These include other formulations of fertilizer (e.g. slow-release fertilizer) and the use of Plant Growth Promoting Rhizobacteria (PGPR).

PGPR can exert their effect in both direct and indirect way. The indirect pattern comprises exercise of biocontrol of pathogens and deleterious microorganisms. The best documented example of PGPR acting in a direct plant growth promoting way is phytostimulation. Various bacteria genera are capable of producing plant growth stimulating factors (auxins, cytokinins, etc.) and when colonizing the roots of plants, they promote root growth. This assures a better uptake of water and nutrients by the plants and can result in higher crop yields.

GM *Azospirillum* increases nitrogen uptake

It is known that *Azospirillum* strains can promote plant root development and increase nitrogen uptake through the produced by them phytohormones. However, the mechanisms by which, and the conditions under which, these bacteria produce phytohormones as well as the interaction between bacteria and plant roots, are still not defined and require a better understanding.

To elucidate these mechanisms several important questions/approaches should be addressed:

- The genetic and biochemical grounds of the synthesis of indole-3-acetic acid (IM, the plant growth promoting hormone produced by *Azospirillum*;
- The construction of genetically modified *Azospirillum* stains with known production levels of IAA (i.e. IAA-minus , IAA-attenuated, IAA-over producers;
- Testing the effect of these genetically modified bacteria on plants (growth promotion, nitrogen uptake) and on the environment (interaction with resident microbial flora, survival and spread) under field conditions.

At present GM *Azospirillum* strains with these basic features are available. Research studies with these strains are focused on their impact on resident microbial populations, plant growth and nitrogen uptake rates from soil. These studies are being conducted in lab experiments (i.e. growth cabinet and glasshouse studies) in order to gain vital information on the way GM strains are likely to behave under field conditions. The experiments are conducted with a range of crops, soil types and climate conditions, representing the agricultural parameters existing within

Europe. Despite of the advancement of these research studies extensive and careful testing under containment is required before the GM *Azospirillum* can be considered for field release,

GM *Rhizobium* strains with increased competitiveness

Legume inoculation with highly efficient nitrogen fixing bacteria is a widely used approach to increase productivity of leguminous crops. This inoculation is not always successful since native soil bacteria with low nitrogen-fixing efficiency can out-compete the introduced strains in terms of nodulation initiation. Critical for the successful use of rhizobial inoculants is their competitiveness, i.e. the ability to dominate nodulation. Thus, inoculant strains are modified in a way that they occupy a sufficient number of root nodules to provide high rates of nitrogen fixation for the plant host.

Experiments with *Sinorhizobium meliloti* strains from diverse geographical origins regarding their competitiveness for alfalfa roots have shown that in all cases this property has been enhanced by genetic manipulation. The said genetic manipulation comprises modification of the expression of the *nifA* gene which is responsible for the control of all the rest nitrogen-fixation (*nif*) genes. When thus GM *S. meliloti* strains were mixed with wild-type ones, the former occupy most of the nodules on the alfalfa roots. The precise mechanism of this improvement is not understood yet but it is speculated that *nifA* regulates the expression of genes different from the *nif* cluster resulting in an advantage during nodule formation and development.

The ability of *Rhizobium* strains to efficiently recognize the plant root is another feature that contributes to their nodulation competitiveness. This is very important because the efficient inoculation means lower doses of the bacterial strain. Furthermore, the movement of the inoculation strain towards the plant roots is another factor influencing competitiveness. Experiments with GM *Rhizobium leguminosarum* strains, engineered to express β-glucuronidase, reporter gene (*gusA)*, showed that the percentage of the nodules induced by the GM *gusA*-labeled strain compared to the nodules induced by a flagella-deficient non-motile strain is higher. In this way it was proven that the functional flagella are required for effective competition for nodulation.

All these data provides valuable information regarding the mechanism of root attraction allowing the development of *Rhizobium* strains with enhanced nodulation competitiveness and increased host specificity.

Impact of GM *Rhizobium* strains on arbuscular mycorrhizal fungi

Arbuscular micorrhizal fungi are important group of fungi that form symbiotic relationships with plants. A major question is whether the application of GM *Rhizobium* strains with increased competitiveness leads to increase of the colonization and nodulation of the plant root or it interferes the beneficial symbiotic relationship.

In lab and green-house experiments it has been established that GM *Sinorhizobium meliloti* strain, with improved nodulation ability, did not interfere with any aspect of mycorrhiza formation by the representative AM fungi *Glomus mosseae.* On the contrary, GM S. *meliloti* increased the number of AM colonization units and the nutrient acquisition ability of the mycorrhizal plant.

GM *Rhizobium* strains: field release

Several *Rhizobium* species have been GM either to improve nitrogen fixation, or to study their survival making use of marker genes through field trials.

Thus, a Tn5-marked R. leguminosarum strain introduced into a field as an inoculant for peas and cereals persisted for 5 years in the plots where peas were grown. The persistence of the strain was attributed to the soil type, the cultivation of the proper host plants, and the climate conditions. Potential non-target effects on the microbial ecosystem were not studied.

The use of an improved R. meliloti strain, with additional copies of nifA and dctABC, resulted in an increase of alfalfa yield of 12.9% in a field study. However, at sites with high nitrogen concentrations or native rhizobial populations alfalfa yield did not increase.

The fate of a Tn903-marked R. meliloti strain introduced into alfalfa-planted field plots was studied and it was found that the cell numbers decreased rapidly after inoculation. One year after introduction, numbers of introduced cells had dropped to below the numbers of indigenous rhizobia.

In a contained field experiment a GM S. meliloti strain with enhanced competitiveness for nodule occupancy was released in the rhizosphere of alfalfa. Effects of the GMM and the wild type on the indigenous microbial communities were studied by restriction fragment length polymorphism (RFLP) and temperature gradient gel electrophoresis (TGGE). Inoculation of wild type and GMM had only limited effects. It appeared that alfalfa plants had a greater influence on the microbial community than the inoculated strains.

Both the fate and ecosystem effects of a Luc-marked S. meliloti in a field experiment with Medicago sativa were studied. The bacteria were detected up to 12 weeks after introduction. No effects of the strains on carbon and nitrogen concentrations in the soil could be detected, and there were no differences in the total number of colony forming units of indigenous microorganisms. Over a thousand bacterial isolates obtained from the plots were further studied by ARDRA, and the dominant groups were identified by 16S rRNA sequencing. In the rhizosphere of M. sativa numbers of Alcaligenes and Pseudomonas were reduced as a result of the inoculation. Molecular analysis by studying SSCP banding profiles revealed shifts confirming the effect of the inoculum on the native microbial population.

In China wild type and GM Alcaligenes faecalis isolates have been introduced into rice fields at a large scale to improve crop productivity. A. faecalis, a non-nodule-forming nitrogen-fixing isolate, was GM by insertion of a constitutively expressed nifA regulatory gene. Nitrogen fixation appeared to be 15-20% higher and yield was 5-12% higher compared to the non-treated fields. The possible ecosystem effects of the introduction of this GM strain by DGGE of amplified 16S rDNA in a microcosm experiment was studied. The introduced GM strain survived well in the rhizosphere. DGGE banding profiles of samples treated with the modified strain closely resembled profiles of untreated samples throughout the 40 days of the experiment, suggesting that there are no obvious effects on the bacterial community. Overall, the survival of the strain and the increase in crop yield indicate that this derivative of A. faecalis is a good candidate for commercial application, since its ecosystem effects seem very limited.

The impact and fate under field conditions of GM *Rhizobium* strains were investigated in a field trial with a model system comprising different GM *Rhizobium leguminosarum* v. *viciae* strains, marked with the *lacZ* gene and HgCb resistance genes *(mer* genes) inoculated in the rhizosphere of pea plants. Three modified strains were used:

- 1110 strain containing plasmid pDG3 carrying genes for resistance to HgCb *(mer* genes) and *lacZ* whose expression is under the control of the l*acZ -lacO* system
- 1111 strain carrying the plasmid pDG4 in which the *lacZ* gene is constitutively expressed at high levels;
- 1112 strain containing a copy of *mer* genes and a regulated *lacZ* gene inserted into the chromosome.

Wild-type *R. leguminosarum* v. *viciae* 1003 was used as a control.

These strains were monitored according to the reporter *lacZ/mer* system along with the soil metabolic activity plus nitrogen transforming capacity.

The field experiments showed that all tested strains colonized the rhizosphere to the same extent; similar values were determined for the respiration rate and soil metabolic activity as well as for the nitrogen transforming capacity of all tested strains. These results indicate that although the presence of the plant had a considerable impact on carbon mineralization in soil, the impact of GM *Rhizobium* strains is indistinguishable from the impact of the wild-type strain and also suggest that the impact of the plant on microbial activity is considerably greater than the impact of GM inoculants compared with wild-type strains.

In spite of the fact that the field trials with GM bio-fertilizers are limited the initial results about their use are promising in respect to the improved performance in agricultural applications. GM bio-fertilizers have been introduced with an encouraging success regarding the survival and the activity of the inoculants, which is dependent on the environmental conditions. So far, non-target effects of GM bio-fertilizer strains that have been reported are small and insignificant compared to natural variations, such as differences between populations of different plant species..

However, our knowledge on the benefits, fate and effects of GM strains in the environment is still quite limited and partial.

Questions that have to be solved include: how and when (at what physiological state) bacteria survive best in soil; what is their effect on the natural microflora; how can be mix microbial community structured and optimized for use in agriculture. And last but not least – what is the ecosystem effects of GM strains, especially on non-target organisms.

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PRODUCTION OF BIOFERTILIZERS

Contents

INTRODUCTION

Environmental issues, for example, freshwater contamination, energy saving, and soil erosion are compelling the farmers to present developmental strategies that have a lower polluting impact. The utilization of environmentally friendly practices is advanced by voluntary certification schemes (e.g., GlobalGAP or organic farming schemes) as well as by legally binding regulations (e.g., the EU Directive 2009/128 aiming at the implementation of sustainable pest management practices). In this context, the diminished utilization of chemical fertilizers with expanded use of organic fertilizers is viewed as compulsory route to improve the pressure on the environment derived from rural practices. In recent year's history, the chemical pesticides and fertilizers have assumed an essential part in boosting the rural development; however, they have a short history in modern agriculture. Their immediate action and low cost succeeded to bring them rapidly in to the centre of attention. On the other hand, their toxic effects on environment, plant, animal and human life diverted the focus on eco-friendly plant protection. Moreover, the development of resistance in insects against common pesticides has not been solved yet. Thus, practices such as Integrated Pest Management (IPM) have gained more importance.

Biofertilizers are vital segment of the IPM. They can be of extraordinary financial significance: they can in part replace different agrochemicals which are turning out to be increasingly costly and their improvement is in light of expanding requests for all the more ecologically agreeable farming practices. The term “biofertilizer” commonly refers to a product containing soil microorganisms applied to plants to promote their growth. However, it has often also been wrongly used as a synonym for a wide range of products such as green or animal manure, intercropping, or organic-supplemented chemical fertilizer. Vessey (2003) defined a biofertilizer as “a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant”. The microorganisms they contain are also called plant growth promoting rhizobacteria (PGPR) and result in benefits to the plant hosts after inoculation.

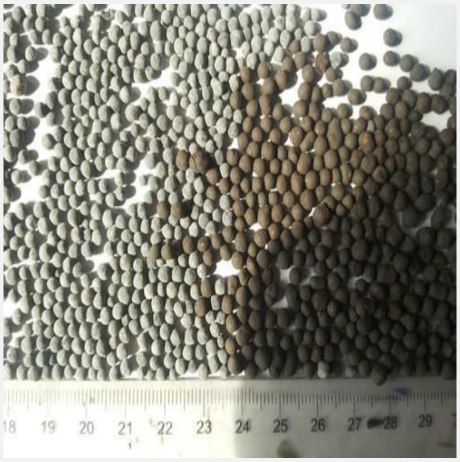
The enthusiasm for the utilization of these products is ascending due to the improvement in nutrient uptake efficiency and society demands for more green technologies and increased costs of agrochemicals. Moreover, biofertilizers and phytostimulators have optional helpful impacts that would increase their usefulness as bioinoculants. Indeed, microorganisms such as Rhizobium and Glomus spp. have been shown to also play a role in reducing plant diseases. The practice of inoculating plants with PGPM can be followed back to 20th century, when a product containing Rhizobium sp. was patented. Mycorrhizal fungi, even though utilized as biofertilizers since couple of decades, were reported to promote plant growth through P uptake since the late 1950s. Since then, research endeavours in these fields have consistently expanded, resulting in the selection of various strains demonstrating several beneficial characteristics.

The policies supporting sustainable rural development and broad research that has enhanced the adequacy and consistency of microbial inocula have resulted in the enrolment of several strains for both biocontrol and biofertilization, with mycorrhizal and PGPR preparations being marketed in several countries. Yet, a wider use of microbial inoculants, especially those acting as phytostimulators and biofertilizers, has been frequently hindered due to the variability and inconsistency of results between laboratory, greenhouse, and field studies. The explanation behind these discrepancies lies in the fragmented comprehension of the complex relationships established between the components of the system: the plant, the microorganisms, and the environmental conditions, particularly that of soil. In addition, the lack of correct formulations and the costly and tedious procedures of registration are also among the factors holding back the use of PGPM on a more extensive scale.

The real commercialization of PGPR began in 1995 in the USA and UK with the inoculation of legumes with rhizobia. However, the enthusiasm for other PGPR has been increased over time and a range of new products have been developed more recently. Most of the nonrhizobial PGPR inoculants currently available contain bacteria from the genus *Azospirillum* (free living N_2-fixing bacteria) or *Bacillus* (phosphate-solubilizing bacteria (PSB) and biocontrol agents. Products containing arbuscular mycorrhizal fungi (AMF) are also becoming increasingly applicable worldwide. However, the diversity of PGPR and AMF populations potentially available in soil and the range of their modes of action are very broad and, for the vast part, incompletely understood and thus underexploited. It is also recognized that the various mechanisms involved in plant promotion may be host plant-specific and strain-specific and that the advantageous impacts may vary extraordinarily under various natural conditions. In addition, once introduced to the soil, microorganisms face competitive and often harsh conditions that may severely reduce their beneficial effects.

The four main types of formulation that have been used up to now are liquid, peat, granules, and freeze-dried powders (Fig.1). Their success relies on target crop, cost, market availability, environmental constraints, and usability. One of the real difficulties for the inoculant industry is to develop an improved formulation that combines all the above characteristics and that are suitable for use under field conditions. Moreover, while a microorganism may seem promising in laboratory, producing it commercially in order to obtain similar results under a wide range of field conditions is a difficult step. Some manufacturers included at least two types of microorganisms (e.g., rhizobia and AMF, rhizobia and PSB, various strains of AMF or PSB) in a single product, thus augmenting the subsequent benefits for the host plants. However, only a few reviews reported the positive effects of these co-inoculants. Their efficacy was not proven and their production and commercialization pose a number of technical difficulties. The most important aspect during inoculant development is assurance of the quality in a way that guarantees the reliability of the products with maximal chances for success. The absence of consistency in results obtained under field conditions because of conflicting quality has enormously influenced the commercialization of biofertilizers.

C **D**



C **D**

Fig. 1. Types of biofertilizers formulations: A – liquid; B - peat, C - granules, and D – encapsulated freeze-dried powders.

PRODUCTION OF INOCULANTS

Development of an effective inoculant represents a multistep procedure comprising the attachment of one or more strains of microorganisms in a particular carrier together with sticking agents or other additives which assure the protection of the cells during storage and transportation. Since the inoculants are often stored under non-optimum conditions (e.g., high temperature, light exposure), they must have an extended shelf life, i.e., the microorganism should be either robust or to have greater capacity to survive in high numbers under harsh conditions. A good formulation will also provide effective introduction of microorganisms in the soil and will enhance their activity in order to obtain the maximal benefits after inoculation to the host plants. To be easily accepted by the farmers, an inoculant must be cost effective and simple to deal with and use, to guarantee that the microorganisms are delivered to the target plant in the most suitable way and form. Formulation is a crucial issue and limited investigations were performed in this subject. Available data showed that since the 1980s, most *rhizobial* research are concentrated on the bacterial genetics and physiology and less than 1 % - on formulation aspects of rhizobia inoculants. In any case, there is a real need for improved formulations of inoculants, to develop and commercialize new biofertilizers that will be more successful, more stable over time, of better quality, and addressing agricultural needs.

The ideal formulation does not exist and obviously every type has its own particular advantages and constrains. However, there are some critical steps which must be precisely considered during the biofertilizers production. The choices made at these steps can lead to the success or the failure of the inoculation. The decision of the microorganisms to be inoculated is of crucial importance. Some of the most important desirable characteristics of the inoculant strain (bacterial or fungal) include its genetic stability, its ability to be beneficial for the target crops, to be competitive to the indigenous populations, to migrate from inoculation site to the hosts, and to survive in hostile soil without the presence of the host. Other important features sought during production is the ability of the strain to grow in laboratory conditions (exception is made for AMF which cannot grow without a host plant), grow or survive in carriers (during curing or storage), on seeds and in soil and to be compatible with agrochemical products that might be applied on seeds. The live inoculant must also be able to overcome the various technological processes during production and maintain its functional properties. Bacterial inoculants are generally cultivated in liquid medium to reach high biomass yields. The composition of the media and growth conditions (temperature, pH, agitation, aeration, etc.) are directly related to the physiology-biochemical properties of the particular strain and the kind of inoculant that is to be produced. Obtained bacterial cultures are then used to inoculate the different carriers (encapsulation or impregnation of peat and granules), or after addition of various additives liquid formulations could be produced. The large-scale production of bacteria in pure cultures using bioreactors is wildly spread common practice (Fig. 2).

Fig.2. Mass-production of *Azolla*

In this way, once the specific strain/s for the inoculum has been chosen, an industrial standardized procedure of production can be defined. However, for biofertilizers, dissimilar to biopesticides, the cost of production is an important limitation. This is due to the fact that the price of the biofertilizer shall not exceed that of the conventional ones. Hence, several cheap raw materials (e.g., whey, water sludges, composts, etc.) have been utilized as growth media for PGPM. Another approach to diminish the production costs is by using agro-industrial residues enriched with rock phosphate. During composting or fermentation, free or immobilized microorganisms that produce organic acids are added to the matrix, enhancing the solubilisation of phosphate and thus making it more available to plants.

Recently, the use of biofilms has also been applied as possible means to produce effective plant inocula. A biofilm comprises of microbial cells embedded into a self-produced polymeric matrix (known as an extracellular polymeric substance—EPS) and adherent to an inert or living surface, which provides structure and protection to the microbial community. Three major types of biofilms are observed in the soil: bacterial (including Actinomycetes), fungal, and fungal-bacterial biofilms). Both bacterial and fungal biofilms are formed on abiotic surfaces, while fungi act as the biotic surface in formation of fungal-bacterial biofilms. The majority of plant-associated bacteria found on roots and in soil are forming biofilms. Therefore, applying PGPM strains that form biofilms could be a successful strategy in formulation and production of biofertilizers. While ectomycorrhizal fungi can be produced under fermentation conditions, the production of AMF inocula is much more difficult due to the need of a plant host for the multiplication of the mycorrhizal fungi. The first attempts in AMF production are based on pot cultures with soil mixtures, or aeroponics. However, the development of monoxenic cultures in the late 1980s has allowed the production of AMF under strictly controlled conditions. A method was developed for production of spores by using split-plate cultures and Ri T-DNA transformed roots of carrots. However, although the method allows production on average of 15.000 spores per Petri dish in 4-5 months after beginning the production cycle, it has been used mainly for physiological and laboratory studies. The improvement of this method was achieved through replacing the media in the distal compartment every 2 months with parallel replenishing the carbon source in the proximal compartment with glucose. Obtained results lead to the production of about 65.000 spores in 7 months. Yet, such methods are mainly used for experimental batch production of spores or for maintenance of gene banks. The reason is that the estimated annual cost for producing of one spore is up to 30–50 USD, depending on the method utilized. Recently, a large-scale in vitro production of mycorrhizal fungi, feasible for implementation on a commercial scale, has been proposed. It is based on several key points: selection of appropriate Ri T-DNA transformed host roots for different AMF species, selection and maintenance of optimal growth medium, and application of quality assurance procedures.

However, commercial inoculants containing AMF species are still produced mainly by growing host plants in controlled conditions, with the addition to the inoculant of various fungal structures (spores, mycelium hyphae) and containing mycorrhizal roots residues from the plants used as the propagating material (i.e., sorghum, maize, onion, or *Plantago lanceolata*) (Fig. 3). This could be considered a classical method where substrates of sand/soil and/or other materials (e.g., zeolite, perlite) are used to mass-produce AM fungal inoculum in pots, bags, or beds, for large-scale applications. Critical issues in this production strategy are:

(i) usage of known AMF species,

(ii) selection of host species with a short life cycle, adequate development of the root system, a good colonization level by a large range of AM fungi, and tolerance to relatively low levels of phosphorus,

(iii) control of mineral nutrients level in soil,

(iv) suitable combination of AMF species and host plant.

With this technique, it is possible to achieve inoculum densities of 80–100 thousand propagules per liter. This implies the need of diluting the inoculum with a carrier for the preparation of a commercial product.

Fig.3. *Plantago lanceolata* root nodules

Considering that microbial associations between bacteria and mycorrhizal fungi occurring naturally in the soil can promote the mycorrhizal symbiosis, it could be suggested that formulations including two or more species of different PGPM would have enhanced beneficial effect on plants. Microbial consortia can stimulate plant growth through a range of mechanisms that improve nutrient uptake and suppress fungal plant pathogens. The different approaches proposed to explain such growth stimulation are based on the increased rate of nutrients cycling. The last is due to the greater microbial content and biodiversity found in the soil where mycorrhizal plants are grown. Simultaneous inoculation with different PGPR and/or AMF often resulted in increased growth and yield, compared to single inoculation through improved nutrient uptake. Indeed, the interactions between bacteria and AM fungi have positive effect on nutrient uptake, particularly when PGPR and N2-fixing bacteria are combined. Inoculation of maize and ryegrass with *A. brasilense* and AMF resulted in N and P contents comparable to plants grown with fertilizer. Co-inoculation with different AMF species is generally more effective due to the lack of AMF fungi colonization specificity for define plant species/cultivars. Synergistic interaction between AM fungi and several PGPR, including *Azospirillum*, *Azotobacter*, *Bacillus*, and *Pseudomonas* species, has also been reported as favourable for plant growth. Improved root colonization by AMF was observed when mycorrhizal fungi were co-inoculated with such PGPR. Four times higher nodule number was reported when plants were inoculated with a mixture containing *Glomus deserticola* and *Rhizobium trifoli*, in comparison to single *R. trifoli*, inoculation, and enhanced mycorrhization and nodulation was observed with co-encapsulated *R. trifoli* and *Yarrowia lipolytica*. Inoculation with nodule-inducing rhizobia and AM fungi resulted in increasing both P and N uptake efficiency. Application of PGPM as commercial biofertilizers containing consortia of different microorganisms often leads to diminishing the infection rate, better mineral nutrition, and increased plant growth. All these examples are are indicating the convenience and higher adequacy of biofertilizers composed by more species having different mechanisms of growth promotion. The possibility for testing of several strains of PGPR and AMF in different crops species and under different field conditions should allow the definition of consortia suitable for commercial uses.

CARRIERS

The carrier is the delivery material of live microorganisms from the processing plant to the field. It represents the major element (by volume or weight) of the inoculant and has a crucial significance in the delivery of the correct number of viable cells in good physiological condition. It provides a momentarily protective niche to microbial inoculants in soil: physically by provision of a protective surface of pore space (creating protective microhabitats) and nutritionally by provision of a particular substrate. Ideally, a good carrier possesses the following features:

✓ Provision of appropriate microenvironment to the target microorganism(s).

✓ Possession of appropriate physical and chemical properties: moisture absorption capacity (high water holding capacity), pH buffering capacity, and easy adjustable pH.

✓ Stability during the process: the carrier should be chemically and physically stable. It should be sterile or easy to sterilize (autoclaving or other methods), be free of protuberance materials, easily grinding and mixing with other substances (nutrients, adjuvants) using standard machinery equipment. It should also be applicable for as many bacterial or fungal species and strains as possible and simple to deal with and handle.

✓ Easy storage and inoculation: a good carrier should guarantee an adequate time span of usability (at least 2–3 months at room temperature), adhere well to and survive on seeds, and permit quick and controlled release of the microorganisms into the soil near the roots of the host.

✓ Economically and environmentally sustainable: that suggests a low cost and and reliable accessibility and quality. The carrier should be free of toxic materials, biodegradable, and non-polluting and minimize environmental risks (dispersal of cells to the atmosphere or ground water).

Selection of a carrier defines the physical form of the inoculant and clearly there can't be a perfect and widespread carrier for all microorganisms (Table 1). The carriers can be of various origins (organic, inorganic, or synthetic) and can be classified into four main categories:

- ✓ Soils: peat, coal, clays, lignite, inorganic soil
- ✓ Plant waste materials: charcoal, composts, farmyard manure, cellulose, soybean meal, soybean and peanut oil, wheat bran, press mud, corn cobs
- ✓ Inert materials: vermiculite, perlite, ground rock phosphate, bentonite, calcium sulfate, polyacrylamide gels, alginate beads
- ✓ Plain lyophilized microbial cultures and old dried bacteria: can be later incorporated into a solid carrier or used as they are

It is also possible to obtain carriers made of a combination of the above: mixture of soil and compost, of soil, peat, bark, and husks among others. Four dispersal forms are generally used: dry inoculant (powders), slurries (powder-type inoculants suspended in liquid), granules, and liquids. Peat is the most commonly used carrier, especially for bacterial inoculants. However, it is not easily accessible worldwide and its use has a undesirable impact on the environment and ecosystem from which it is extracted. This highlights the need of development of new formulations using alternative materials to compete with the existing inoculants.

Dry inoculants (powders)

Dry inoculants are delivered using soil, organic, or inert carrier. In many parts of the world, inoculants are formulated using peat (soil carrier). Peat is made of partially decomposed flora accumulated over the years. It provides a nutritive and defensive growth environment of an extensive variety of microorganisms which can develop and form microcolonies both on the surface of the particles and in fissures. To be appropriate for inoculant use, peat must be nontoxic (for microorganisms, plant, animals, and human), highly adsorptive and easily sterilized, have a high organic matter content and water-holding capacity, and be available locally at a reasonable cost. Peat has been principally utilized because it is widely available. However, its processing is expensive as it requires several steps before it can be used as carrier for inoculant. Harvested peat must be drained and sieved to remove coarse material before it is slowly dried to around 5 % moisture. This drying step is of crucial significance since it can prompt to the formation of toxic compounds. The drying should be carried out at the lowest possible temperatures and certainly never surpass 100°C. Air drying is the preferable method instead of oven drying. The type of peat and the particle size desired defines the extent of drying. However, the moisture content must be decreased adequately to guarantee that the subsequent addition of liquid culture brings the final moisture content of the inoculant to the sought level. Once dried, peat is ground, commonly to pass through at least a 250-µm sieve. Generally, the peat deposits have a low pH, which must be corrected to pH 6.5–7.0. The peat is then sterilized and an adequate amount of liquid inoculum is added to it.

In the case of bacterial inoculant, a final moisture content of 40–55 % is generally acceptable. Inoculated peat is incubated for a certain period to allow bacteria multiplication in the carrier. This step, also called maturing or curing is of major importance since it improves the bacteria survival rate during storage and on seeds. Peat can also be used for AMF and ectomycorrhizal inoculants though the latter are not broadly utilized, except for forest regeneration. Ectomycorrhiza generally are grown in glucose containing medium and produced spores are used for inoculation. Pure mycelia cultures are preferred as they suppress growth of pathogens and contaminants. Ectomycorrhizal inoculants may be formulated using a carrier made of vermiculite and 5–10 % peat moisturized with salts and glucose nutrient medium. This formulation provides a strong buffering capacity (keeping pH below 6) and enhances the production of fulvic acid that stimulates growth.

Table 1. Advantages and limitations of the most common carriers

Carrier	Benefits	Restrictions
Peat	➢ Suitable for a wide range of microorganisms: bacteria, AMF, ectomycorrhizal ➢ Protective nutritive environment ➢ Moisture content can be adjusted to improve growth and survival of bacteria during curing, storage, and inoculation ➢ Strong buffering capacity	➢ Not readily available ➢ Strong negative impact on the environment and the ecosystems ➢ Costly investment for extraction ➢ Toxic compounds released during drying and sterilization ➢ Highly variable in composition and quality depending on the origin ➢ Seed application: contact with other chemical compounds (toxicity)
Liquid	➢ Easy to handle and apply ➢ Easy addition of additives to improve growth or survival of the cells ➢ Composition easily defined and controlled ➢ High cells concentration → low application rates	➢ Lack carrier protection: low viability during storage and on seeds ➢ Cool temperatures for storage (4 °C) ➢ Limited shelf life ➢ More sensitive to stressful conditions
Granules	➢ Easy to store, handle, and apply ➢ Less dusty than peat ➢ Application rate easily assessed ➢ Soil application: no direct contact with the other chemical compounds (no toxicity) ➢ Especially efficient under stressful environmental conditions	➢ Bulky: high transport and storage costs ➢ Higher application rates ➢ Often nonsterile carriers
Lyophilized encapsulated cells	➢ Suitable for all types of cells (all sizes) ➢ Cells protected in a nutritive shell against mechanical and environmental stresses and against predators ➢ Slow and controlled release of the microorganisms when the shell is degraded ➢ Wide variety of polymers: nontoxic, biodegradable ➢ High concentration of cells/shell → limited space for storage ➢ Storage at room temperature (dried capsules)	➢ High production cost ➢ More handling work at the industry level ➢ Specific equipment required ➢ Physiological, morphological, and metabolic changes occurring in the shell ➢ Several applications needed if strains cannot establish in soil ➢ No commercial product available

Inoculated peat is typically applied on-site on the seeds just before sowing. The required amount of product is relatively small. However, the quantity of microorganisms used per seed is not well controlled as they are in direct contact with the other chemicals which may have been covered on the seeds. The seed coating can be done by machines (large dough, cement mixers, and mechanical tumbling machines). This procedure allows the inoculation of a large number of seeds. The significant disadvantage of peat originates from the variability in its quality and composition, which are source-dependent. Peat is an undefined and complex material and different sources will vary in their ability to support cell growth and survival. Toxic compounds might also be released during sterilization, negatively influencing the growth and survival rate of desired microorganisms. This may bring about challenges to guarantee reliable quality and results in the field, as well as to identify the optimal storage conditions, or usage instructions. Regardless of these restrictions, peat remains the standard by which every other material is judged.

Coal, clays, and inorganic soils (i.e., lapillus, volcanic pumice or diatomite earths) are available in different areas and could be utilized as carriers. Their microbial load depends on the deriving place (about 102-103 CFU g−1), but it is generally lower than in organic carriers. Vermiculite, perlite, and bentonite are also available in different countries, but their application in general is restricted due to the difficulties in preparing an effective formulation. In reality, the impact of these carriers on bacteria viability and growth is dependent on the pH, ion strength, and the electrolyte in solution. Expanded clay has been tested as a carrier for AMF and mycorrhized roots mixed with soil are also used for AMF inocula. Among other inorganic compounds, glass beads have also been proposed for AMF inocula. A mixture of organic and inorganic materials has been demonstrated successful in increasing activity and shelf life of *Burkholderia sp*. The majority of the previously mentioned carriers depend on the absorption of the microorganisms by the substance/matrix of the carrier. This strategy for incorporation has some disadvantages, especially in relation to the survival of the microorganisms and their protection during transport, storage, and handling. Nevertheless, some procedures with different carriers using such approach have been patented:

(i) the Belgian patent no. 521.850 for use of diatomaceous earth and colloidal silica for Rhizobium,

(ii) the British patent no. 1.777.077 for the use of bentonite for *Rhizobium*,

(iii) French Patent no. 1.180.000 using a must juice, to which substances with an adsorbing action are added, such as cellulose, bone meal, kaolin, or silica gel, in the manufacture of preparations rich in bacteria of the *Azotobacter* group,

(iv) United States Patent no. 4956295 for the stabilization of dried bacteria extended in particulate carriers, where dried viable bacteria are mixed in a particulate carrier composed primarily of an inorganic salt of low moisture absorbing capacity together with a minor proportion of a silica gel absorbent. The inorganic salts may be sodium or calcium carbonates, bicarbonates, sulfates, or phosphates.

Granules

To overcome the disadvantages in application of peat, the interest in other types of formulations and especially in granular inoculants is increasing. Granules are made of peat pill or small marble, calcite, or silica grains that are wetted with an adhesive material and then mixed with a powder-type inoculum. Thus, the granules are coated or impregnated with the target microorganism(s). The size of the granules varies, however the relation between initial microbial population density and finished product quality is direct: the better the initial microbial population, the better the product. Granules have many advantages over peat. They are less dusty and easier to handle, store, and apply. The placement and the application can be easily controlled and the limitations of seed applications are overcome: the inoculant is placed in a furrow near to the seed to facilitate lateral–root interactions but is not in direct contact with the chemicals or pesticides potentially toxic for the microorganisms. Limits in granules applications are related to the fact that they are bulkier and the transport and storage costs are therefore higher.

The prevalence of *rhizobial* granular inoculants over peat and liquid inoculants has been evaluated in several studies and obtained results are variable. A few reviews demonstrated that granular application of rhizobia did not display predominant nodulation or biological N_2 fixation compared with the other formulations (peat and seed coating), while other studies on inoculation of legumes showed that granular formulations are superior to peat-based products and liquid inoculants in terms of number of nodule formation and weight, N accumulation, N_2 fixation (% Ndfa), and total biomass generation. The benefits of using granular inoculants are particularly advantageous under soil stress conditions like high acidity, moisture stress, or cool, wet soils.

Liquid inoculants

Liquid inoculants are based on aqueous (broth cultures), mineral or organic oils, oil-in-water or polymer-based suspensions. Liquid products have been elevated as being simpler to handle and apply either on seeds or in soil. So, their ubiquity has expanded in the most recent decade. They are currently popular and have been applied for legume inoculation (in the USA and Canada for instance) due to their high cell concentrations. This characteristic allows the application of a lower quantity of inoculant for a similar efficiency. However, a number of limits blocked their utilization: inoculants based on liquid cultures lack carrier protection and quickly lose viability on the seed. They require more particular storage conditions (cool temperatures) and generally have a limited shelf life. It was additionally revealed that liquid inoculants were more sensitive to environmental stresses and poorly survived in the carrier. Application of some other components (sucrose, glycerol, gum arabic, PVP) may improve survival of microorganisms in liquid inoculants.

Polymer-based carriers (cell immobilization)

The advance made in formulation improvement has led to new types of microorganism entrapment and immobilization processes that seem particularly promising. Immobilization encompasses the different forms of cell attachment or entrapment into a matrix. These include flocculation, adsorption on surfaces, covalent binding to carriers, cross-linking of cells, and encapsulation in a polymer gel. Encapsulation has proven to be the most promising technique for development of microbial carriers. Once encapsulated, the living cells are protected in a nutritive shell (or capsule) against mechanical and environmental stresses (such as pH, temperature, organic solvent, or poison) and predators. When placed into the soil, soil microorganisms slowly degrade the capsules and the target cells are gradually released in large quantities. Usually this happens during the time of seed germination or seedling emergence. Different kinds of cells could be encapsulated, including bacteria, fungal spores, or small hyphal segments. In this way, the encapsulation procedure represents a promising technology for development of single and multiple strain products, such as PSB–AMF or rhizobia–AMF-based ones.

Different kinds of polymers may be used for encapsulation: natural (polysaccharides, protein material) or synthetic (polyacrylamide, polyurethane) and homo-, hetero-, or co-polymers. There are more than 1,350 possible combinations of polymers which can be applied for encapsulation. Selection generally is made on the basis of their chemical composition, molecular weight (too low or too high molecular weights being considered as a disadvantage), and their ability to interact with other components. Polyacrylamide and alginate are the most commonly used polymers for cell encapsulation. However, alginate is preferred since polyacrylamide requires more specific handling precautions due to its toxicity. Alginate is a natural, biodegradable and nontoxic substrate which forms a 3D porous gel when mixed with multivalent cations (Ca^{2+}). To form beads, microorganism cells are dispersed into the polymer matrix and the mixed solution is simply dropped in the cationic solution. Nutrients and other supplements can be included to prolonged shelf life and inoculation efficacy. The beads are then dried for simplicity of packaging and handling. Different technologies are applied (including spray drying, extrusion, emulsion technique, coacervation, solvent extraction/evaporation, thermal gelation, pre-gel dissolving technique) to control the size, the shape, and the texture of the beads. Smaller beads of 10–100 μm (microencapsulation) are preferred since they offer direct contact with seeds, while macroencapsulation (larger size, extending from a few millimeters to centimeters) requires the released cells to move through the soil toward the plants.

Inclusion of bacteria in alginate beads has been used for various species, either spore forming or not. Different AMF have also been entrapped into alginate matrixes or in beads formed with different polymers. Spores of mycorrhizal fungi were entrapped in alginate film formed in a PVC coated fiberglass screen. Roots of leek seedlings inoculated with this alginate film containing *G. mosseae* spores were heavily colonized after few weeks of growth in greenhouse conditions. Similar results were obtained with spores obtained from monoxenic cultures embedded into beads. Inclusion of filamentous microorganisms such as Aspergillus and Actinomycetes has been also proved possible.

Several positive effects over free cells (conventional formulations) have been reported. Besides the cell protection provided by the shell, different studies under numerous conditions have revealed that encapsulation has numerous advantages during storage and field applications. This process is not stressful to cells, aseptic conditions minimize contamination, and the carriers are biodegradable and nontoxic. As the beads can be highly concentrated, their volume is very low, and thus, limited space for storage is required and transportation and handling are facilitated. They have an extended shelf life, can even be stored dried at room temperatures for relatively long periods, are easy to use, and are of consistent quality. When are microencapsulated the cells are distributed uniformly to the targeted site, even on small seeds, thus enhancing the application efficacy. As a result, the cell movement through soil and the possibility of off-site drift during application are significantly reduced. It was also demonstrated that encapsulation of PSB microorganisms increased their P solubilization capacity and their potential to promote plant growth compared to free cells. Limitations include a high production cost, more handling work at the industry level, and special equipment requirements. It was also mentioned that physiological, morphological, and metabolic changes may occur in encapsulated cells and that repeat applications of beads may be required since cells may not establish outside of beads.

Even though encapsulation seems to have a relative success, the vast majority of the research was performed in laboratory conditions and up to now no commercial bacterial product is available on the market. One of the explanations of the non-adoption of the technology by the inoculant industry might be the high production costs and technical handling. New technologies must remain affordable and cost effective to be easily implemented by manufacturers and farmers.

Reducing the cost of the production process and improving the quality of the beads were achieved by encapsulation and air-drying of bacteria into a mixture made of alginate (3%), standard starch (44.6%), and modified starch (2.4%). This process permits to obtain beads that after drying have a water content of 7%, size of 4 mm, and a mechanical resistance of about 105 Newton (features like that of grain seeds). Encapsulated bacteria can be stored at room temperature or at 4◦C without losing their viability - they are able to survive up to six months maintaining a final population size of about 108 CFU g−1 (corresponding to about 105 CFU bead−1). However, with this composition, some problems can arise when standardizing and automating the beads formation due to the viscosity of the mixture and the need of a continuous agitation of the stock medium. Recently, a new procedure was proposed, using starch industry wastewater as a carbon source for the production of *Sinorhizobium meliloti* with simultaneous addition of alginate and soy oil as emulsifier. Results obtained showed a cell viability of more than 109 CFU mL−1 after 9 weeks of storage. Addition of synthetic zeolite to the alginate mixture did not improve the survival of the embedded microbial cells, nor the physical structure of the beads.

Different other polymers have also been tested with AMF. Carrageenan was used to encapsulate AMF communities while hydroxyethylcellulose was used as a gel carrier. Two patents have also been registered:

(i) French Patent application no. 77.10254 (corresponding to U.S. Patent no. 4.155.737) which makes use of a polymer gel based on polyacrylamide gel or a silica gel for different microorganisms,

(ii) the US patent 5021350 on the process for inclusion of mycorrhizae and actinorhizae in a polymer gel matrix based on at least one polymer from the polysaccharide group, with at least partial crosslinking of the polymer.

Other carriers

An extensive variety of materials, both natural and artificial, have been tested and assessed as alternative carriers for diverse microorganisms. The principle drivers for the utilization of another carrier appear to be its supply and cost rather than a requirement for better quality and that works against their more widespread adoption.

Several cheap organic matrixes including water sludge, composts, sawdust, sugarcane bagasse, whey, or enriched agro-industrial residues have been proposed. Sludge wastewater might be an appropriate carrier but it contains heavy metals and this pose legal problem in respect to its utilization. Good alternative to peat is the compost from the cork industry. It is better in maintaining the survival of different rhizospheric bacteria during 6 months of storage as well as survival on seeds. However, organic composts may not be applicable for AMF formulations as they can decrease the mycorrhization rate.

Coal, clays, and inorganic soils (lapillus, volcanic pumice, or diatomite earths) can be used where available, though microbial concentration is lower than in organic carriers. In Madagascar, AMF production was done using Pouzzolane, a volcanic rock. Utilization of perlite as an inoculant gave variable outcomes. It is a suitable carrier but less efficacious than cork- and peat-based inoculants. Its effectiveness was increased when sucrose was employed as adhesive.

Gels of various chemical compositions (including magnesium silicate, fluidized bed or cellulose-based gel) is regarded as having a potential but none of them have been adopted on-farm till now.

Promising New Technologies for Carriers Development

Water-in-oil emulsions seem to be a good, yet underutilized, method for storing and delivering microorganisms through liquid formulations. The oil traps the water around the organism and, therefore, slows down water evaporation once applied. This is especially helpful when microorganisms sensitive to desiccation are used or in case of horticultural crops where irrigation systems are in place. Water-in-oil emulsions permit the addition of substances to the oil and/or aqueous phases. In this way both cell viability and release kinetics are improved. However, cell sedimentation during storage is a major issue to be considered. Several studies are carried out trying to solve this problem through application of nanomaterials. Thickening the oil phase using hydrophobic silica nanoparticles essentially diminished cell sedimentation and enhanced cell viability during storage.

Recently, a new procedure for encapsulation of virus formulations based on the application of supercritical fluid properties has been proposed. Same idea could also be applied to prepare bacterial inocula. The process, named PGSS (Particles from Gas Saturated Solutions), is carried out at low temperatures and uses carbon dioxide as a supercritical fluid. Main advantages of proposed technic would be lack of negative effects on the microorganisms' viability, and the low cost of production. The final product of the process is almost spherical particles that form a free-flowing powder which can be suspended in water. The possibilities of the PGSS process have already successfully been demonstrated for several solids and liquids.

Another interesting innovation is the exploitation of the natural production of bacterial biofilms as a possible carrier. It could be applied not only for the production of the bacterial inoculum but also for fungi-bacteria consortia. Biofilms are already obtained for different industrial applications (e.g., wastewater treatment, production of chemical compounds). Two types of biofilms are considered: biofilms growing onto inert supports (charcoal, resin, concrete, clay brick, and sand particles) and biofilms that are formed as a result of aggregate formation. In the first case, microorganisms grow all around the particles, and the size of the biofilm grows with time usually to several mm in diameter. Biofilms formed by aggregation is called granular biofilms and their formation may take from several weeks to several months.

There are four phases in the development of a mature biofilm: i) initial attachment, ii) irreversible attachment, iii) early development, and iv) maturation. Particularly critical is the irreversible attachment when cells bind to the surface and extracellular polymeric substances (EPS) are generated. Thus, microorganisms are protected from the surrounding environment. EPS generally are composed form polysaccharides, proteins, nucleic acids, or phospholipids. A typical EPS excreted by bacterial cells in biofilms is the exopolysaccharide alginate (Fig. 4 and 5).

The rate of biofilms formation and maturation is affected by surface, cellular, and environmental factors. Rough surfaces, porous, and less hydrophobic materials tend to improve the biofilm formation. Biofilms tend to form more readily in the presence of optimum nutrients availability, particularly of phosphorous which increases the adhesion ability of cells. Other factors positively influencing the biofilm formation are high temperature, EPS production, and surface adhesion. Biofilm reactors can be assembled in a number of configurations including batch, continuous stirred tank, packed bed, trickling bed, fluidized bed, airlift reactors, up flow anaerobic sludge blanket, and expanded bed reactors.

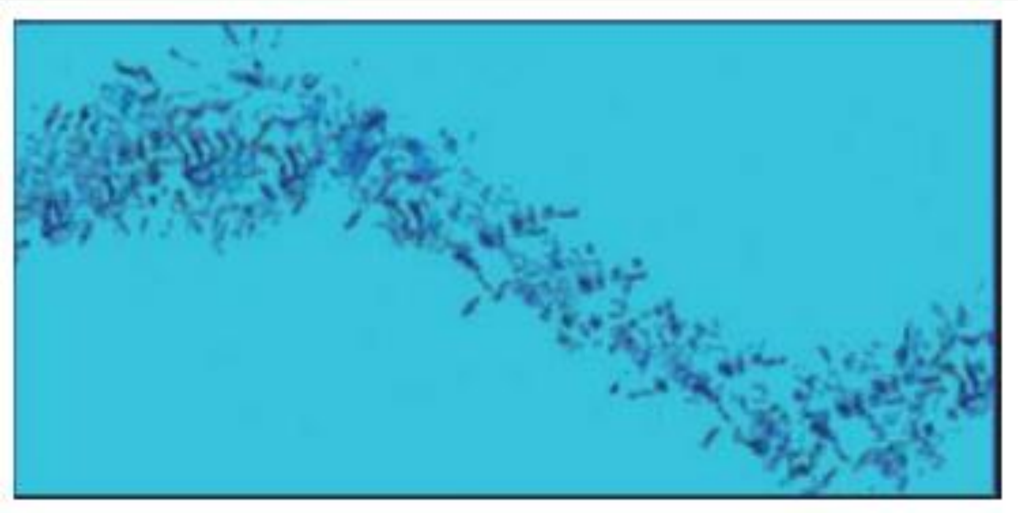


Fig. 4. Fungal –bacterial biofilm (FBB)

Fig. 5. A fungal–rhizobial biofilm (FRB) on a wheat root.

Recently, with good practical efficacy for nonlegume species biofilms were used that were developed in in vitro cultures containing both fungal and bacterial strains. Application of this biofilmed fungalrhizobia consortium led to significantly increased N2 fixation in soybean compared to a traditional rhizobium inoculant. Wheat seedlings inoculated with biofilm-producing bacteria exhibited an increased yield in moderate saline soils. Moreover, experimental data showed that biofilms protect microorganisms and assure their survival even under stress conditions. The last issue is from key importance for the effectiveness of PGPM inoculation under agricultural conditions. It was reported that biofilmed inocula allow rhizobia strains to survive at high salinity (400mM NaCl) by 105-fold compared to rhizobial monocultures. Interestingly, it was observed that beneficial endophytes in biofilms produce higher acidity and plant growth-promoting hormones than their mono- or mixed cultures.

Another new frontier in the development of carriers for PGPMs is production of hybrid materials for inoculating microorganisms. Silica has appeared as a promising host for encapsulation: technic is based on dispersing of bacterial population into a silica gel and its immobilization. Cell can be either entrapped into alginate microbeads coated with silica membranes or into macrocavities created inside the silica matrix. Such hybrid material improves the mechanical properties of the alginate bead, reduces cell leakage, and enhances cell viability.

The application of bionanotechnologies could also provide new directions in the development of carrier-based microbial inocula. Nanoparticles made of inorganic or organic materials are employed in dimensions 100 nm and less. The integration of whole cells within hybrid nanostructures have numerous applications in many fields including agriculture. Already macroscopic filters, made of radially aligned carbon nanotube walls, able to absorb Escherichia coli, were fabricated. This technology was applied to collect bacterial cells from fermentation processes and deliver them to the plant. The physical stability and the high surface area of nanotubes, together with the ease and cost-effective fabrication of these membranes, may also expand in the production of biofertilizer.

The use of nanoformulations may improve the stability of biofertilizers and biostimulators with respect to desiccation, heat, and UV inactivation. The addition of hydrophobic silica nanoparticles of 7–14 nm to the water-in-oil emulsion formulation of the biopesticide fungus *Lagenidium giganteum* reduces the desiccation of the mycelium. The physical features of the formulation are improved and the microorganism are still viable and active after 12 weeks of storage at room temperature.

STICKERS

Often in peat sticking agents are incorporated thus enhancing its uniformity of coverage on seed. The adhesives used in current agricultural practices are different polymers: polysaccharides (such as gum arabic or carboxymethylcellulose), polyalcohol derivatives, or caseinate salts. Important prerequisites are:

- nontoxic to seed or microorganisms,
- easily dispersible in water
- offering a better adhesion and survival to microorganisms on seed.

They have been for the most part for their ability to maintain the viability of rhizobia on the legume seed. However, little is known about the exact mechanisms responsible for the assurance of the enhanced survival by these polymers. The significant disadvantage is that, when applied with stickers, more peat is retained on the seed coat, resulting in a more extended time of contact between the bacteria and the toxic compounds of the coat.

ADDITIVES

Other materials added to the inoculant formulation include macro- and micronutrients, carbon or mineral sources, hormones, and even fungicides. The aim is to supply microorganisms with protective and/or a nutrient source, to assure better adhesion to seed thus improving the inoculant quality, to make the product more stable, to inactivate the toxins, or to enhance the strain(s) survival during storage and after exposure to environmental stress conditions (high temperature, desiccation). There is a critical interrelation between the strains survival rate and used additives. Some of them (such as glycerol) improve cell viability by protecting cells from desiccation through holding considerable amounts of water. Thus, the drying rate is significantly reduced. Each additive should be selected for individual strain in order to provide maximal performance. Moreover, their chemical nature should be complex to prevent them from rapid degradation. Several components have been tested, such as clay and skim milk, xanthan, or sodium alginate with variable results on strain(s) survival during storage and field application. Furthermore, certain signaling molecules added in the growth media and inoculants have been shown to provoke desired physiological activities of used microorganisms. Recently, it was reported that some *rhizobial* metabolites enhance the performance of *Bradyrhizobium spp*. and *Azospirillum brasilense* inoculants when soybean and maize are treated. These metabolites include mainly lipochitooligosaccharides (LCOs also called Nod factors) but also exopolysaccharides and plant hormones. Nod factors were shown to be produced by most rhizobia and are mandatory for the root legume infection and nodule formation. To our knowledge, the use of signaling molecules for improving the crop performance is still limited. However, several legume inoculants containing stimulators of nodulation (flavonoids or Nod factors) are commercially available in North and South America. Stimulators of the mycorrhizal symbiosis have also been identified. Strigolactones are of fundamental and practical interest as they are supposed to play a key role in the establishment of the mycorrhizal symbiosis. It was reported that they act as hormones in plants, and they may also have a role in the presymbiotic growth of AMF. Application to crops could result in beneficial effects on plant development. However, more investigations are needed to assess the potential of these stimulators for the development of a new generation of mycorrhizal inoculants.

PACKAGING

Packaging material is another important issue to be consider when biofertilizer is produced as it can affect inoculant quality. It must allow some exchange of oxygen but restrict the passage of water. Particular care must be taken when choosing a material for a product that is supposed to be sterilized. Some materials are suitable for autoclaving but might break during irradiation and vice versa.

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QUALITY CONTROL OF BIOFERTILIZERS

Contents

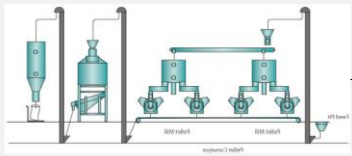
GENERAL CONCEPT OF QUALITY CONTROL

Quality assessment of inoculants has been a matter of interest for years. While examining peat-based rhizobial inoculants for moisture, viable counts, contaminants, and effectiveness using plate counts and MPN it was found that rhizobial counts were variable but that contaminants were present in most of the inoculants, even exceeding the number of rhizobia and affecting inoculation effectiveness. Similar results were obtained with a wide range of inoculants produced and used in different parts of the world. It was stated that inoculants prepared with nonsterile peat contained 100-fold fewer rhizobia than those made with sterilized peat. In an information bulletin on production and quality control of legume inoculants, it was indicated that most of the products tested in India contained suboptimal level of rhizobia (<10^8 rhizobia/g of inoculant) together with a large quantity of nonrhizobial organisms. Other autors found that rhizobia counts were inversely related to contaminants level. After analysis of 40 rhizobial inoculants produced in North America it was reported a constantly high level of contaminants (10^8 to 10^{10} cells/g of product), outnumbering the rhizobia in all the products but one. In some products, rhizobia could not even be detected. These results were confirmed with another study with 60 more samples among which the majority of the products contained more contaminants than they did rhizobia. Similar tests were run on commercial soybean inoculants from Argentina and showed that out of 18 products, 17 were highly contaminated, with rhizobia being outnumbered by contaminants in 14 of them. More recent studies report comparably alarming results on rhizobial inoculants but also on products containing PSB or free N_2-fixing bacteria. Moreover, among the isolated contaminants, several strains were found to be opportunistic pathogens for human, plant, or insects. Evaluation of the quality of AMF inoculants showed that they generally contain a very low quantity of viable propagules and a reduced (or an absence of) host infection and colonization potentials, resulting in highly inconsistent performance under field conditions.

There are a number of factors influencing the quality and the efficacy of an inoculant during production and after inoculation into soil. The main are presented in Fig. 1.

QUALITY CONTROL OF BIOFERTILIZERS

Large-scale production



- Composition of media
- Growth conditions (pH, t^0C, O_2, ..)
- Purity
- Cost

Formulation

- Type of carrier
- Sterilization of the carrier
- Peat : water content
- Granules: speed of drying
- Stickers and other additives
- Level of contaminations

Storage and transport

- Water content (drying)
- Competition (if contaminated)
- Temperature (4^0C)
- Time (< 12 months)

Inoculation in the field

- Concentration of inoculum (the more the better)
- Mode of application:
  - Seed coating
  - Seed inoculation
  - Soil inoculation
- Competition with better adapted populations

Fig. 1. Main factors affecting the quality of the inoculants from production to inoculation

In this respect, many technical difficulties related to a large-scale production of inoculant must be overwhelmed. For example, media and growth conditions (temperature, pH, time) for bacteria must be optimal in order to ensure that the cells are in good physiological conditions. For AMF, hosts might be chosen on the basis of strain–host specificity providing possibilities of AMF strain(s) to multiply. The type of cultivation and the corresponding required space are the major disadvantages for large-scale production of AMF. In all cases, the provision of competent and well-trained operators is of critical importance, thus assuring implementation of the right methodologies. Other important factors are minimization of the production cost and maintenance of the pure microbial culture throughout the process. In this way, better quality of the product is ensured.

Other important step in quality provision of biofertilizers is the formulation. New carriers are needed to overcome the limitations of peat (availability, environmental impact, toxicity) and provide a more suitable environment for the microorganisms. They should maintain microbial viability and fitness during storage, as well as on seed and in soil after inoculation.

One of the critical stages in biofertilizers production is the inoculation of the carrier. It has been broadly perceived that the utilization of a sterile carrier offers a few favourable fetures over nonsterile ones. These are higher populations of the target strain(s) and a longer shelf life. Moreover, contaminating microorganisms are generally able to grow faster than the target ones (especially in the case of rhizobia), thus easily replacing them in a short period of time. They compete for space and nutrients and may also produce toxic compounds reducing the growth of other cells, or be pathogenic for plants, humans, or environment. Sterility is generally obtained by using a steam (autoclaving) or gamma irradiation. The last one is considered as slightly better to steam sterilization but is more expensive and slow, requiring specific costly and not easily available equipment. Other technologies such as electron acceleration have also been developed, but they are economically unjustified as well.

Other major aspect important for quality assurance is the maintenance of cell viability during transport and storage. It is affected by many factors. The moisture is of primary importance for peat-based products and generally reaches 45 to 60 % on a wet weight basis. For the granular inoculants, the speed of drying was shown to be of great importance. Slow drying affects less severely the cells than fast drying. Addition of substances providing higher desiccation tolerance (such as osmoprotectants) could permit the production of biofertilizers more resistant to severe storage conditions. Low temperatures (4 °C) are generally recommended as the best storage conditions. However, it was shown that temperatures during both storage and transport can be above 26 °C and sometimes even 40 °C. These conditions are detrimental for rhizobial strains. It is very important to note that effects of water content, temperature, and time are not mutually exclusive. Several studies have reported that over time microbial populations in inoculants decline, leading to a lower inoculation efficiency and increased contaminant strains. This is especially true for products that have not been stored under optimal conditions. Generally, inoculant expiry date is about 12 months after production, but some products are likely to be older when used.

Another detected problem is that most of the literature reports evaluating the quality of biofertilizers (or strain selection) are made under controlled conditions but not under field ones. Available studies generally reported variable performances (even of very promising products under controlled conditions) due to interactions between the target plant, microorganisms, soil and environmental conditions. Other factors such as the mode of application (seed coating, on-site seed application, or soil inoculation) may also affect inoculation efficiency depending on the kind of crop (size and fragility of the seeds) and anterior seed treatments. The type and the density of the native populations in the soil can be major barriers for successful inoculation. This is due to the fact that recently introduced cells must not only survive in the new potentially harmful conditions, but compete for protective niche and nutrients, dominating over the indigenous, better-adapted populations. In this aspect, the success of the inoculation is related to the persistence of the introduced strain, i.e., its ability to establish high population levels despite of the unfriendly environment and to live as a continuing member of the soil microflora even in the absence of its host plant.

A better understanding of these complex interactions is highly required since it significantly influences the effectiveness of the inoculants and their perseverance in soil. Up to now, the variability and the unpredictability of the results from crop to crop, place to place, and from season to season have restricted a wider use of inoculants.

Successful commercialization of new inoculants principally depends on the on the cooperation between the research (to formulate the best inoculant, using the right strain for the right crop in the right conditions), the private sector (to scale up the production, establish an economically viable and sustainable market chain), and the acceptance by farmers. The need for farmers' education is great. If the end users are convinced of the efficacy of the biofertilizers on their crops, they will be more willing to buy and use them instead of expensive and harmful chemical fertilizers. To accomplish that, the improvement of the biofertilizer quality is a critical issue. Demonstration trials with high-quality products and regular training of the farmers for the use of inoculants would lead to a greater confidence from the farmers and a significant increase in the use of biofertilizers.

QUALITY CONTROL PARAMETERS

Microbial Functions

Numerous soil bacteria which reside in the plant rhizosphere and which may grow in, on, or around plant tissues, stimulate plant growth. These bacteria are known as plant growth promoting rhizobacteria (PGPR).

Some of PGPR can promote growth by acting as both biofertilizer and biopesticides.

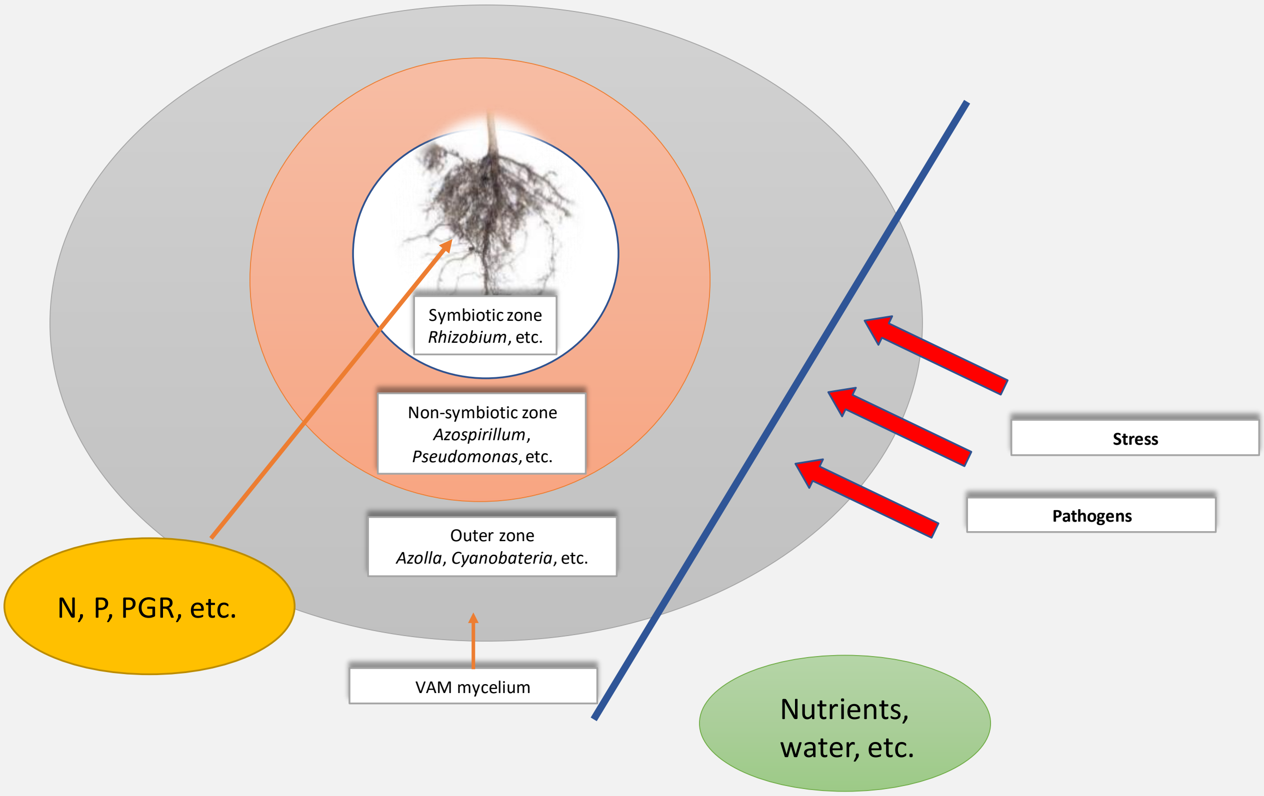


Fig. 2: Integrated microbial actions in soils.

The screening for PGPR and investigation of their activities are expanding at a fast pace as endeavors are made to exploit them commercially as biofertilizers.

The most valuable activities of PGPR include fixing N_2, increasing the availability of nutrients in the rhizosphere, positively influencing root growth and morphology, and promoting another beneficial plant-microbe symbiosis. The blend of these modes of actions in PGPR is also addressed, as well as the difficulties facing the broader usage of PGPR as biofertilizers.

Two types of materials are used in agriculture, fertilizer or pesticide. It can be assumed that fertilizer is required for nourishment, and pesticide for medication of plants in conventional agriculture. On the other hand, biofertilizer and/or biopesticide represent respectively both materials in sustainable or environmentally friendly system (Fig. 2).

The main sources for biofertilizer are nitrogen fixing bacteria, phosphate solubilizer, and mycorrhizae. Similar to the functional foods, like restoratives and/or adjuvant, who are required for human health care; plant growth promoting rhizobacteria may be one of the compatible substances for better crops yield.

However, several limitations exist in the use of biofertilizer for agricultural system. Primarily, the efficacy for most biofertilizer is not reliable. This is due on the scarce data available about the mechanism of action of different biofertilizer in promoting plant growth. However, research into biofertilizer is increasing, trying to manage these issues.

Moreover, different parameters should be also assessed, such as: soil type, managements practices, and weather effect on biofertilizer efficacy. Furthermore, there is a block in biofertilizer development. It is difficult to test inoculant in field as routine experiments, as shown in Figure 3.

Isolation of microbes from roots or soils

Laboratory screening of microbes for plant growth

Greenhouse screening of microbes to promote growth in potted soil

Field screening of most effective microbes in cropped soil (Crop variety and different soil types examined)

Refinement of inoculum

Environmental impact test and substantiation of microbes

Production

Fig. 3: Experimental process for biofertilizer testing.

QUALITY CONTROL OF BIOFERTILIZERS

Properties of Microbial Products

The microorganisms used for development of biofertilizers are bacteria of genera *Bacillus*, *Pseudomonas*, *Lactobacillus*, photosynthetic bacteria, nitrogen fixing bacteria, fungi of *Trichoderma* and yeast. Among the microbes, the most employed microorganism is Gram (+) endospore-forming bacteria from genus *Bacillus*. Usually, several species of microbes are used in microbial products with an available period of by- products of about 1~2 and/or 2~3 years.

Biofertilizers can be solid or liquid. Carriers used in solid type biofertilizers are generally clay mineral, diatomaceous soil, and white carbon as mineral. Other materials used are rice, wheat bran, and discarded feed as organic matter. However, the effects of carriers and/or supplements on microbial growth are of great importance and should be seriously consider in the control of microbial products. In fact, often farmers misunderstood this carrier effect as microbial action.

As displayed by producers, microbial products stimulate plant growth, decrease pest occurrence, stimulate composting and ameliorate the soil. However, the main effect generally is the plant growth stimulation. Nevertheless, in 40 % of the commercial biofertilizers manufacturers declare presence of multiple effects.

In this respect controlling the quality of biofertilizer is one of the most important factors. Thus their success or failure and acceptance or rejection by end-user, the farmers will be assured. Principally, quality represents the number of selected microorganism in the active form per gram or milliliter biofertilizer. Up to now quality standards are developed only for *Rhizobium*. Moreover, specifications of biofertilizer differ from country to country and maybe comprise parameters like: microbial density at the time of manufacture, microbial density at the time of expiry, the expiry period, the permissible contamination, the pH, the moisture, the microbial strain, and the carrier. Quality has to be monitored at different production stages (during pre-culture stage, carrier selection and preparing, broth formulation, mixing of broth and culture, packaging and storage). Main quality parameters to be respected during biofertilizer production are summarized in Table 1.

QUALITY CONTROL OF BIOFERTILIZERS

Table 1: Key quality parameters of biofertilizers

Forms	Liquid	Powder	Granular
Appearance of living target bacteria	**Without strange smell**	**Brown or black**	**Brown**
Fast-growing Rhizobium	>0.5×10^9/ml	>0.1×10^9/g	>0.1×10^9/g
Slow-growing Rhizobium	>1.0×10^9/ml	>0.2×10^9/g	>0.1×10^9/g
N fixation bacteria	>0.5×10^9/ml	>0.1×10^9/g	>0.1×10^9/g
Si bacteria **P bacteria**	>1.0×10^9/ml	>0.2×10^9/g	>0.1×10^9/g
Organic P	>0.5×10^9/ml	>0.1×10^9/g	>0.1×10^9/g
Inorganic P	>1.5×10^9/ml	>0.3×10^9/g	>0.2×10^9/g
Multi-strain biofertilizer	>1.0×10^9/ml	>0.2×10^9/g	>0.1×10^9/g
pH	5.5-7.0	6.0-7.5	6.0-7.5
Water content (%)		20-35	10
Non-target bacteria Contamination (%)	<5	<15	<20

QUALITY MANAGEMENT

Quality management is very important process, and must be performed repeatedly to monitor the microbial products in favor of the customers.

The current guidelines used for evaluating quality of biofertilizers are restricted to controlling the: density of the available microorganisms, their viability and preservation. However, it is also important to set control points that do not contain available microorganisms, but are focused on the consistency of the other compositions in the final microbial products. Also, it is highly desirable that the biofertilizer demonstrates the major effects for quality management of the final biofertilizer products. It is a crucial requirement to discriminate between the role of the available microorganisms and the supplementary compositions on the effects of the biofertilizer guaranteed by the suppliers. If the final results of the two experimental schemes (microorganisms / supplements) are the same or cannot be confirmed statistically, then the product is only an organic matter. This means that the effects of microbial products should resulted from the activity of the guaranteed microorganisms, and the target of the substances should be presented in detail as a prescription. It is important to assess precisely the functions under the given usage manifested by the end-user (Fig. 4).

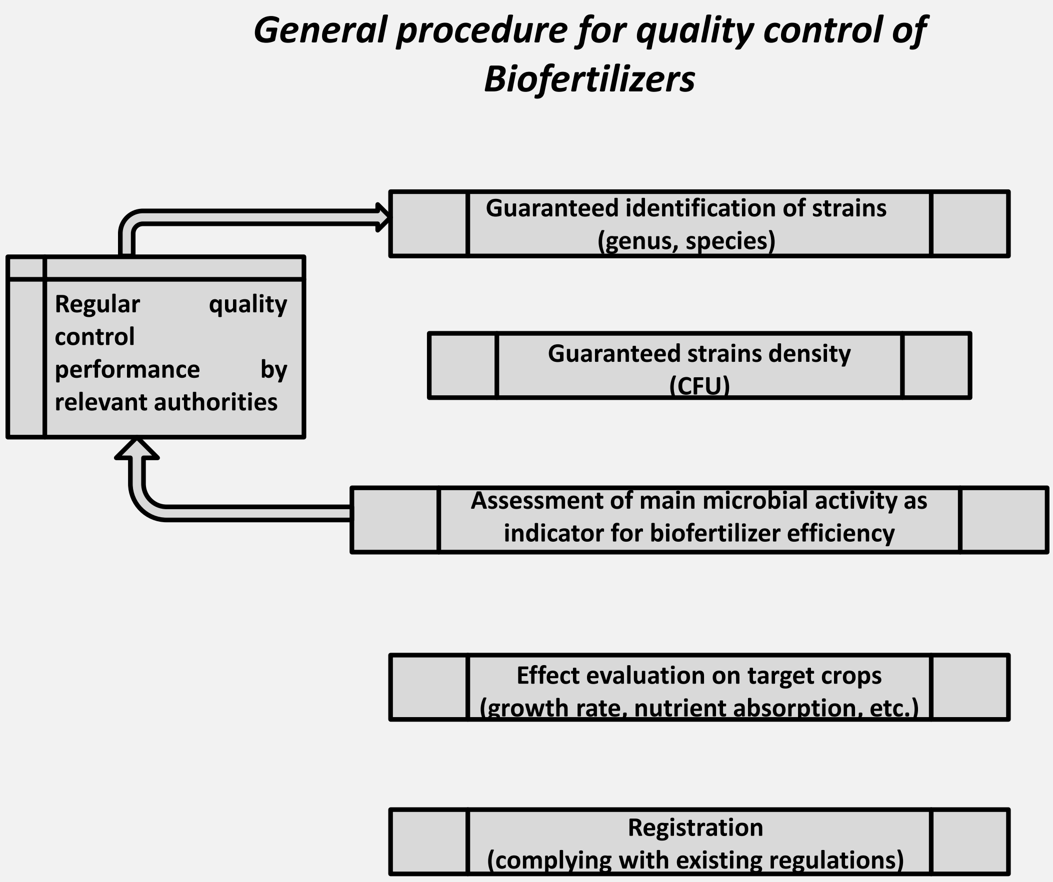


Fig. 4: Procedure of biofertilizer quality control.

Biofertilizers, being microbial products, supply soil with nutrients, diminish the agricultural burden and conserve the environment. Good soil condition is imperative to improve crop yields, as well as to assure human and/or animal health welfare. That's why, the materials, as biofertilizers, used to sustain good soil condition, are treated as environmental matters. However, as mentioned earlier, there are still some problems to be met on the use of microbial products.

QUALITY CONTROL OF BIOFERTILIZERS

More accurate quality control must be performed in favor of the customers. With this in mind, the need to develop better production techniques and to improve the management system for microbial products is defined.

Although the effects of biofertilizers vary in different geographical regions due to the peculiarities in climate and soil conditions, the importance of biofertilizer on environmental preservation in the 21st century must not be ignored. In the same time, development of various biotechnological approaches should be considered in order to increase the biofertilizer effects with concern for the environment.

PROCEDURES FOR QUALITY CONTROL OF BIOFERTILIZER

Rhizobium

Quality checks on *Rhizobium* biofertilizer can be divided into three parts:

- Pre - culture test
- Broth test
- Peat test

Before producing *Rhizobium* biofertilizer, the pre-culture should be checked on the following parameters:

- Growth
- Purity
- Gram stain
- Broth composition test
- pH

Cell morphology

Rhizobial are stained for observation of shape and size of the cells. Cells of rhizobia are rod-shaped, with one or two cells sticking together. Microscopical check for contaminates is performed.

Viable count

The number of living cells is counted by spread plate or drop plate methods in YMA + CR medium. Plates are incubated in incubator (28 - 30^{0} C) or at room temperature for 7 days.

QUALITY CONTROL OF BIOFERTILIZERS

Peat test

For the peat inoculant, the following quality parameters are checked:

- pH
- Moisture content
- Viable number
- Plant infection method (MPN)
- pH

The optimal pH for the inoculant is the neutral. Since peat is acidic the pH has to be adjusted with $CaCO_3$. The optimum moisture content of peat-inoculant is between 40 - 50 %. At low moisture rhizobia will die rapidly. If moisture is high, inoculant may stick to the plastic bag and, thus to compromise the rhizobial growth.

Plant Infection Analysis using Most Probable Number Method (MPN)

This is an indirect method of assessing plant infection on nodulation. It is widely used when peat is not sterile. It takes more time than spread plate method as to grow plants is required. This method is based on the assumptions that: if a viable rhizobia is inoculated on its specific host, nodules will develop on that roots. Nodulation on that inoculated plant is a proof of the presence of infective rhizobia.

Non-symbiotic N_2-fixer

In research aspect, microbial growth may be represented by the augmentation in cell mass, cell number or any cell constituent. Growth of the organism could be also assessed by the utilization of nutrients or accumulation of metabolic products. Growth, therefore, can be determined by various methods based on one of the following assays: (a) cell count, directly by microscopy or by an electronic particle counter, or indirectly by colony count, (b) cell mass, directly by weighing or measurement of cell nitrogen, or indirectly by turbidity; and (c) cell activity, indirectly by relating the degree of biochemical activity to the size of the population.

The growth rate of *Azospirillum* is expected to have reached its maximum at 3-5 days after inoculation. The recommended counting technique in this case uses the drop-plate method. Proper aseptic procedures should be observed, otherwise contaminants may be accidentally introduced during the injection of the broth culture and during serial dilution and plating. These contaminants are also detectable on the utilized indicator media and their number should be reported together with the number of viable cells as additional measure of the quality.

QUALITY CONTROL OF BIOFERTILIZERS

Mycorrhiza - the arbuscular mycorrhizal fungi, AMF

Quality control in the formulation of AMF inoculum is essential for product uniformity, reliability and reproducibility. This is applied to the laboratory, preparation room, growth room, storage room and the greenhouses, taking care into the design, to achieve the most efficient control in inoculum production.

Laboratory quality control

Laboratory quality control is applied in respect to the production of bacterial spores. They are extracted from monospecific spore cultures in the preparation room. The spores are transported in petri dishes to the laboratory and placed in a refrigerator before examination under stereoscopic microscopes. Spores from each petri dish is described and records are prepared.

Preparation room quality control

This room has to be isolated from the greenhouse and growth room, and unsterilized soil or media samples should not be stored in it. Materials (cultures; sterilized growth media) are clearly labeledand placed in specific containers. Floor should always be clean without presence of dust. All surfaces should be clean and disinfected. Containers are surface-sterilized with 10% sodium hypochlorite.

Growth room quality control

The growth room should be temperature controlled (22 °C). Air is exhausted to the outside and no recycling is applied. Surfaces should be painted with anti-microbial paint and sterilized periodically e.g. monthly. All samples are checked for contaminants and pathogens. Watering is done manually, avoiding cross-contamination.

Storage room quality control

All samples stored are placed in plastic bags, with proper labelling, and surface of bags should be cleaned before usage. Floors and surfaces are clened regularly, preventing generation of dust.

Phosphate Solubilizers

Phosphate solubilizers (PS) contain phosphate solubilizing bacteria or fungi. Commercially produced PS biofertilizers (PSB) are certified in respect to the guaranteed components such as type of strains, microbial density, and biological activity. If possible the rate of phosphorus absorption of target crops is also determined. The procedure shown in Fig. 5 could be used for the quality control of PSB (Fig. 5).

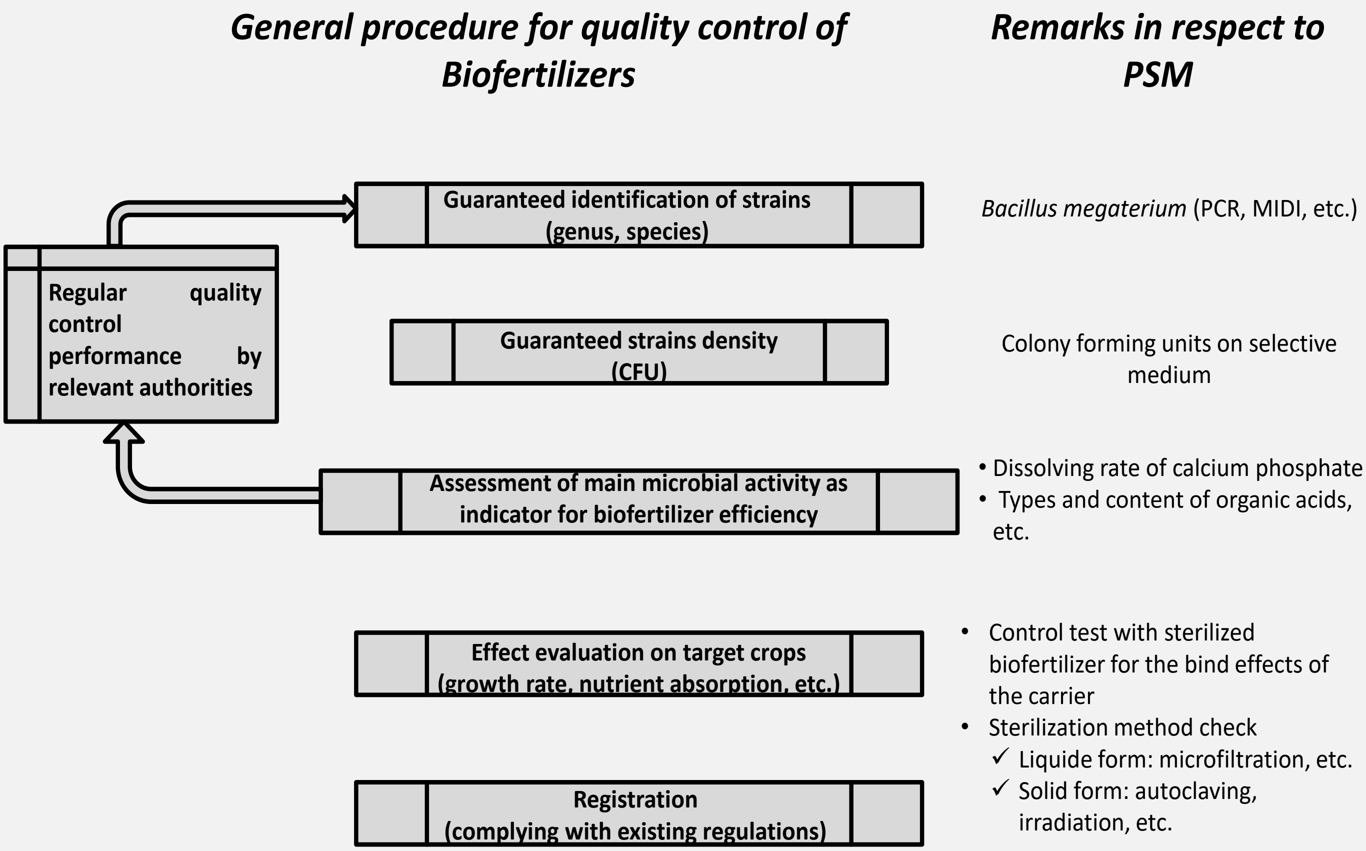


Fig. 5: General procedure for quality control of PSB.

Phosphate solubilizing microorganisms play an important role in plant nutrition through increasing the available phosphate for plant. Accordingly, great attention should be paid to investigations and formulation of new combinations of phosphate solubilizing bacteria and other plant growth promoting rhizomicrobes for improved crop yields.

QUALITY STANDARDS FOR *RHIZOBIUM* AND *AZOTOBACTER*

Several quality standards have been formulated for *Rhizobium* and *Azotobacter* inoculants. These specifications are shown in Table 2.

Table 2: general standards specified for *Rhizobium* and *Azotobacter* biofertilizers

Parameters	*Rhizobium* Biofertilizer	*Azotobacter* Biofertilizer
Cell no. at the time of manufacture	10^8/g carrier within 15 days of manufacture	10^7/g carrier within 15 days of manufacture
Cell no. at the time of expiry date	10^7/g carrier within 15 days before expiry date	10^6/g carrier within 15 days before expiry date
Expiry date	6 months from the date of manufacture	6 months from the date of manufacture
Permissible contamination level	No contamination at 10^8 dilution	No contamination at 10^7 dilution
pH	6.0–7.5	6.5–7.5
Strain	Should be checked serologically	Nothing specific. But *A. chroococcum* species is mentioned
Carrier	Should pass through 150–212 microns IS sieve	Should pass through 160 microns IS sieve
Nodulation test	Should be positive	—
Nitrogen fixation	Above 20 mg/g of glucose	Not less than 10 mg/g of sucrose

The variability in quality standards specified for *Rhizobium* in various countries are as follows (Table 3).

Table 3: Quality standards of commercial Rhizobium culture in different countries

Country	Cells/gm of culture (total viable count on Congo-red agar)		
	Very satisfactory	**Satisfactory**	**Doubtful**
U. S. A.	10^9	–	$10^6 – 10^7$
Australia	–	2×10^8	$10^6 – 10^7$
Russia	10^9	–	–
India	More than 10^9	10^7–10^9	Less than 10^7

QUALITY CONTROL OF BIOFERTILIZERS

Although quality control standards for biofertilizer *Azospirillum* and PSM has not been in force, the proposed standard specification of PSM and *Azospirillum* are given in Table 4.

Table 4: Proposed standard specifications of PSM and *Azospirillum*

No.	Parameter	PSM	*Azospirillum*
1.	Base	Carrier (Lignite/ Charcoal)	Carrier (Lignite/ Charcoal)
2.	Carrier	>100 micron	>100 micron
3.	pH	6.5–7.5	7.0–8.0
4.	Moisture	35–40%	35–40%
5.	Viable count at manufacture	10^7/g carrier	10^7/g carrier
6.	Viable count at expiry	10^7/g carrier	10^7/g carrier
7.	Level of contaminant	No at 10^4 dilution	No at 10^4 dilution
8.	Growth in Pikovskaya medium	+ve	–
9.	Growth in S. S. Malate medium	–	+ve
10.	P Solubilization zone	1mm	–
11.	Pellicle formation	–	+ve
12.	Shelf life	6 months	6 months
13.	P Solubilization	30–50%	–
14.	N-fixation	–	15 mg/g of malic acid

QUALITY CONTROL MEASURES

The biofertilizer should be evaluated for the following quality standards:

1. Inoculant should be carrier based or liquid based.
2. The inoculant should contain minimum of 10^8 viable cells of bioinoculant/g of carrier on dry weight basis when it is stored at 25–30°C.

3. The inoculant should have at least 6 months shelf life from the manufacturing date in case of carrier based and 9 months in case of liquid based.
4. The pH of inoculant should be between 6.0 and 7.5.
5. Inoculant should show effective nodulation/nitrogen fixed on particular crop before expiry date.
6. The carrier material should be in the form of powder, i.e. peat, lignite, peat soil, and humus, etc.
7. Inoculant should be packed in 50–75 microns low-density polythene bags.
8. Each package should be marked legibly to give the information about name of the product, name of microbial inoculants, activity of bioinoculant, intended crop, name and address of manufacturer, type of carriers, batch and code numbers, date of manufacture, date of expiry, net quantity meant for 0.4 hectare, and storage instructions.
9. It should be free from any contaminant/contamination with other microorganisms.

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APPLICATION OF BIOFERTILIZERS

Contents

Biofertilizers can be inoculated on seeds as well as in the roots of different crop plants under ideal conditions. They can also be applied directly to the soil. There are certain approaches to the application of biofertilizers as described below:

METHODS OF APPLICATION

Seed inoculation OR seed treatment

This is the most common practice of applying biofertilizers. In this method, the biofertilizers are mixed with 10% solution of jaggary. The slurry is then poured over the seeds spread on a cemented floor and mixed properly in a way that a thin layer is formed around the seeds. The treated seeds should be dried in the shade overnight and then they should be used. Generally, 750 grams of biofertilizer is required to treat the legume seeds for a one-hectare area.

Seedling root dip

The seedling roots of transplanted crops are treated for half an hour in a solution of biofertilizers before transplantation in the field. In this method, the seedlings required for one acre are inoculated using 2–2.5 kg biofertilizers. For this, a bucket having adequate quantity of water is taken and the biofertilizer is mixed properly. The roots of the seedlings are then dipped in this mixture so as to enable the roots to get inoculum. These seedlings are then transplanted. This method has been found very much suitable for crops like tomato, rice, onion, cole crops and flowers.

Main field application

This method is mostly used for fruit crops, sugarcane and other crops where localized application is needed. At the time of planting of fruit trees, 20 g of biofertilizer mixed with compost is to be added in the ring of one sapling. The same quantity of biofertilizer may be added in the ring soil of the seedling after it has attained maturity. Sometimes, biofertilizers are also introduced in the soil but this may require four to ten times more biofertilizers. Before use, the inoculants should be incubated with the desired amount of well decomposed granulated farmyard manure (FYM) for 24 hours. The FYM acts as nutrition medium and adjuvant (carrier) for biofertilizers.

Self-inoculation or tuber inoculation

This method is exclusively suitable for application of *Azotobacter*. In this method, 50 liters of water is taken in a drum and 4–5 kg of *Azotobacter* biofertilizer is added and mixed properly. Planting materials required for one acre of land are dipped in this mixture. Similarly, if we are treating potato, then the tubers are dipped in the mixture and planting is done after drying the materials in the shade.

LIQUID BIOFERTLIZER APPLICATION

Seed Treatment

Seed treatment is the most common method adopted for all types of inoculants. The seed treatment is effective and economic. For small quantities of seeds (up to 5 kg), the coating can done in a plastic bag. For this purpose, a plastic bag sized 21” x 10” or larger can be used. The bag should be filled with 2 kg or more of seeds. The bag should be closed in such a way so as to trap the air as much as possible. The bag should be squeezed for 2 minutes or more until all the seeds are uniformly wetted. Then the bag is opened, inflated again and shaken gently. The shaking can stop after each seed gets a uniform layer of culture coating. The bag is opened and the seeds are dried in the shade for 20–30 minutes. For large amounts of seeds, the coating can be done in a bucket and the inoculant can be mixed directly by hand. Seed treatment with *Rhizobium*, *Azotobacter*, *Azospirillum*, along with PSM can be done.

The seed treatment can be done with any of two or more bacteria. There is no side (antagonistic) effect. The important things that have to be kept in mind are that the seeds must be first coated with *Rhizobium*, *Azotobacter* or *Azospirillum*. When each seed gets a layer of these bacteria, then the PSM inoculant has to be coated as an outer layer. This method will provide a maximum number of all bacteria required for better results. Treatments of seeds with any two bacteria will not provide a maximum number of bacteria on individual seeds.

Root dipping

This method is used for application of *Azospirillum*/ /PSM on paddy transplanting/ vegetable crops. The required quantity of *Azospirillum/* /PSM has to be mixed with 5–10 liters of water at one corner of the field and the roots of seedlings have to be dipped for a minimum of half-an-hour before transplantation.

Soil application

Use 200ml of PSM per acre. Mix PSM with 400 to 600 kgs of cow dung FYM along with ½ bag of rock phosphate if available. The mixture of PSM, cow dung and rock phosphate has to be kept under any tree or in the shade overnight and 50% moisture should be maintained. The mixture is used for soil application in rows or during leveling of soil.

Some recommended liquid biofertilizers and their method of application and quantity to be used for different crops are as follows:

APPLICATION OF BIOFERTILIZERS

Crop	Recommended Biofertilizer	Application method	Quantity to be used
Field crops **Pulses** **Chickpea, pea, groundnut, soybean, beans, lentil, alfalfa, berseem clover, green gram, black gram, cowpea and pigeon pea**	*Rhizobium*	Seed treatment	200 ml/acre
Cereals **Wheat, oat, barley**	*Azotobacter/ Azospirillum*	Seed treatment	200 ml/acre
Rice	*Azospirillum*	Seed treatment	200 ml/acre
Oil seeds, mustard, sesame, linseeds, sunflower, castor	*Azotobacter*	Seed treatment	200 ml/acre
Millets **Pearl millet, finger millet, kodo millet**	*Azotobacter*	Seed treatment	200 ml/acre
Maize and sorghum	*Azospirillum*	Seed treatment	200 ml/acre
Forage crops and grasses **Bermuda grass, Sudan grass, Napier grass , paragrass, star grass etc.**	*Azotobacter*	Seed treatment	200 ml/acre
Other misc. plantation crops **Tobacco**	*Azotobacter*	Seedling treatment	500 ml/acre
Tea, coffee	*Azotobacter*	Soil treatment	400 ml/acre
Rubber, coconuts	*Azotobacter*	Soil treatment	2–3 ml/plant
Agro-forestry/fruit plants **All fruit/agro-forestry (herbs, shrubs, annuals and perennials) plants for fuel wood, fodder, fruits, gum, spice, leaves, flowers, nuts and seeds**	*Azotobacter*	Soil treatment	2–3 ml/plant at nursery
Leguminous plants/ trees	*Rhizobium*	Soil treatment	1–2 ml/plant

Note: Doses recommended when count of inoculum is 1 x 10^8 cells/ml; then doses will be ten times more. Besides the above-said nitrogen fixers, phosphate solubilizers and potash mobilizers at a rate of 200 ml/acre could be applied for all crops.

APPLICATION OF DIFFERENT TYPES OF BIOFERTILIZERS

Nitrogen biofertilizer application:

- *Rhizobium* for legume crops.
- *Azotobacter* and *Azospirillum* for non-legume crops.
- *Acetobacter* for sugarcane only.
- Blue-green algae (BGA) and *Azolla* for low-land paddy.
- *Frankia* for *Casuarina* and *Alnus*.

Rhizobium

Table 1. *Rhizobium* spp. suitable for different crops

Rhizobium sp.	Crops
R. leguminosarum	Pea (*Pisum*), *Lathyrus*, *Vicia*, lentil (*Lens*)
R. trifoli	Berseem clover (*Trifolium*)
R. phaseoli	Kidney bean (*Phaseolus*)
R. lupine	*Lupinus*, *Ornithopus*
R. japonicum	Soybean (*Glycine*)
R. meliloti	*Melilotus*, alfalfa (*Medicago*), fenugreek (*Trigonella*)
Rhizobium spp.	Cowpea, clusterbean, greengram, blackgram, redgram, groundnut, mothbean, dhaincha, sunn hemp, *Glyricidia*, *Acacia* etc.

Methods of application of Rhizobium inoculants:

Seed treatment has been found to be the suitable method of *Rhizobium* inoculation. Some adhesive is used to make proper contact between seeds and inoculants (bacteria). About 900 g soil base culture is sufficient to inoculate the seeds for an area of one hectare in case of legumes. A 10% jaggery (gur) solution is used as sticker for *Rhizobium* cells to seed. First, the solution is spread over the seeds and mixed to build up a thin coat over the seeds. After ascertaining the proper coating of slurry over the seeds, the inoculant is sprinkled over the seeds and the content is again mixed thoroughly. Then the content is dried in the shade by spreading thinly on a polythene sheet for at least overnight.

APPLICATION OF BIOFERTILIZERS

Azotobacter

Field experiments carried out on *Azotobacter* indicated that this is suitable when inoculated with seeds or seedlings of crop plants like onion, aubergine, tomato and cabbage under different agro-climatic conditions. *Azotobacter* inoculation curtails the requirement for nitrogenous fertilizers by 10 to 20% under normal field conditions.

Azospirillum

Azospirillum inoculation helps to improve the vegetative growth of the plants, cutting back on nitrogenous fertilizers by 25–30%. So far, only four species of *Azospirillum* have been identified. They are *A. lipoferum*, *A. brasilense*, *A. amazonense* and *A. iraquense*. In Indian soils, *A. brasilense* and *A. oferum* are very common.

Acetobacter

Under field conditions, the yield of sugarcane increases after *Acetobacter* inoculation. Productions of auxins and antibiotic type substances have also been observed after its application.

Blue-green algae

The blue-green algae inoculum is applied after transplantation of rice crops in the main field. The inoculum required is 10 kg/ha. For higher nitrogen fixation, 3 to 4 t/ha of farmyard manure and 200 kg/ha of superphosphate are applied.

Azolla

Azolla is applied to the main field as a green manure crop and as a dual crop. As a green manure crop, *Azolla* is allowed to grow on the flooded fields for 2 to 3 weeks before transplanting. Later, water is drained and *Azolla* is incorporated by ploughing in. As a dual crop, 1000 to 5000 kg/ha of *Azolla* is applied to the soil one week after transplanting. When a thick mat forms, it is incorporated by trampling. The leftover *Azolla* develops again and is trampled in as a second crop. For better growth of *Azolla*, 25 to 50 kg/ha of superphosphate is applied and standing water of 5 to 10 cm is maintained continuously in the rice fields.

Frankia

Frankia inoculation enhances the growth, nodulation, nitrogenase activity of nodules and nodule dry weight of *Casuarina* and *Alnus* plants.

Phosphorus biofertilizer application

Phosphobacteria are a type of biofertilizer. Phosphorus is a major nutrient for plants, inducing vigorous growth and also contributing to plant disease resistance. Phosphorus helps in root formation and plant growth. The plants utilize only 10–15% of the applied phosphate. The balance 85–90% remains in insoluble form in the soil. The bio-promoters have highly efficient phosphate-solubilizing bacteria (*Bacillus megaterium*) that grow and secrete organic acids, which dissolve this unavailable phosphate into soluble form and make it available to the plants. Thus, the residual phosphate fertilizers in the soil can be well utilized and the external application can be optimized.

The broth is prepared in flasks and inoculum from a mother culture is transferred to flasks. The culture is grown under shaking conditions at 30±2°C as submerged culture. The culture is incubated until maximum cell population of 1010 to 1011 cfu/ml is produced. Under optimum conditions, this population level could be attained within 4 to 5 days for *Rhizobium*; 5 to 7 days for *Azospirillum*; 2 to 3 days for phosphobacteria and 6–7 days for *Azotobacter*. The culture obtained in the flask is called starter culture. For large-scale production of inoculant, inoculum from starter culture is transferred to large flasks/seed tank fermenter and is grown until the required cell count is reached.

The recommended dosage of *Azospirillum* is adopted for phosphobacteria inoculation; for combined inoculation, both biofertilizers as per recommendations are to be mixed uniformly before use.

Inoculum preparation for phosphorus biofertilizer

Prepare appropriate medium specific to the bacterial inoculant in 250 ml, 500 ml, 3 liter and 5 liter conical flasks and sterilize. The media in 250 ml flasks are inoculated with an efficient bacterial strain under aseptic conditions. Keep the flasks at room temperature in a rotary shaker incubator (200 rpm) for 5–7 days. Observe the flasks for growth of the culture and estimate the population, which serves as the starter culture. Using the starter culture (at log phase) inoculate the larger flasks (500 ml, 3 liter and 5 liter) containing medium, after obtaining growth in each flask. The above medium is prepared in large quantities in a fermenter, sterilized well, cooled and kept ready.

The medium in the fermenter is inoculated with the log-phase culture grown in the 5-liter flask. Usually 1–2% inoculum is sufficient; however, inoculation is done up to 5%, depending on the growth of the culture in the larger flasks. The cells are grown in the fermenter by providing aeration (passing sterile air through a compressor and sterilizing agents like glass wool, cotton wool, acid etc.) and giving continuous stirring. The broth is checked for the population of the inoculated microorganism and contamination, if any, during the growth period. The cells are harvested with a population load of 10^9 cells ml^{-1} after the incubation period. There should not be any fungal or any other bacterial contamination at a 10^{-6} dilution level. It is not advisable to store the broth after fermentation for periods longer than 24 hours. Even at 4°C, the number of viable cells begin to decrease.

PSB can be used for all crops, including paddy, millets, oilseeds, pulses and vegetables.

APPLICATION OF BIOFERTILIZERS

The methods recommended for application are:

1. Seed treatment;

2. Seedling dipping;

3. Soil application.

In addition to these, combined use of bacterial biofertilizers can also be done. Bacterial inoculants should not be mixed with insecticide, fungicide, herbicide and fertilizers. Seed treatment with bacterial inoculant is to be done at last when seeds are treated with fungicides.

Compost application

The quality of compost depends principally on the feedstock and the right composting process. Compost is used in two ways in agricultural practice. One is to improve cultivated soil and the other is to manufacture substrates for growth of horticultural and floricultural plants. Adding mature compost in the soil has positive effects due to the increase in soil organic matter, which means an improvement of some physical and chemical characteristics such as porosity, air/water ratio, cation exchange capacity (CEC), pH, available amount of nutrient elements, etc.

Application of compost in gardens

Compost is used in the following cases:

1. As a soil enrichment material in ornamental plant nurseries and plant exteriors of hotels, instead of peat;
2. In filling new gardens, when mixed with the soil in a 1:3 ratio (compost: soil);
3. In new grass plants instead of turf, but must be free from weed seeds, otherwise they may cause problems;
4. In old degraded lawns due to intensive use by customers, the so-called "cap" applies, i.e. surface-spread sifted compost and then re-sown.

The compost is applied in the gardens of the hotels especially before the start of season, when new gardens are renewed or built. The quantities used annually in the gardens depend on the scope of work renovations performed.

Benefits from the use of compost are:

1. The soil is enriched with organic matter.

2. The structure and properties of soil are improved as considered of importance.

3. The nutrients are recycled from the plants by pruning back through the compost.

4. It is cheaper material than the humus trade.

However, there are some drawbacks:

1. The presence of weed seeds can carry weeds to clean regions and infect them.

2. The non-standardization of the compost in small sacks limits its use.

3. Lack of screening for the presence of large pieces of the raw materials used. So there are increased costs due to higher number of workers for their removal.

Nano-fertilizer inoculation

A few studies have suggested that nanoparticles delivered at a safe dose may help in promoting plant growth and overall yield. Multi-walled carbon nanotubes (MWCNTs) have been reported to have the ability to increase the seed germination and growth of tomato and to enhance the growth in tobacco cells and mustard plants.

On the basis of germination index and relative root elongation, oxidized MWCNTs have been shown to be more effective at lower concentrations than non-oxidized MWCNTs. Moreover, nano-silver performs better than silver nitrate in improving the seed yield and preventing leaf abscission in borage plants. The plant hormone ethylene plays a key role in leaf abscission, and silver ions have been shown to inhibit ethylene by replacing copper ions from the receptors.

Employing the foliar spray method, both nano-silver and silver nitrate were sprayed on different sets of plants, and it was observed that nano-silver was effective at a lower concentration than silver nitrate. The effect of biosynthesized silver nanoparticles on emergence of seedlings and various plant growth parameters of many economically important plant species were studied by Namasivayam and Chitrakala (2011). Mahajan et al. (2011) used the agar plate method to test the effect of nano-ZnO particles on the growth of *Vigna radiata* and *Cicer arietinum*. Evidence of nanoparticles adsorbed on the root surface was provided using correlative light and scanning electron microscopy. Inductively coupled plasma/atomic emission spectroscopy (ICP-AES) studies revealed the absorption of ZnO nanoparticles by seedlings. Using the foliar spray method, Burman et al. (2013) studied the effect of ZnO nanoparticles on the growth and antioxidant system of chickpea seedlings. They found that lower concentration (1.5 ppm) of ZnO nanoparticles has a positive effect on chickpea seedling growth.

Moreover, seedlings treated with ZnO nanoparticles showed improved biomass accumulation, which may be due to lower reactive oxygen species (ROS) levels as evident from lower malondialdehyde (MDA) content. Similarly, Prasad et al. (2012) observed that treatment with nano-zinc at lower concentration (1,000 ppm) had positive effects on plants, but caused toxicity symptoms at higher concentration (2,000 ppm) pointing out the importance of their meticulous use. Furthermore, during field experiments, they reported usage of a 15 times lower dose of ZnO nanoparticles compared to the recommended dose of $ZnSO_4$ and recorded 29.5% higher pod yield.

Likewise, ZnO nanoparticles showed root elongation in *Glycine max* at a concentration of 500 ppm but reduction in size at higher concentrations of ZnO. A study aimed to investigate the effects of ZnO and CeO_2 nanoparticles (400 ppm) on *Cucumis sativus* fruit quality showed that both these nanoparticles resulted in increased starch content and could alter the carbohydrate pattern.

Lu et al. (2002) showed the productive effect of a mixture of SiO_2 and TiO_2 nanoparticles in *G. max* with an increase in water and fertilizer uptake capacity and stimulation of nitrate reductase and antioxidant activity. Studies demonstrating the effect of nano-TiO_2 in promoting photosynthesis and growth in spinach have also been conducted, in which enhancement of the photosynthetic processes under both visible and ultraviolet light has been reported due to the pivotal role of TiO_2 (Leiet al. 2007). Zheng et al. (2005) reported that TiO_2 nanoparticles have 73% higher dry weight, threefold higher photosynthetic rate and a 45% increase in the chlorophyll *a* content after seed treatment in spinach.

As suggested, the enhanced photosynthetic rate may be due to the increase in the absorption of inorganic nutrients which enhance the utilization of organic substances and the quenching of oxygen free radicals. Unlike most of the studies showing negative impact of nanoparticles at higher concentrations, Mahmoodzadeh et al. (2013) reported that up to 2,000 ppm of TiO_2 nanoparticles leads to increased seed germination and seedling vigour in *Brassica napus*. Shah and Belozerova (2009) studied the effect of different metal nanoparticles, such as silicon (Si), palladium (Pd), gold (Au) and copper (Cu), on lettuce seed germination. They reported that nanoparticles showed positive influence at different concentration ranges: Pd and Au at lower concentrations, Si and Cu at higher concentrations and Au and Cu in combined mixture. Likewise, in a field study, Quoc Buu et al. (2014) reported an increased seed germination rate in *G. max* as compared to control when treated with nanocrystalline powder of iron, cobalt and copper at an extra-low concentration. In addition, a marked increase was observed in the chlorophyll index, number of nodules and crop yield. Arora et al. (2012) reported that foliar spray of gold on *Brassica juncea* plants in field experiments showed a positive effect, as it resulted in increased plant height, stem diameter, number of branches, number of pods and seed yield.

Interestingly, gold nanoparticles also improved the redox status of treated plants. Suriyaprabhaet al. (2012) reported that treatment with SiO_2 nanoparticles in maize plants significantly enhanced the plant dry weight and also enhanced the levels of organic compounds such as proteins, chlorophyll and phenols.

Genetically engineered microbes application

There are many biotechnological applications of genetically engineered microorganisms that potentially may fall under the purview of the Toxic Substances Control Act (TSCA), including a number of uses that are relevant to agriculture. These include intergeneric microorganisms used as biofertilizers such as symbiotic nitrogen-fixers, e.g. *Sinorhizobium meliloti* and *Bradyrhizobium japonicum*. Field tests of numerous intergeneric rhizobia have gone through review under TSCA, and one particular strain of *S. meliloti*, RMBPC-2, was approved in 1997 for limited commercialization.

APPLICATION OF BIOFERTILIZERS

In the future, there could be more submissions for more rhizobia for increased nitrogen-fixation ability, or perhaps, for enhanced nodulation efficiency. In addition, applications for other symbiotic nitrogen fixers, such as the actinomycete *Frankia*, which is a Gram positive bacterium that forms symbiotic relationships with certain plants such as woody angiosperms referred to as actinorhizal plants, are a possibility. There may also be submissions for free-living nitrogen-fixing microorganisms. In addition to nitrogen-fixing intergeneric microorganisms, other biofertilizer applications that would be reviewed under TSCA include phosphate-solubilizing microorganisms, mycorrhizal fungi or other endophytic microorganisms that aid in nutrient absorption, plant hormone production, or act by other mechanisms that may increase plant productivity.

TIPS TO GET GOOD RESPONSE TO BIOFERTILIZER APPLICATION

- Biofertilizer products must contain an appropriate population of good effective strains and should be free from contaminating microorganisms.
- Select the right combination of biofertilizers and use before the expiry date.
- Use the suggested method of application and apply at appropriate time as per the information provided on the label.
- For seed treatment, adequate adhesive should be used for better results.
- For problematic soils, use corrective methods like lime or gypsum pelleting of seeds or correction of soil pH by use of lime.
- Ensure supply of phosphorus and other nutrients.

PRECAUTIONS BEFORE BIOFERTILIZER APPLICATION

- Biofertilizer packets need to be stored in a cool and dry place away from direct sunlight and heat.
- Right combinations of biofertilizers have to be used.
- As *Rhizobium* is crop specific, one should use it for the specified crop only.
- Other chemicals should not be mixed with the biofertilizers.
- When purchasing, one should ensure that each packet is provided with all necessary information like name of the product, name of the crop for which it is intended, name and address of the manufacturer, date of manufacture, date of expiry, batch number and instructions for use.

APPLICATION OF BIOFERTILIZERS

- The packet has to be used before its expiry, only for the specified crop and by the recommended method of application.
- Biofertilizers are live products and require care in their storage.
- Both nitrogenous and phosphate biofertilizers are to be used to get the best results.
- It is important to use biofertilizers along with chemical fertilizers and organic manures. Biofertilizers are not a replacement of fertilizers but can supplement plant nutrient requirements.

ENVIRONMENTAL LIMITATIONS FOR APPLICATION OF BIOFERTILIZERS

- Unavailability of suitable carrier resource constraint
- Market level constraints and lack of awareness of farmers
- Lack of quality assurance and limited resource generation for biofertilizers production
- Seasonal and unsure requirement
- Soil and climatic factors and inadequately experienced staff
- Native microbial population, faulty inoculation techniques and mutation during fermentation

IMPACTS ON HUMAN HEALTH

While fertilizers cause relatively little harm to wildlife at least in comparison to the damage caused by pesticides, they are hazardous, in certain circumstances, to human health. These include:

- High nitrate concentrations in drinking water, which can result in clinical methaemoglobinaemia (often referred to as the blue baby syndrome);
- Dust exposure, which is the main occupational health problem in fertilizer manufacture;
- Ingesting of nitrate, which is implicated in a number of serious diseases, like gastric, bladder, esophageal cancer.

APPLICATION OF BIOFERTILIZERS

Occupational health and safety (OHS) needs to be properly managed. A farmer's OHS system helps ensure effective control of OHS risks, prevent work-related illness or injury and achieve compliance with regulations and standards.

Particularly appropriate for the new economic and occupational structure of farmer work, practitioners, researchers and other stakeholders are interested in assessing and managing the existing OHS risks. The goals concern:

1) The identification of effective practices in OHS risk management, and

2) Using a simple framework of good practice.

Products (or material) safety data sheets (MSDS) (appendix) serve two purposes, as they inform those concerned in handling chemicals of the hazards involved and they also provide the basis for risk assessments. Safety data sheets should be provided at all stages in the distribution chain and some countries have required their use under legislation.

In addition to the normal production properties, MSDS are required to provide health hazard and eco-toxicological information, which is generally difficult to obtain and interpret.

Hazards for Farmers

Farmers using biofertilizers may be exposed to many hazards:

HEAT

Heat-related illness can be deadly. Every year, thousands of workers become sick from exposure to heat, and some even die. These illnesses and deaths are preventable.

Workers exposed to hot and humid conditions are at a high risk of heat illness, especially if they are doing heavy work tasks or using bulky protective clothing and equipment. New workers may also be at greater risk than others if they have not built up tolerance to hot conditions. Employers must take steps to help workers become acclimated.

Heat-related illnesses, while potentially deadly, are easily preventable. When working in hot conditions, remember "WATER, REST and SHADE." Drink water every 15 minutes, even when not thirsty. Wear a hat and light-coloured clothing. Rest in the shade. Be sure to watch out for fellow workers and know your location in case you need to call for assistance. Get help right away if there are any signs of illness.

MUSCULOSKELETAL INJURIES

Workers in agricultural operations for crop productions typically use repetitive motions in awkward positions, which can cause musculoskeletal injuries.

Ergonomic risk factors are found in jobs requiring repetitive, forceful or prolonged exertions of the hands; frequent or heavy lifting, pushing, pulling or carrying of heavy objects; and prolonged awkward postures. Vibration and cold may intensify these conditions.

Ergonomic protections. Some methods for reducing musculoskeletal injuries include proper tools, padding to reduce vibration and fewer activities with high repetition.

LADDERS & FALLS

Deaths and injuries from falls remain a major hazard for farm workers.

VEHICLE HAZARDS

Injuries from vehicle accidents are serious and debilitating to farm activities.

HAZARDOUS EQUIPMENT AND MACHINERY

Farm workers routinely use knives, hoes and other cutting tools; work on ladders; or use machinery in their shops. However, these simple tools can be hazardous and have the potential for causing severe injuries when used or maintained improperly.

1. All tools should be maintained in good condition and used according to the manufacturers' instructions.
2. Power tools must be properly grounded or double insulated and all guards or shields must be in place.
3. Farm workers should wear proper personal protective equipment (PPE) and make sure that clothing has no strings or loose ends that could be caught by machinery. Long hair should be tied back to prevent entanglement.
4. In addition, shops should be well lit and have clear walkways to eliminate slips, trips and falls.

GRAIN BINS AND SILOS

While safety issues surrounding grain bins and silos are sometimes overlooked on farms, they pose many dangers. Farm workers are exposed to suffocation or engulfment hazards when working with grain bins and silos, as well as grain dust exposures and explosions. Suffocation is a leading cause of death in grain storage bins.

Suffocation can occur when a worker becomes buried (engulfed) by grain as they walk on moving grain or attempt to clear grain built up on the inside of a bin. Moving grain acts like "quicksand" and can bury a worker in seconds. "Bridged" grain and vertical piles of stored grain can also collapse unexpectedly if a worker stands on or near it.

UNSANITARY CONDITIONS

The lack of drinking water, sanitation facilities and/or hand washing facilities can lead to many health effects. Farm workers may suffer heat stroke and heat exhaustion from insufficient intake of potable water, urinary tract infections due to urine retention from inadequate availability of toilets, agrichemical poisoning resulting from lack of hand washing facilities, and infectious and other communicable diseases from microbial and parasitic exposures.

RESPIRATORY DISTRESS

Respiratory hazards. Respiratory hazards in barns, manure pits, machinery and silos range from acute to chronic air contaminants. Farmworkers' most common respiratory hazards are bio-aerosols, such as organic dusts, microorganisms, and endotoxins and chemical toxicants from the breakdown of grain and animal waste. Inorganic dust, from silicates in harvesting and tilling, is prevalent but less significant.

Respiratory protection. Control of aerosols might include the enclosure and ventilation of tractors, applying moisture to friable material, and respirators.

NOISE

Thousands of workers every year suffer from preventable hearing loss due to high workplace noise levels, and research has shown that those who live and work on farms have had significantly higher rates of hearing loss than the general population. In fact, farming is among the occupations recognized as having the highest risks of hearing loss.

Tractors, forage harvesters, silage blowers, chain saws, skid-steer loaders, grain dryers, squealing pigs and guns are some of the most typical sources of noise on the farm. Studies suggest that lengthy exposure to these high sound levels have resulted in noise-induced hearing loss to farmworkers of all ages, including teenagers. Hearing loss is neither as dramatic nor as sudden as an injury from a tractor overturn or machine entanglement, but it is permanent.

Employers can achieve noise reduction in several ways – usually related to the maintenance of the equipment:

1. Worn, lose or unbalanced machine parts can increase decibel levels during operation. Regular lubrication and parts replacement (bearings, mufflers, silencers, etc.) reduce friction and lower noise levels.
2. Larger engines that can be operated at lower speeds reduce noise levels, and may even save fuel.
3. Vibration isolation pads may be installed under the legs of noisy equipment to reduce noise generated by the equipment vibrating on a cement floor.
4. Newer chainsaws and leaf blowers have flexible mountings to reduce vibration-induced noise as well.
5. Tractor and skid-steers can be purchased with sound-reducing cabs and tightly fitted cab doors and windows to reduce how much outside noise reaches the operator.

6. Acoustical materials may be installed on walls and ceilings to enclose sound.

In addition, employers may provide workers with personal protective equipment (PPE) but must train them in using the PPE correctly. OSHA's Safety and Health Topics Page on PPE describes proper use of personal protective equipment.

The best state of health, safety and well-being for farmers cannot be reached at once. Effective systems are based on the principle of "Plan – Do – Check - Act" (Deming, 1982). In OHS terms for companies this will require to develop a policy on what is intended to achieve, then a plan of how and when it will be done, including any necessary arrangements. Next comes the "doing" phase, when plans are implemented and then a check is made that you have done what you planned to do and that it is effective in controlling risks. Any deficiencies found need to be acted upon and rectified, so that the system performance improves continually (Smith, 2008).

According to the ISO 31000:2009 standard, risk depends both on the probability or frequency of an adverse outcome, and also on the severity of that outcome. Risk has similarly been defined generally as "the potential for realization of unwanted, negative consequences of an event" (Moraru and Băbuţ, 2010). More quantitatively (Sage and White, 1980), risk is defined as "the probability per unit time of the occurrence of a unit cost burden", and it is stated that it "represents the statistical likelihood of a randomly exposed individual being adversely affected by some hazardous event". Thus, risk has been defined at many different levels of detail. The usage of the word ‘risk’ usually has negative connotations and risks are regarded as something to be minimized or avoided.

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CROP RESPONSE TO BIOFERTILIZERS

Contents

APPLICATION OF *RHIZOBIUM* INOCULANT

The effect of inoculants on the growth and yield of legume crops depends on the quality of inoculant, soil properties and application techniques. Generally, inoculants should be used according to the specification on the package and when a legume is introduced into a new area or when the legume is known to have a nodulation problem. The main purpose of inoculation is to nodulate the host legume with a selected rhizobial strain. The inoculant should be of good quality at the time of application.

Commonly, two application methods are used in the inoculation of rhizobial biofertilizers to legumes. This is direct inoculation, where the inoculant is placed in direct contact with the seeds (seed-applied inoculant), and indirect inoculation, whereby the inoculant is placed alongside or beneath the seeds (soil-applied inoculant).

Inoculant is applied to seeds in the following ways:

a) Dusting: With this method, the inoculant is mixed with the dry seeds directly. This may lead to poor adherence of rhizobia to the seeds; the method is least effective.

b) Slurry: The inoculant can be mixed with wetted seeds, or diluted with water and some stickers, e.g. 25% solution of molasses or 1% milk powder. In some cases, gum Arabic, sucrose of methyl ethyl cellulose can be used as stickers.

c) Seed coating: The inoculant can be made into slurry and mixed with the seeds. The seeds are then coated with finely ground lime, clay, rock phosphate, charcoal, dolomite, calcium carbonate or talc. The method has several advantages, such as protection of rhizobia against low pH soil, desiccation, acidic fertilizers, fungicides or insecticides.

In the indirect application method, the inoculant is applied to the soil beneath or alongside the seeds. The method is used when seeds are treated with fungicide or insecticide, and when a high amount of inoculant is needed to outcompete the indigenous rhizobial population. The simplest inoculation is to prepare the liquid formulation of the inoculant and spray to the soil or directly over the seeds after placement. In this case, a high amount of inoculant is needed. Some disadvantages of this method include loss of viability of rhizobia, short storage period and difficulty in the distribution of inoculant.

APPLICATION OF NON-SYMBIOTIC NITROGEN FIXERS INOCULANT

Azospirillum

Application of biofertilizers from associative nitrogen-fixing bacteria

Benefits of Biofertilizers

In general, biofertilizers from associative nitrogen-fixing bacteria could be used especially for cereal crops such as rice and wheat, but also for cash crops such as vegetables, fruits, flowers, tobacco, cotton, oilseed, tea and medicinal or herbal crops. BIO-N in the Philippines is a microbial-based fertilizer for rice, corn and other agricultural crops like tomatoes, pepper, aubergine, okra, lettuce, peach and ampalaya. It is a breakthrough technology that promises very significant impact on the country's farmers in terms of increasing farm productivity and income as well as saving the country's dollar reserve due to decreased importation of inorganic nitrogenous fertilizers. It is mainly composed of microorganisms that can convert the nitrogen gas into available form to sustain the nitrogen requirement of host plants. The active organisms (bacteria) were isolated from the roots of Talahib, a grass relative of sugar cane. These bacteria, once associated with the roots of rice, corn, sugar cane and some vegetable plants, can enhance their root development, growth and yield.

In China and other FNCA countries, associative nitrogen-fixing bacteria biofertilizers have increased the yields by 10–30% and reduced the use of chemical N fertilizer by 15–25%. It is reported that application of biofertilizer with associative nitrogen-fixing bacteria could enhance the maturation of crops, shorten the vegetation period by 5–10 days and improve the soil quality and soil fertility.

The benefits of biofertilizers with associative nitrogen-fixing bacteria can be seen as follows:

1. Enhance the shoot growth and root development;
2. Improve the yield of host plants;
3. Replace 30–50% of the total amount of N requirement;
4. Make plants resistant to drought and pests;
5. Reduce the incidence of rice tungro and corn earworm attack;
6. Increase the yield and milling recovery of rice.

Application in Cereal crops:

The liquid form is good for rice. At transplanting, immerse rice roots into liquid biofertilizer for 10–15 min before transplanting and spread on paddy soil at the regreening stage at a rate of 1.5–3.0 L per ha. For wheat, immerse the seeds into liquid biofertilizer overnight before sowing, and spread onto wheat leaves at a rate of 1.5–3.0 L per ha with water.

Vegetables:

Solid biofertilizer is spread, band-spread and hole-applied as basal or top dressing. For leaf vegetables such as celery, spinach and cabbage, apply at a rate of 3.75–15.0 kg per ha. For fruit vegetables such as cucumber, aubergine, tomato and melon apply at a rate of 7.5 kg per ha. For root vegetables such as sweet potato, potato, ginger and garlic, apply at a rate of 3.75–15.0 kg per ha.

Fruits:

For fruit trees, 10–20 g, 20–30 g or 30–50 g per plant will be applied to those, respectively, with plant yield less than 50 kg, 50–100 kg and over 100 kg.

CROP RESPONSE TO BIOFERTILIZERS

Tobacco:

Rates of 6.25 kg per ha are applied. For those where biofertilizer with associative nitrogen-fixing bacteria is applied, the N-fertilizer should be reduced by 20–25%. Mixed application with organic manure should be encouraged because organic manure will benefit microbes.

Corn:

1. Place seeds in a suitable container and moisten with water. Pour a sufficient amount of inoculant, 1 packet of BIO-N for every 3 kg of seeds.
2. Mix thoroughly until the seeds are evenly coated; (a drop or two of sticker, e.g. Tween 20 or APSA may be mixed with water to enhance adsorption of BIO-N onto the seeds).
3. Sow the coated seeds immediately. Be sure not to expose the inoculated seeds to direct sunlight.
4. Depending on the soil analysis, very marginal soils may require a basal application of at least a bag or two of 14-14-14 to a hectare as side dress.

NOTE:

The basal application of organic fertilizer is highly recommended to provide a whole array of other nutrients for a balancing effect. Split application of the recommended inorganic macro-elements has been found effective, e.g. second application of 14-14-14 NPK is done before tasseling.

Rice:

As solid inoculant for direct-seeded rice:

1. Soak seeds overnight in clean water
2. Pre-germinate the seeds in gunny sacks or a suitable container.
3. When radicles (embryonic roots) come out, place the germinants in a suitable container.
4. Pour the required amount of BIO-N and mix thoroughly until the germinants are evenly coated.
5. Sow directly over field or on prepared beds.

As liquid inoculant for dapog bed:

Suspend the required amount of Bio-N in sufficient volume of clean water (e.g. 1 packet Bio-N to 1 gallon water) and evenly drench the seed/seedling-lined dapog bed.

As slurry for transplant seedling:

1. In a suitable container, mix BIO-N with clean water to form a slurry or thick preparation.

2. Prune the roots of seedlings into uniform length and dip for at least 30 min or 1 h before transplanting.

Procedures for Growing Corn using Biofertilizer Inoculated Seeds

A) Seeds

- Use the best seeds for certain locations as recommended by the Department of Agriculture.

B) Land Preparation

- The land is ploughed with a tractor with a depth of 15–20 cm, and then hoed.
- Clear the land from weeds and prepare seedbeds.

C) Seeds Inoculation

- Check the instructions on the biofertilizer pack. For example, one pack of biofertilizer for corn (200 g for 2000 m) and 3 kg of seeds.
- Inoculation is done step by step. Prepare one clean bucket or plastic bag to hold the seeds that are being inoculated. Prepare slurry by mixing a sticker with the inoculant. If sticker is not available, use vegetable oil.
- Mix the slurry thoroughly with corn seeds and let them dry.
- When inoculating seeds, avoid making them too wet. See the procedure on the pack.
- Sweet-corn seeds are commonly coated with fungicide. Use a larger amount of inoculant and plant immediately after inoculation.
- Inoculated seeds are ready to sow. Put the inoculated seeds under shade.

D) Sowing

- Sow the seeds at a planting distance of 75cm x 25 cm.
- To protect seedlings against infestation by seed flies, insecticide is applied to seed holes.

E) Fertilization

- Basal fertilizer, 66 kg/ha of N (urea), 150 kg/ha of SP-36 and 100 kg/ha of KCl are applied at 10 days after planting (DAP), banded in a depth of 5 cm and applied 7 cm in front of plant rows.
- Second N fertilization, 33 kg/ha of urea is applied banded at 10 cm in front of plant rows.

F) Weeding

- Weeding is done before fertilizer application.
- At the second N fertilizer application, the soil and weeds are returned back to plant rows.

G) Pest Management

- Spray the plants with suitable insecticide at the recommended dose as soon as the symptoms of infection appear.

H) Watering

- Corn needs sufficient water at sowing, flowering and grain filling.
- Drainage is made to avoid flooding.

I) Harvesting

- Harvesting could be done at around 96 DAP for corn varieties, and 70 DAP for sweet corn.

APPLICATION OF MYCORRHIZAL INOCULANT

1. The application rate of VA Mycorrhiza biofertilizer is 10 g or 1 spoonful per plant.

2. VA Mycorrhiza biofertilizer can be used at any stage of plant growth. However, for maximum benefits, it should be applied during the seedling stage or placed at the base of plant holes before planting. After two weeks of application, other suitable fertilizers can be applied.

3. For planting by stem cutting, the growth media are mixed with VA Mycorrhiza biofertilizer prior to planting. The cutting stocks can be transferred to the field one month after roots have developed.

4. For transplanting, simply sprinkle VA Mycorrhiza biofertilizer adjacent to the plant roots and cover with soil.

5. For grown trees, the soil under the plant canopy is trenched or the leaf litter under the tree is removed. About 10 g (1 spoonful) per plant of VA Mycorrhiza biofertilizer is applied to the root hair system and then covered with soil.

6. VA Mycorrhiza biofertilizer can be used in combination with several types of biofertilizers (e.g. Rhizobium biofertilizer, or PGPR).

APPLICATION OF PHOSPHATE SOLUBILIZERS INOCULATION

Generally, biofertilizers in powder form are applied like organic matter onto the soil. This type is very convenient for users in the management of biofertilizers. Some biofertilizers are costly products for farmers, so their use would be restricted by the specific conditions of agronomy. Microorganisms are generally supplied by producers of biofertilizers, so it is only necessary for the users or farmers to follow the application method recommended by the manufacturers. However, the popular application method is regarded as the next procedure.

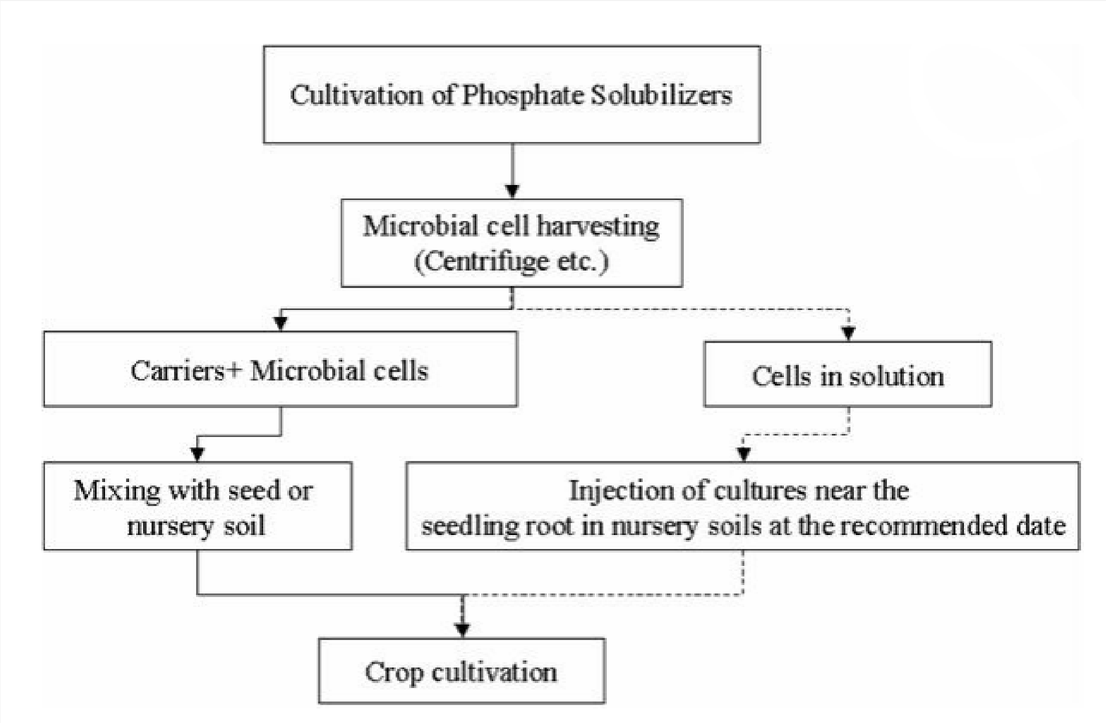

Fig. 1. Inoculation method of phosphate solubilizers

Two weeks before spore inoculation, the desired seedlings (e.g. oil palm, vegetable, pasture grass) are prepared in suitable containers filled with sandy loam soil.

Improvement of phosphate solubilizers:

An alternative approach for the use of phosphate-solubilizing bacteria as microbial inoculants is the use of mixed cultures or co-inoculation with other microorganisms. Evidence points to the advantage of the mixed inoculations of PGPR strains comprising phosphate-solubilizing bacteria. The effect of combined inoculation of *Rhizobium*, a phosphate-solubilizing *Bacillus megaterium* ssp. *phospaticum* strain-PB and a biocontrol fungus *Trichoderma* spp. on the growth, nutrient uptake and yield of chickpea were studied under glasshouse and field conditions. Combined inoculation of these three organisms showed increased germination, nutrient uptake, plant height, number of branches, nodulation, pea yield and total biomass of chickpea compared to either individual inoculations or an inoculated control.

On the other hand, it has been postulated that some phosphate-solubilizing bacteria behave as mycorrhiza helper bacteria. It is likely that the phosphate solubilized by the bacteria could be more efficiently taken up by the plants through a mycorrhizal pipeline between roots and surrounding soil that allows nutrient translocation from soil to plant. Considerable evidence supports the specific role of phosphate solubilization in the enhancement of plant growth by phosphate-solubilizing microorganisms. However, not all laboratory or field trials have offered positive results. Therefore, the efficiency of the inoculation varies with the soil type, specific cultivars and other parameters.

APPLICATION OF BIOFERTILIZERS IN IRRIGATED CROPS

Application of Biofertilizers on Rice

The biofertilizers used for rice crops are *Azospirillum*, phosphobacteria, blue-green algae, *Azolla* and mycorhizae.

Methods of application of biofertilizers:

Application of *Azospirillum Bacteria:*

- Seed treatment: 600 g/ha of *Azospirillum* culture are to be mixed with water where the seeds are soaked one night before sowing in the nursery bed.
- Seedling inoculation: A slurry can be prepared by mixing *Azospirillum* at 1000 g/ha in 40 litres of water and the root portion of transplanted rice seedlings is dipped in bacterial suspension for 15–30 minutes and then they are transplanted.

- Main field: 2000 g/ha of *Azospirillum* with 25 kg farmyard manure and 25 kg of soil are mixed uniformly and broadcasted in the main field before transplanting.

Uses:

- *Azospirillum* thrives in the root zones of rice and is capable of fixing more atmospheric nitrogen, which is absorbed by the plants. Root exudates of the crops provide nutrients for survival and multiplication of the bacteria.
- *Azospirillum* also solubilizes phosphorus and silicon to some extent required by rice.
- It renders plants drought-tolerant when irrigation or rainfall is delayed.
- By adopting *Azospirillum* application, 30% of the inorganic nitrogen usage can be reduced.

Application of Blue-Green Algae

Blue-green algae (BGA) can also be artificially cultured.

Beds sized 20 x 2 m are prepared in a ploughed land banded on all sides and water is let into the field to a height of 10 cm and maintained at 2–5 cm depth. Then, 5 kg of algal inoculum with 100 g of lime are sprinkled for one cent plot (1 cent = 0.01 acre). After 30 days, without drainage of water, the plot is dried and, hence, an algal mat settles over the soil. Drying, it peels off like flakes and is collected and distributed for rice field application at a rate of 10 kg/ha, 10 days after transplanting.

Otherwise, algal flakes can be powdered, mixed with 25 kg of farmyard manure and 25 kg of soil and can be broadcasted. At the time of application, a thin film of water is to be maintained.

Uses:

- The nitrogen fixed by BGA is about 15 kg/ha over a season.
- BGA produce vitamin B12 and growth factors that make plants grow vigorously.
- BGA oxygenate the water impounded in the field.
- BGA excrete organic acids that render phosphorus solubilization.
- The algal mat in paddy fields also protects against loss of moisture from the soil.

Application of *Azolla*

Azolla can be multiplied by constructing nurseries with 10 cm deep standing water and adding superphosphate at 8 kg/ha of P_2O_5in small plots. Inoculation can be done at 8 kg/m^2. *Azolla* can be used immediately after harvest.

It can be applied as green manure prior to rice planting or can be grown as a dual crop with rice. About 10 tons of fresh *Azolla* per hectare is equivalent to 30 kg/ha of N.

Uses:

- *Azolla* excretes organic nitrogen in water during its growth and also immediately upon trampling.
- Fern fronds are soft and rapidly decomposed.
- *Azolla* absorbs traces of potassium from irrigation water.
- It provides nitrogen, potassium organic carbon etc.
- It prevents weed growth in rice field water.

Application of Phosphobacteria

This is applied at the same dose in the same manner as *Azospirillum*. Bacteria like *Bacillus megatherium* var. *phosphaticum*, *Pseudomonas fluorescens*, fungi like *Pencillium digitatum*, *Aspergillus niger* have been found to have a strong phosphate-dissolving ability.

Uses: 25 to 50 of the recommended phosphorus can be reduced depending upon the native phosphorus content of the soil.

Biofertilizers could offer an opportunity to increase rice yields, productivity and resource use efficiency. Moreover, the increasing availability of biofertilizers in many countries and regions and the sometimes aggressive marketing brings ever more farmers into contact with this technology. However, rice farmers get little advice on biofertilizers and their use from research or extension because so little is known on their usefulness in rice.

The study of Nino Paul Meynard Banayo et al. tested different biofertilizers in an irrigated lowland rice system in the Philippines during four seasons. In all four seasons and across the biofertilizer treatments, the grain yield increased with increasing the amounts of applied biofertilizer. However, this increase was not always statistically significant and the yield increase varied considerably between seasons.

Generally, low yields in that season were due to a typhoon that caused considerable damage through flooding of the experimental field and lodging of the crop. For this reason, the crop was harvested prematurely by about 1 week, which further reduced the attainable yields. The grain yields in the other three experimental seasons were similar. The biofertilizer achieving the highest average grain yields across all four inorganic fertilizer treatments and in all four seasons was BN (*Azospirillum lipoferum*, *A. brasilense*). Statistically significant interactions between biofertilizer treatment and inorganic fertilizer treatment could not be detected in any season (at $p \leq 0.05$), suggesting that the effect of the biofertilizer was independent of the inorganic fertilizer rate. However, there was a trend towards higher yield increases due to biofertilizer use at low to medium inorganic fertilizer rates. This trend was most obvious for the BN biofertilizer, whereas the performance of the BS (*Trichoderma parceramosum*, *T. pseudokoningii* and a UV-irradiated strain of *T. harzianum*) and BG (rhizobacteria) biofertilizers was less consistent.

CROP RESPONSE TO BIOFERTILIZERS

The grain yield increases due to biofertilizer use ranged from 200 to 300 kg/ha for the best biofertilizers, when the BN treatment had an almost 800 kg/ha better grain yield than the control. In relative terms, the seasonal yield increase across the fertilizer treatments was between 5% and 18% for the BN biofertilizer, for the BS (*Trichoderma parceramosum*, *T. pseudokoningii* and a UV-irradiated strain of *T. harzianum*) biofertilizer (up to 24% for individual treatment combinations), and between 1% and 9% for the BG (rhizobacteria) biofertilizer (up to 28% for individual treatment combinations). For the calculation of the relative yield increase, only average values could be compared and no statistical analysis could be conducted.

The tested biofertilizers did increase the grain yield significantly, and especially the BN biofertilizer did so consistently. Even in seasons in which no significant effect could be detected due to the yield variability between plots, the grain yield with biofertilizer was usually better than that without it. The seasonal yield increase across fertilizer treatments was between 5% and 18% for the BN biofertilizer, which is within the 5–30% range reported for *Azospirillum* inoculums and non-rice crops.

Similarly, the observed yield increase for the *Trichoderma*-based BS (3–13%) was close to the 15–20% rice yield increase described by the trend of yield increases between the different inorganic fertilizer treatments, which was not so clear across seasons but the yield increases were often lower at higher inorganic fertilizer rates. The absolute grain yield increases due to biofertilizer were usually below 0.5 t/ha. The study was conducted to evaluate the effect of different biofertilizers on the grain yield of lowland rice and to investigate possible interaction effects with different inorganic fertilizer amounts.

The results showed significant yield increases for all products tested in some seasons but the most consistent results were achieved by the *Azospirillum*-based biofertilizer. In most cases, the observed grain yield increases were not huge (0.2 to 0.5 t/ha) but could provide substantial income gains, given the relatively low costs of all biofertilizers tested. The positive effect of the tested biofertilizers was not limited to low rates of inorganic fertilizers and some effect was still observed at grain yields up to 5 t/ha.

However, the trends in our results seem to indicate that the use of biofertilizers might be most helpful in low- to medium-input systems. The results achieved can already be used to specify better advice for farmers on biofertilizer use in lowland rice, but several important questions remain. In particular, biofertilizers need to be evaluated under conditions with abiotic stresses typical for most low- to medium-input systems (e.g. under drought or low soil fertility) and with a range of germplasm because their effect might also depend on the variety used. More upstream-oriented research would be needed to better understand the actual mechanisms involved, which, in turn, could also contribute to making the best use of biofertilizers in rice-based systems.

APPLICATION OF BIOFERTILIZERS ON COTTON

The study of Achieves of Agronomy and Soil Science testing selected strains of *Azotobacter*, *Acetobacter*, *Azospirillum* and *Pseudomonas* on two varieties of cotton (American H1098 and Desi HD123) continuously for two years (2000–2001 and 2001–2002) under field conditions. These two varieties of cotton are genetically different. HD123 is a Desi cotton variety, which is diploid, with less nutrient uptake and lower susceptibility to pests. H1098 is a tetraploid American cotton variety, which has high nutrient uptake ability and is highly susceptible to pests.

As cotton is a summer crop and the temperature in the summer rises up to 48 °C, the selected cultures were mostly high temperature tolerant. *Azotobacter* has the property of forming cysts. This enables it to survive at high temperatures. Several reports have suggested that PGPRs (plant-growth-promoting rhizobacteria) also stimulate plant growth by facilitating the uptake of minerals such as N, P, K and other important micronutrients (Barea et al., 1976; Dobbelaere et al., 2003). This uptake is suggested to be due to a general increase in the volume of the root system. Higher amounts of IAA affect the seed emergence of wheat primarily because of the production of growth regulators by bacteria.

Better performance is attributed to the high temperature tolerance of some cultures during the cotton crop season. It is also due to the better proliferation, survival, ability to fix more nitrogen, antifungal properties of the inoculant strains and growth-promoting substances which are also likely to contribute to the beneficial effects on crops. The *Azotobacter* strains used in this investigation have also been tested for the above-mentioned properties and it has been observed that they have the ability to excrete ammonia, produce IAA, siderophores, have antifungal properties and are capable of fixing nitrogen.

Higher seed yield, plant growth and survival of the bio-inoculants may be attributed to many factors, most important being the favourable influence exerted by root exudates, which contain acids, organic acids, carbohydrates and growth hormones like indole acetic acid. IAA synthesized by bacteria is taken up by the plants and can stimulate cell proliferation. Nitrogen fixation and solubilization of insoluble phosphate also contribute significantly to plant growth. Phosphate solubilizers can exert considerable influence on nutrient uptake.

Therefore, the use of phosphate-solubilizing, IAA-producing *Azotobacter chroococcum* may augment the efficiency of applied and native P_2O_5 by reducing fixation by the soil fraction. Therefore, selection of isolates with high temperature tolerance, phosphate solubilization, phytohormone production and high nitrogen fixation has expanded the possibilities of applying free-living nitrogen fixers to cereals and other non-legume crops. Our studies suggest that microbial inoculants can be used as an economic input to increase crop productivity and lower the fertilizer level along with harvesting more nutrients from the soil. However, a lot of research work is still left to be done on aspects of phytohormone production and increased nutrient uptake, which is an important parameter in plant–microbe interactions.

APPLICATION OF BIOFERTILIZERS ON DRYLAND CROPS

Cereal Crops

Biofertilizers that are used are:

•*Azotobacter*

•*Azospirillum*

•Phosphotika

In the following CEREALS:

MAJOR CEREALS: paddy, wheat, maize

MINOR CEREALS: barley, oats, millets, sorghum, etc

Methods of application

- **Seed treatment**

Suspend 200 g of *Azotobacter* or *Azospirillum* + 200gm of Phosphotika in 300–400 ml of water and mix thoroughly. Mix this with 10–12kg of seeds with hands till all the seeds are uniformly coated. Dry the coated seeds in shade and sow immediately.

- **Seedling root dip treatment**

Mix 1 kg *Azotobacter* and 1 kg Phosphotika in sufficient quantity of water and dip the roots of seedlings to be transplanted in 1 acre in this suspension for 30 minutes or more and transplant them immediately. In case of paddy (low land), prepare a small seedbed in the field and fill with 3–4 inches of water. Put 2 kg of *Azospirillum* + 2 kg Phosphotika in this water and mix. Dip the roots of the seedlings to be planted in 1 acre in this suspension for 8–12 hours (overnight) and transplant them.

CROP RESPONSE TO BIOFERTILIZERS

Benefits

- Increase crop yield by 20–30%.
- Replace chemical fertilizers by 25%.
- Restore natural fertility.
- Provide plant nutrients at very low cost.
- Have no harmful effects on soil fertility and plant growth.
- Hasten seed germination, flowering and maturity in crops.
- Helps in recycling/decomposition of organic waste.
- Provide residual effects for subsequent crops.
- Pollution-free and eco-friendly.

The effect of PGPR (plant-growth-promoting rhizobacteria) on cereals growth, development and yield has been examined by Yasin M. et al. Normally, PGPR enhance the availability of unavailable nutrients and also increase the nutrient absorption capacity of crop plants. Nitrogen-fixing and phosphorus-solubilizing bacteria have synergistic effects on the growth and development of cereal crops. Plant-growth-regulating rhizobacteria have normally been used in non-leguminous crops such as paddy, maize and wheat. Inoculation with *Bacillus* species has shown positive yield response in paddy, sorghum, barely and maize. Wheat seed treatment with PGPR has shown optimistic increase in wheat yield due to high nutrient assimilation capacity of roots. The bacterial genera involved in PGPR include *Azotobacter*, *Bacillus* and *Azospirillum*.

Seed treatment of wheat and barley with *Bacillus* species has shown an increase in crop yield. In the same way, wheat seed treatment with *Bacillus* sp. enhanced the root growth and also improved the soil structure and the plant development. Collective seed treatment with nitrogen-fixing and phosphorus-solubilizing bacteria is more effective than single application. Biofertilizers inhibit the harmful soil pathogens and also enhance the availability of essential nutrients for crop plants. Joint application of nitrogen-fixing and phosphorus-solubilizing bacteria promotes the yield in sorghum and barley in contrast to only treatment with nitrogen-fixing or phosphorus-solubilizing bacteria.

Wheat seed treatment with *Pseudomonas putida* and *Baccilus lentus* increases the germination of seeds, the growth of seedlings and the wheat yield. Wheat seed inoculation with *Azotobacter* increases all yield parameters and the final yield of the crop both separately and mutually with phosphorus-solubilizing bacteria. Use of nitrogen-fixing bacteria (*Azotobacter chroococcum*) as a source of biofertilizer increases the biological yield of wheat. Joint application of *Azotobacter chroococcum* and *Bacillus magatherium* gives more positive results in plant growth when utilized as a source of biofertilizer in wheat than single application of *Bacillus magatherium*.

Inoculation of wheat cultivars with PSB and nitrogen-fixing bacteria gives good results over the control treatment: increase of 10% in the yield of non-leguminous crops has been observed due to the inoculation of *Azotobacter chroococcum* and round about 15 to 20% increase in the yield in cereal crops. *Azotobacter* is widely used in agricultural crops as an inoculant due to its unique ability to fix atmospheric nitrogen and make it available for crop plants. Combined seed treatment of flax with nitrogen-fixing bacteria along with phosphorus-solubilizing bacteria including *Bacillus* sp. enhances the production of growth-promoting substances which help the multiplication of plant cells and cell enlargement and finally increase all the growth parameters.

APPLICATION OF BIOFERTILIZERS ON DRYLAND LEGUMES

The biofertilizer used for legume crops is rhizobial.

Generally, inoculants should be used according to the specification on the package and when a legume is introduced into a new area or when the legume is known to have a nodulation problem. The main purpose of inoculation is to nodulate the host legume with a selected rhizobial strain. The inoculant should be of good quality at the time of application.

Commonly, two application methods are used in the inoculation of rhizobial biofertilizers to legumes. This is direct inoculation, where the inoculant is placed in direct contact with the seeds (seed-applied inoculant), and indirect inoculation, whereby the inoculant is placed alongside or beneath the seeds (soil-applied inoculant).

Inoculant is applied to seeds in the following ways:

a) Dusting: With this method, the inoculant is mixed with the dry seeds directly. This may lead to poor adherence of rhizobia to the seeds; the method is least effective.

b) Slurry: The inoculant can be mixed with wetted seeds, or diluted with water and some stickers, e.g. 25% solution of molasses or 1% milk powder. In some cases, gum Arabic, sucrose of methyl ethyl cellulose can be used as stickers.

c) Seed coating: The inoculant can be made into slurry and mixed with the seeds. The seeds are then coated with finely ground lime, clay, rock phosphate, charcoal, dolomite, calcium carbonate or talc. The method has several advantages, such as protection of rhizobia against low pH soil, desiccation, acidic fertilizers, fungicides or insecticides.

In the indirect application method, the inoculant is applied to the soil beneath or alongside the seeds. The method is used when seeds are treated with fungicide or insecticide, and when a high amount of inoculant is needed to outcompete the indigenous rhizobial population. The simplest inoculation is to prepare the liquid formulation of the inoculant and spray to the soil or directly over the seeds after placement. In this case, a high amount of inoculant is needed. Some disadvantages of this method include loss of viability of rhizobia, short storage period and difficulty in the distribution of inoculant.

APPLICATION OF BIOFERTILIZERS ON VEGETABLES

For vegetables, the biofertilizers commonly used are *Azotobacter* and phosphate solubilizers.

There are four methods for application of biofertilizers in vegetables:

- Seed treatment;
- Cut-piece/set treatment;
- Seedling treatment;
- Soil application.

- **Seed Treatment**

1. About 200 g of biofertilizers is required to treat 10–14 kg of seeds.
2. Suspend one packet of 200 g in approximately 400 ml water and mix it thoroughly.
3. Pour this mixture on seeds and mix with hands to obtain uniform coating on each and every seed.
4. Spread the seeds in shade for drying for 10–15 minutes then sow them immediately.

- **Set treatment**

1. Prepare a culture suspension by mixing 1 kg of culture in 50–60 litres water.
2. The cut pieces of planting material required for 1 acre are kept immersed in the suspension for 10–15 minutes.
3. Then bring out these cut pieces and allow to dry for some time before planting.
4. The cut-pieces method is applicable for crops like potato.

- **Seedling treatment**

1. Seedling treatment is recommended for tomato, chilli pepper, onion etc.
2. Prepare the suspension by mixing 1 kg of culture in 10–15 litres of water.
3. Get seedlings required for 1 acre and make small bundles of seedlings.
4. Dip the seedlings in the suspension for 15–20 minutes.
5. Transplant these immediately.
6. Generally, the ratio of inoculants and water should be 1:10 approximately, i.e. a 1 kg packet in 10 litres of water.

- **Soil Application**

1. Prepare the mixture of 2–3 kg of biofertilizer in 40–60 kg of soil/compost.
2. Broadcast the mixture in one acre of land, either at sowing time or 24 hours before sowing. The application of phosphate solubilizers is very common.

Application of biofertilizers on tomato crops

The recommended biofertilizers for tomato are *Azotobacter* in combination with PSB. Mycorrhizal inoculation gives additional benefit for mobilizing nutrients and overcoming soil moisture stress. Biofertilizers are applied by seed coating, seedling root dip and soil application.

- **Seed treatment:**
  - Keep the seeds required for sowing one acre of land in a heap on a clean-cemented floor or polyethylene sheet.
  - Prepare culture suspension by mixing one packet (200 g) each of *Azotobacter* and PSB biofertilizer in approx. 800 ml of water.
  - Sprinkle the culture suspension on the tomato seeds and mix.
  - Spread the seeds to dry under shade for some time and then sow.

An alternate method involves 10% sugar solution or 10% solution of gum Arabic sprinkled on the seeds serving as a sticker for biofertilizers to seeds. Dry the seeds by spreading them under shade for some time and then sow. Add the contents of the inoculant packet uniformly over sticker-coated seeds and simultaneously mix the contents. Prepare the suspension by mixing 1 kg (5 packets) each of *Azotobacter* and PSB culture in 15–20 litres of water. Get the tomato seedlings required for one acre of land. Dip the root portion of seedlings in the suspension for 30 minutes and transfer to the main field.

- **Soil application method:**

- Mix 2–3 kg each of the *Azotobacter* and PSB culture packets with 100 kg of well decomposed cattle manure/compost for one acre of land and sprinkle water to the mixer.
- Keep the mixer overnight for curing.
- Broadcast into soil at the time of planting or at the time of irrigation.

Mycorrhizal Application in Tomato:

- Apply mycorrhizal culture in the tomato nursery at 100 g/m^2 three centimeters below the soil.
- For planting out, apply 20 g mycorrhizal culture per seedling into the planting pit and cover with soil.
- For existing plants, apply mycorrhizal culture at 20 g near the root zone along with other fertilizers.

APPLICATION OF BIOFERTILIZER ON FRUIT CROPS

The use of biofertilizer, even though not spread on a wide scale for all crops, has witnessed growing awareness among the farmers that production can be increased by the use of biofertilizers in case of cereals, pulses, oil seed and some cash crops like vegetables and sugarcane. Biofertilizers are a recent concept in horticultural crop practices.

Generally, fruit crops have now received more attention than vegetables and ornamental crops. *Glomus fasciculatum*, *Glomus mosseae*, *Azospirillum*, *Azotobacter* and PSB are found useful for different horticultural crops. Use of biofertilizers, particularly inoculation with *Azotobacter*, could substitute 50% of the nitrogen requirement of banana and could produce higher yields over full doses of nitrogen application. The absorption of mobile nutrients like nitrogen also increases in association with VAM fungi.

Beneficial effect of *Azotobacter* and *Azospirillum* in enhancing the productivity of banana has also been reported. VAM fungi are responsible for more than two-fold increased acquisition of the less mobile nutrient elements like P, Ca, S, Zn, Mg and Cu from the rhizosphere. The high efficiency of *AzospiriIlum* for fixing nitrogen and better mobilization of fixed phosphorus by VAM even at high temperature can make these highly suited for mosambi (sweet lime). The percent of wilting in VAM-treated trees of guava has been recorded to be lower as compared to that of untreated trees. The content of N, P, K and also of Fe, Mn, Zn and Cu increases due to VAM inoculation. Studies on biofertilizers along with chemical fertilizers have been undertaken for assessment of their effect on the growth, yield and quality in mosambi.

The role of biofertilizers in fruit crops are discussed below.

CROP RESPONSE TO BIOFERTILIZERS

EFFECT OF BIOFERTILIZERS ON GROWTH CHARACTERISTICS

- VAM significantly increase the growth of plants compared to non-mycorrhizal control and are also effective in increasing the nutrient uptake by plants.
- VAM influence the growth-related characteristics and the yield-related component. About 50% cut-back on the use of phosphorus can be achieved through the use of VAM.
- VAM fungi have been found to be effective in papaya in increasing the plant height, stem girth, petiole length and the number of leaves.
- Mycorrhizal treatment is superior to non-mycorrhizal treatment in pomegranate.
- The *Glomus epigaeum* (GE) + *G. mosseae* + *Gigaspore calospora* mixture has been reported to give the maximum height, root length, number of leaves, dry weight of shoots and roots and mycorrhizal dependency percentage in pomegranate.
- The response of VAM on apple seedlings in combination with VAM, *Azotobacter* and inorganic fertilizers.
- Dual inoculation with *Glomus fasciculatum* and *Azotobacter chrococcum* produces larger plants which have a larger leaf area. In addition, the plant vigour is improved with inoculation of *Azospirillum* on peach seedlings of cv. 'Nemaguard' as compared to control.
- The treatment also leads to increase in plant height, stem diameter, leaf number, plant dry weight and leaf area.
- Greatest percentage increase has been found in seedling height of mango, seedling diameter and number of leaves by treatment with 49 g N, *Azotobacter* + 48 g N, 32 g N or *Azotobacter* alone as compared to control.
- Both soil and foliar application of nitrogen in combination with *Azotobacter* increase the plant height, plant girth, the number of hands, bunches and the number of fingers/hand significantly in banana cv. 'Robusta'.

EFFECT OF BIOFERTILIZERS ON YIELD

- Significant increase in the bunch weight and yield of banana has been achieved with *Azotobacter* and organic manures supplements over 100% fertilizer.
- *Azotobacter* also enhances shooting and shortens crop duration.
- The application of *Azospirillum* + 150 kg/ha of N can increase the yield in strawberry by 54%, the number of fruits per plant and the clump weight compared to treatment with 150 kg N alone.

CROP RESPONSE TO BIOFERTILIZERS

- The microbial inoculants in combination with inorganic manures have been shown to augment the yield and nutrient uptake in several crops.
- Application of biofertilizers (*AzospiriIIum*, phosphobacteria and VAMF) and organic manure (FYM) increase the bunch weight by 15.3 kg in hill banana var. Virupakshi along and with 75% NPK.
- Nitrogen-fixing bacteria improved the pseudostem circumference and the number of fingers/hand and advanced the flowering time in banana.
- Apple trees treated with phosphorene, active dry yeast and nitrobein at different concentrations showed effective improvement of fruit yield. The improvement was greatest with phosphorus biofertilizers.
- Increase in the number of fruits per plant, total weight of fruits and average fruit weight in strawberry as compared to the control has been achieved by the application of *Azotobacter*, *Azospirillum* and phosphate-solubilizing bacteria.
- The yield of sapota is greatly increased due to the application of 75 kg FYM + 1500 g N + 1000 g P_2O_5 + 500 g K_2O + 12.5 g PSB.
- The benefit–cost ratio is also high as compared to other fertilizer combinations. The inoculation of bacteria (*Azotobacter chrococcum* as a nitrogen fixer and bio-stimulant) along with N fertilizers between 80–100% favour banana development.
- The use of vermi compost, FYM and biofertilizers like *Azotobacter*, *Azospirillum*, VAM increase the production in citrus.

EFFECT OF BIOFERTILIZERS ON SOIL CHARACTERISTICS

- Plants inoculated with *Azotobacter* and *Azospirillum* derive benefits in terms of enhancement in the uptake of NO_3^-, NH_4^+, $H_2PO_4^-$, K^+ and Fe^{2+}, enhanced nitrate reductase activity in plants and production of antibacterial and antifungal compounds.
- The combined application of inorganic fertilizers and biofertilizers in banana cv. ‘Barjahaji’ significantly increases the available NPK status, organic C and microbial biomass and dehydrogenase activity in soil after harvest.
- VAM inoculation, either singly or in combination, significantly increases the root or shoot dry weight as well as the P-uptake over non-mycorrhizal treatments.
- Combined inoculation of *Acaulospora calospora* + *G. mosseae* + *G. margarita* and single inoculation of *G. mosseae* are superior in increasing the dry weight of ber seedlings as compared to other tested inoculation treatments.
- Application of VAM fungi in peach helps in better accumulation of Zn in their tissue.

CROP RESPONSE TO BIOFERTILIZERS

- The quantities of beneficial microorganisms in the soil increase considerably due to the use of *Azotobacter* mycorrhiza and phosphorins in banana.
- The commercial yield is also increased by 25–30% and a 50% cut-back on the use of inorganic fertilizers is achieved.

EFFECT OF BIOFERTILIZERS ON QUALITY PARAMETERS

- The treatment combination of P + VAM + N is the best treatment for producing better growth and yield of high quality fruit. This treatment also influences the plant height, trunk diameter, canopy volume, root growth and biomass production as compared to control.
- The effect of biofertilizers (phosphorene, active dry yeast, rhizobacteria and nitrobein) on fruit set and productivity has been investigated on Red Roomy grape vines.
- The use of phosphorene has been found to improve the fruit set and yield as well as the physical and chemical properties of fruits compared to control.
- A fairly high TSS and reducing sugar content have been reported in fruits harvested from *Azotobacter*-inoculated banana plant cv. 'Giant Governor'.
- The effect of inoculation with *Azospirillum* and phosphobacteria on the fruit quality of banana (Musa MA) cv. ‘Giant Governor’ by manipulating the doses of nitrogen and potassium fertilizers has been studied. The results show that inoculation of biofertilizers along with application of the recommended dose of fertilizer proves most effective in improving the fruit quality of Dwarf Cavendish banana cv. ‘Giant Governor’.
- The plant growth, yield and fruit quality of strawberry are significantly increased with the application of biofertilizer and nitrogenous fertilizers.
- Maximum TSS content has been observed with *Azotobacter* inoculation along with 80 kg/ha of N. Inoculation to fruit plants has proved the possibility of curtailing about 50% P fertilizers without reducing the crop yield.
- Nitrogen-fixing biofertiIizers mainly *Azospirillum* and *Azotobacter* can fix 20–40 kg N/ha and produce growth-promoting substances like IAA.
- The use of microbial inoculants not only is a low-cost technology, but also takes adequate care of soil health and environmental safety.

Generally, the effect of biofertilizers on fruits and yield is not as striking as that of chemical fertilizers.

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BIOFERTILIZERS APPLICATION FOR SUSTAINABLE ECONOMIC DEVELOPMENT: ADVANTAGES AND CONSTRAINTS

Contents

ADVANTAGES OF USING BIOFERTILIZERS IN AGRICULTURE

Biofertilizers are defined as formulations containing either living or latent cells of efficient strains of microorganisms that facilitate the uptake of nutrients form crop plants. They execute this pivotal role through interactions in the plant rhizosphere when applied through seed or soil.

BIOFERTILIZERS APPLICATION FOR SUSTAINABLE ECONOMIC DEVELOPMENT: ADVANTAGES AND CONSTRAINTS

Biofertilizers accelerate certain microbial processes in the soil which supplement nutrients in a form easily assimilated by plants. Biofertilizers supply nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus and stimulating plant growth through the synthesis of growth-promoting substances. Currently, biofertilizers are an important component of the integrated nutrient supply system.

Biofertilizers like *Rhizobium*, *Azotobacter*, *Azospirillum* and blue-green algae (BGA) are in use for decades. However, these microorganisms are very often not as efficient in natural surroundings as desired; thus, application of massively multiplied cultures of selected efficient microorganisms is needed to accelerate the microbial processes in soil. Therefore, the use of biofertilizers is strongly recommended by the competent professionals to guarantee good plant growth and higher production yields.

Biological fertilization (or biofertilization) as a process of application of natural inputs including fertilizers offers significant advantages in the efforts of contemporary agriculture to reduce the use of chemical fertilizers and pesticides. The most important advantages can be summarized as follows:

Low cost and easy application techniques

Biofertilizers are cost effective relative to chemical fertilizers. They differ from chemical and organic fertilizers because they do not directly supply any nutrients to crops and constitute cultures of special bacteria and fungi with relatively low installation cost. The use of biofertilizers can improve the productivity per unit area in a relatively short time. They have lower manufacturing costs and reduced use costs, especially regarding nitrogen and phosphorus use. Their easy way of application consumes smaller amounts of energy. This means lower costs associated with the process of fertilization that can be directly translated into profitable benefits for farmers. In this sense, application of biological fertilizers can bring benefits from an economic point of view, since biofertilizers are a cost effective and renewable source of plant nutrients to substitute the chemical fertilizers for sustainable agriculture.

Most commonly biofertilizers are in powder, carrier-based form. The carrier usually is lignite. The lignite has high organic matter content and holds more than 200% water. This high water content enhances the growth of the microorganisms. The application method for this type of biofertilizers is preparation of slurry, which is applied to the seeds. This method was considered universal until recently.

At present, however, another method, dry complex fertilizer for direct soil application, has been developed. It consists of granules (1–2 mm) made from tank bed clay (TBC) and baked at 200 °C in a muffle furnace, which helps to sterilize the material and gives porosity to the granules. The baked granules are soaked in a suspension of desired bacteria grown in a suitable medium overnight. The clay granules are air-dried at room temperature under aseptic conditions. They contain about 10^9 bacteria per gram of granules. These granules are suitable for field application along with seeds. However, the quantity of biofertilizer to be applied is slightly higher than that in seed application.

Increase of the yield with additional 15-35% in most vegetable crops

Biofertilizer is a technological innovation that has the potential to increase crop yield, reduce production cost and improve soil condition.

Biofertilizers can be considered as supplementary to chemical fertilizers. When they are applied as seed or soil inoculants, they multiply and participate in the nutrient cycling, thus benefiting the crop productivity. Biofertilizers have great potential to improve crop yields through environmentally better nutrient supplies. They provide reserve plant nutrients. It is reported that biofertilizers increase crop yields by 20–30% and stimulate plant growth. The efficiency of biofertilizer use is the key characteristic that ultimately contributes to the increase of the crop yield.

There are numerous examples that biofertilizers positively affect the crop yield. For instance, Vital N®, an organic biofertilizer registered with the Philippine FPA, is a powder formulation that induces extensive growth in roots of crops like corn, rice, banana, garlic, orchids and onion. It contains *Azospirillium*, a beneficial bacterium that produces the plant-growth-stimulating substance indole-3-acetic acid (IAA), resulting in higher growth yield.

There are reports that the overall performance of potato crops is positively influenced by application of green manures (cowpea and *Crotolaria* sp.): 30% yield improvements. The increased productivity values verify the efficiency of biofertilizers in agricultural production. On the other hand, some physicochemical properties of the soil are improved and environmental impacts due to the prolonged use of chemical fertilizers are gradually mitigated.

Furthermore, 10% increases in the yield per hectare have been observed for crops treated with arbuscular mycorrhizal (AM) fungi, combined with increased resistance of the plants to the action of pathogenic microorganisms. Additionally, when AM is combined with nitrogen-fixing bacteria or compost extracts, this combined use of biofertilizer on crops provides better yield performance, higher by a factor of two, and better physical characteristics of individual plants.

A trial investigating the feasibility of biofertilizers prototypes based on native bacteria from rice crops reported 10% increases in yield production by using the mixtures, from 7,625 kg/ha to 8,500 kg/ha. The main outcomes deal with the importance of biofertilizers to get higher revenues and increase productivity, in order to achieve, progressively, sustainable agricultural development.

The application of the aquatic fern–cyanobacteria symbiotic association *Azolla–Anabaena* as a biofertilizer in rice paddies of northern Italy allowed obtaining yields close to 40 kg nitrogen/ha during a 3-month period and verifying increases in the growth rate of rice. Furthermore, higher resistance of some of the rice species to the presence of herbicide Propanil was evidenced.

Provision of nitrogen and several growth hormones

Biofertilizers contribute to the maintenance of stable nitrogen (N) concentrations in the soil. They replace chemical nitrogen by 25%. Thus, nitrogen-fixing microorganisms play an important role in nitrogen supply by converting atmospheric nitrogen into organic forms usable by plants. Use of biological N_2-fixation technology can contribute to a decrease in the N fertilizer application and to the reduction of environmental risks. *Azotobacter* (free-living N_2-fixer) plays an important role in the nitrogen cycle in nature due to its diverse metabolic potential. In addition to N_2 fixation, this microorganism has the ability to synthesize and secrete considerable amounts of biologically active substances, among which the vitamins thiamine and riboflavin, nicotinic acid, pantothenic acid, biotin; the plant-growth hormones heteroxins, gibberellins. These biologically active substances help in modification of the nutrient uptake by the plants. Another free-living N_2-fixer, *Azospirillum*, is reported to produce plant-growth-promoting substances indole acetic acid (IAA) and indole butyric acid (IBA) and increase the rate of mineral uptake by plant roots, resulting in the enhancement of plant yield.

It is well known that most plants form symbiotic associations with the arbuscular mycorrhizal fungi (AMF) acting as bio-ameliorators. They have the potential to considerably enhance the rhizospheric soil characteristics. This, in turn, leads to improved soil structure and promotes plant growth under normal as well as stressed conditions. The results revealed that the AMF-induced enhancement in nutrient uptake promotes various biologically important metabolites. Among them of special importance are the plant hormones, including GA and auxin, which play a unique role in plant growth regulation under both normal and stress conditions. The activity of phytohormones like cytokinin and IAA is also significantly higher in plants inoculated with AMF. Higher hormone production results in better growth and development of the plant.

Do not cause atmospheric pollution but increase soil fertility

The use of biofertilizers is not only cost effective; it also augments the problem of environmental pollution. They are environmentally friendly because their use not only prevents damaging the natural resources but also helps to some extent to free the plants of precipitated chemical fertilizers. Biofertilizers promote the reduction of environmental impacts associated with the excessive use of chemical fertilization. Thus, their use in organic farming, sustainable agriculture, green farming and non-pollution farming contribute to implementation of healthy environment policies at national, regional and global level.

BIOFERTILIZERS APPLICATION FOR SUSTAINABLE ECONOMIC DEVELOPMENT: ADVANTAGES AND CONSTRAINTS

All types of crops grown in different agro-ecologies can benefit from the use of biofertilizers. Continuous use of biofertilizers enables the microbial population to remain and build up in the soil and helps in maintaining soil fertility contributing to sustainable agriculture.

Biofertilizers keep the soil environment rich in all kinds of micro- and macro-nutrients via nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant-growth-regulating substances, production of antibiotics and biodegradation of organic matter in the soil. Growing crops using biofertilizers is advantageous in protecting the soil from degradation. Biofertilizers can mobilize nutrients that favour the development of biological activities in soils. In this way, they prevent micro-nutrient deficiencies in plants and guarantee better nutrient uptake and increased tolerance to drought and moisture stress, all factors that strongly contribute to soil fertility.

Excretion of antibiotics and acting as pesticides

The use of biofertilizers can promote antagonism and biological control of phytopathogenic organisms. Thus, positive effect on soil microbiology is exerted: suppression or control through competition of pathogenic populations of microorganisms present on the soil.

Strategies for biological control of fungal species in crops include application of biofertilizers obtained from biological digestion to control target pests and pathogens. Through the siderophores and antibiotics produced by them, biofertilizers are antagonistic to foliar or rhizosphere pathogenic bacteria, fungi and insects.

Arbuscular mycorrhizal fungi (AMF) have the potential to reduce damage caused by soil-borne pathogenic fungi, nematodes and bacteria. Meta-analysis has shown that AMF generally decrease the effects of fungal pathogens. A variety of mechanisms have been proposed to explain the protective role of mycorrhizal fungi. The major mechanism is nutritional, because plants with a good phosphorus status are less sensitive to pathogen damage. Non-nutritional mechanisms are also important, because mycorrhizal and non-mycorrhizal plants with the same internal phosphorus concentration may still be differentially affected by pathogens. Such non-nutritional mechanisms include activation of plant defense systems, changes in exudation patterns and concomitant changes in mycorrhizosphere populations, increased lignification of cell walls and competition for space for colonization and infection sites.

Recently, several fungal endophytes, like *Trichoderma* spp. (Ascomycota) and Sebacinales (Basidiomycota, with *Piriformospora indica* as a model organism), which are distinct from the mycorrhizal species, have attracted scientific attention. These fungi are able to live at least part of their life cycle away from the plant, to colonize its roots and to transfer nutrients to their hosts, using mechanisms that are not clear yet. They are receiving increasing attention, both as plant inoculants easier to multiply *in vitro* and as model organisms for revealing the mechanisms of nutrient transfer between fungal endosymbionts and their hosts.

Trichoderma spp. have been extensively studied and used for their biopesticidal (mycoparasitic) and biocontrol (inducer of disease resistance) potential, and have been exploited as sources of enzymes by biotechnological industries. Now it is speculated (on the basis of convincing evidence) that *Trichoderma* spp. also induce many plant responses. Among the most important of them are the increased tolerance to abiotic stress, nutrient use efficiency and organ growth and morphogenesis.

On the basis of these effects, these fungal endophytes may be regarded as both biopesticides and biostimulants.

Improvement of physical and chemical properties of soil

Biofertilizers contribute to better physical conditions in the soil through improvement of structure and aggregation of soil particles, reducing compaction and increasing the pore spaces and water infiltration. They improve soil structure and allow better tilth; ensure better soil aeration and water percolation, reducing soil erosion. Biofertilizers serve as major food source for microbial populations thus keeping the soil alive. They also contribute to soil chemical conditions through improvement of nutrients availability in the soil, leaving free elements to facilitate their absorption by the root system; improved capacity of nutrients' exchange in the soil resulting in favourable effects on the physico-chemical stability of soils. As a result of the good structure and improved stability provided to the soil, root growth is promoted.

The maintenance of good soil structure in all ecosystems is largely dependent on mycorrhizal fungi. Formation and maintenance of soil structure is influenced by soil properties, root architecture and management practices. The use of machines and fertilizers are considered to be responsible for soil degradation, which is a key component of soil structure. Mycorrhizal fungi contribute to maintain good soil structure through the following processes:

- growth of external hyphae into the soil creates a skeletal structure that holds soil particles together;
- external hyphae create conditions that are conducive to the formation of micro-aggregates;
- enlargement of micro-aggregates by external hyphae and roots to form macro-aggregates;
- directly tapping carbon resources of the plant to the soils. This process influences the formation of soil aggregates, because soil carbon is crucial to form organic materials necessary to cement soil particles. The hyphae of AM fungi are more important in this

process than the hyphae of saprotrophic fungi due to their longer residence time in soil. In addition, AM fungi produce glomalin (12–45 mg/cm^3), a specific soil protein with still unknown biochemical nature. Glomalin has a longer residence time in soil than hyphae, allowing for a long persistent contribution to soil aggregate stabilization. The residence time for hyphae is considered to vary from days to months and for glomalin from 6 to 42 years. Glomalin is considered to stably glue hyphae to soil. The mechanism is the formation of a 'sticky' string-bag of hyphae which leads to the stability of aggregates.

Enhance crop yield even under ill irrigated conditions

Biofertilizers increase the water and nutrient holding capacity of the soil and also increase the drainage and absorption of moisture in soils, especially in those with structural deficiencies or lack of nutrients. They increase the tolerance towards drought and moisture stress. In this way, they increase the crop yield even in plantations that lack sufficient natural water supply or irrigation. For instance, AM association improves the hydraulic conductivity of roots at lower soil water potentials and this improvement is one of the factors contributing towards better uptake of water by plants. Moreover, leaf wilting after soil drying does not occur in mycorrhizal plants until the soil water potential is considerably lowered (approx. 1.0 MPa). Mycorrhiza-induced drought tolerance can be related to factors associated with AM colonization such as improved leaf water and turgor potentials and maintenance of stomatal functioning and transpiration, greater hydraulic conductivities and increased root length and development.

Eco-friendly and pose no danger to the environment

The most important and contributing function of biofertilizers is considerable reduction in environmental pollution and improvement of agro-ecological soundness. Biofertilizers are eco-friendly organic agro-input compared to chemical fertilizers. They cause no harm to ecosystems and are valuable to the environment as they enable reduced use of chemical fertilizers in the production of crops worldwide. Namely due to their eco-friendly characteristics, the demand for biofertilizers is on the increase during the last decade. Their activities influence the soil ecosystem and produce supplementary substances for the plants. Providing continuous supply of balanced micronutrients to the plants and eliminating plantar diseases, biofertilizers enhance the maintenance of plant health and contribute to soil ecology. The provided food supply and impelled growth of beneficial microorganisms contribute to sustain the ecological balance. In the long run, biofertilizers are planned to complement and, where appropriate, replace conventional chemical fertilizers, resulting in economic and environmental benefits.

LIMITATIONS

The term 'biofertilizer' itself means 'live fertilizer'. The quality of biofertilizers demands not only profound study of the microbial characteristics, but also elucidation of the precautions and limitations of their use at laboratory, at production as well as at field level.

Biofertilizers offer a wide range of opportunities for the development of better agro-practices due to the advantages and benefits provided for the soil, crops and farmers. However, there are limitations of these practices that are clearly recognized. These limitations demand feasibility studies to be carried out to find better solutions for each particular case in agricultural activities.

Some of the major limitations are shown below.

Lack of regulatory acts and facilities for testing the samples

Future research on biofertilization should be focused on identifying the options available to tackle the issues and offer valid frameworks for development of environmentally friendly practices around the world that allows improvements on the efficiency and consequent supply of product for the industry in the global economies. What is more, technical tests must be carried out to verify their safety at global scale. Current research of the use of biofertilizers in different regions of the world is necessary to obtain a framework that facilitates the development of future investigations in the agricultural sector and, consequently, promote the reduction of environmental impacts associated with the continuous use of chemical fertilization.

Insufficient popularization of biofertilizers and low level of farmer acceptance

Biofertilizers are a technological innovation that has the potential to increase crop yield, reduce production cost and improve soil conditions. Biofertilization comprises an innovative approach to sustainable agriculture involving scientists, technology developers, policymakers, entrepreneurs and farmers.

Despite having various potential activities, biofertilizers have not yet gained popularity among farmers for adequate acceptance. There are a variety of factors affecting the acceptance of biofertilizers by farmers. By knowing the different constraints or problems faced by farmers in the use of biofertilizers, the extent of acceptance of biofertilizers can be increased by tackling these issues and problems.

BIOFERTILIZERS APPLICATION FOR SUSTAINABLE ECONOMIC DEVELOPMENT: ADVANTAGES AND CONSTRAINTS

Biofertilizers are inexpensive to farmers because of low costs and their ability to help improve soil structure, texture and water-holding capacity in agriculture. However, farmers are not aware of biofertilizers' usefulness in increasing crop yields sustainably. Their lack of awareness about the concentration, time and method of biofertilizer application; about the efficacy of biofertilizers compared to their familiarity with the use of conventional and tested inorganic fertilizers is a serious limitation of their wide-scale application. In addition to these main problems, there are also financial (lack of timely availability of financing and/or lack of subsidies), technical (lack of guidance from expert personnel, non-availability of biofertilizers and inadequate water facilities) and other constraints (lack of interest or confidence in different biofertilizer practices).

Furthermore, entrepreneurs lack knowledge and skills for correct application of biofertilizers and have limited capacity to support considerable marketing strategies about this. The policymakers need to strengthen their efforts in popularization of the adoption and diffusion of biofertilizers, and encouragement of their competition with the well-established inorganic fertilizer industry. The concept behind the government technology promotion policy is to inform the farmers about the broad range of alternative technologies available and proved efficient. Promotion of active farmer participation in adaptive research to enhance product understanding and at the same time to create demand is envisaged.

In order to promote sustainable agriculture, both central and local government authorities have to support extensive application of biofertilizers. In this context, emphasis in attaining higher yield and better quality crops is being given in several directions: the production of inoculants; extension programmes for the farmers to know how to apply inoculants; and demonstration and awareness programmes to show farmers the benefits of inoculated crops.

Possible risks for the safety of consumers and the physicochemical and biological stability of soils

High contents of ammonia can burn the foliage and roots of plants; the presence of manure could increase the amount of weed flora. The presence of heavy metals (e.g. mercury, chromium and lead) pose a threat due to their carcinogenic potential and their capability of bio-accumulation and bio-magnification in the food chain. For this reason, the use of manure to fertilize soils should be well assessed.

BIOFERTILIZERS APPLICATION FOR SUSTAINABLE ECONOMIC DEVELOPMENT: ADVANTAGES AND CONSTRAINTS

Decline in the population of bacteria under certain climate conditions and influence of surrounding microflora and fauna

Biofertilizers, on application to seeds, roots or soil, mobilize the availability of nutrients by their biological activity in particular, and help build up the microflora and in turn the soil health in general. However, their bio-efficacy is dependent on many biotic and abiotic factors. Unfavourable climate conditions (changes in temperature and humidity) can cause a decline in the bacterial populations. Similar negative effects on bacterial quantity can be imposed by the surrounding microflora and fauna, which compete with the introduced beneficial microorganisms for nutrients and other vital factors in the micro-ecological niches. Antagonistic microorganisms already present in the soil compete with microbial inoculants and often do not allow their effective establishment by outcompeting the inoculated population.

Another contributing factor are the non-specific host–inoculant relationships, different physical and chemical edaphic conditions, poor competitive ability against native strains and deficiency of adequate formulations. For instance, the efficiency of plant-associated nitrogen fixation by diazotrophic bacteria may be hampered by a limited supply of energy and substrates.

Requirements for application

Extensive and long-term application may result in accumulation of salts, nutrients and heavy metals that could cause adverse effects on plant growth, development of soil organisms, water quality and human health. Excessive application can generate extreme levels of nitrogen, ammonia and salts that could lead to significant reduction of plant growth and problems for farmers and the soil. Large volumes are required for land application due to low contents of nutrients, in comparison with chemical fertilizers, because main macronutrients may not be available in sufficient quantities for growth and development of plants. Also, there could be some nutritional deficiencies caused by the low transfer of micro- and macro-nutrients.

Thus, the implementation of biofertilization techniques requires monitoring of environmental variables involved in metabolic processes, acquisition of biological inputs, capital investment, time and trained personnel. In order to achieve sustainable agriculture, it is necessary to implement plans, programmes, projects and initiatives directed towards the minimization of environmental impacts and consequent benefits for farmers and producers.

CONSTRAINTS IN BIOFERTILIZER PRODUCTION TECHNOLOGY

An important characteristic common to most biofertilizers is the unpredictability of their performance. It is of vital importance for the consistency of biofertilizers performance to be improved. And the performance is dependent on the biofertilizer production technology.

Although the biofertilizer technology is a low-cost and ecofriendly technology, several constraints limit its application or implementation. These constraints are technological, infrastructural, financial, environmental, human resources unawareness and quality. The different constraints affect the production technology, the marketing and use of biofertilizers.

Technological constraints

Despite significant improvement of biofertilizer technology over the years, the progress in the field of biofertilizer production technology is not satisfactory. Technological constraints faced by both organic and conventional farmers in adoption of organic farming practices are focused on the following aspects:

Strains for production

The use of inappropriate, less efficient strains for production of biofertilizers may lead to insufficient population of microorganisms and is a significant constraint. Lack of region-specific strains is one of the major constraints, as biofertilizers are not only crop specific, but soil specific, too. Additionally, the selected strains should have competitive ability over other strains in a range of environmental conditions, and ability to survive both in broth and in inoculant carriers. Another problem may be the high level of contaminants. Therefore, the good biofertilizer product must contain a good effective strain in an appropriate population and should be free from contaminating microorganisms. Furthermore, in case of problematic soil (acidic, saline and alkaline), biofertilizers cannot be used due to decrease in their efficiency; when the temperature is high, biofertilizer application is also not successful. Poor application of biofertilizers can be expected in case of unfavourable phosphorus in the soil. And finally, biofertilizers tend to mutate during fermentation, thereby raising the production and quality control cost. Extensive research work on this aspect is urgently needed to eliminate such undesirable changes.

Technical personnel

Inadequate and inexperienced staff and not technically qualified one can contribute to technical problems with biofertilizer technology.

Lack of technical information and skills about the biofertilizers application is a big constraint with high intensity, because farmers are not given proper instructions about the application aspects. Poor organization of the application process and lack of spare time for applying biofertilizers at sowing time; lack of knowledge about inoculation technology by the extension personnel and the farmers is another important problem.

The majority of the marketing sales personnel do not know proper inoculation techniques. Biofertilizers, being living organisms, require proper handling, transport and storage facilities.

Quality of production units

Lack of qualified technical personnel in production units may lead to inappropriate manipulations and handling during production.

Quality of carrier material

Unavailability of good quality carrier material or use of different carrier materials by different producers without knowing the quality of the materials can impose serious problems in biofertilizers application efficiency.

Unavailability of a suitable carrier, in which bacteria are allowed to multiply, is a major reason for shortening the shelf-life of biofertilizers. According to the availability and cost at the production site, a choice of carrier material must be made. The good quality carrier must have good moisture-holding capacity, be free from toxic substances, serializable and readily adjustable to pH 6.5–7.0. Under climate conditions where extremes of soil and weather conditions prevail, there is yet no suitable carrier material identified capable of supporting the growth of biofertilizers. Better growth of bacteria is obtained in sterile carrier and the best method of sterilization is gamma irradiation.

In the carrier-based biofertilizers, the microorganisms have a shelf-life of only six months. They are not tolerant to UV rays and temperatures higher than 30 °C. The population density of these microbes is only 10^8 cfu/ml at the time of production. This count decreases day by day. That is why the carrier-based biofertilizers are not very effective and popular among the farmers.

Possible measures to mitigate these disadvantages include use of sterile carriers and installing centralized unit of sterilizing facilities; identification of common carrier materials in different countries based on availability and recommendation to the producers.

The alternative is the so-called liquid biofertilizers. Liquid biofertilizers are special liquid formulations containing not only the desired microorganisms and their nutrients, but also special cell protectants or chemicals that promote formation of resting spores or cysts for longer shelf-life and tolerance to adverse conditions. The shelf-life of the microbes in the liquid biofertilizers is two years with a count as high as 10^9 cfu/ml, which is maintained constant. They are tolerant to high temperatures (55 °C) and UV radiation. Since these are liquid formulations, the application in the field is also very simple and easy. They are applied using hand sprayers, power sprayers, fertigation tanks, etc. Developing suitable alternate formulations, i.e. liquid inoculants/granular formulations for all bioinoculants requires standardizing the media, the method of inoculation etc., for the new formulations.

Quality of inoculants

Production of inoculants without understanding the basic microbiological techniques threatens the inoculants quality, and consequently, their efficiency. Possible removal of the seed coat from the seed due to rubbing the seed with the biofertilizers solution, may result in poor germination. Inadequate formulation of the products can be a serious barrier to the commercialization of biofertilizers. However, the demand for high-quality inputs triggers innovation improvement.

To formulate inoculants of high quality, the following considerations have to be taken in mind: identification/selection of efficient location/crop/soil-specific strains for N-fixing, P, Zn-solubilizing and absorbing (mycorrhizal) to suit different agro-climatic conditions; applying biotechnological methods for strain improvement; exchanging cultures between countries of similar climatic conditions and evaluating their performance for better strains for a particular crop; checking the activity of cultures during storage to avoid natural mutants.

Shelf-life of inoculants

The short shelf-life (usually 6 months) requires efficient storage. This discourages entrepreneurs from producing more than what they could immediately sell as well farmers from buying more than what they immediately need because they could not store the product for a long time. In countries where most biofertilizers in the marketplace are imported, generally they are not tailored to the local conditions in terms of shelf-life and storage environments. For instance, the biofertilizers that require storage in a cool place for an extended shelf-life are not suitable for countries where temperatures are usually quite high. Thus, it is not surprising that such products will not meet the quality standards, probably as a result of loss of viability in the inappropriate storage conditions. That is why product formulation, taking into consideration product shelf-life under variable storage and handling conditions is critical.

BIOFERTILIZERS APPLICATION FOR SUSTAINABLE ECONOMIC DEVELOPMENT: ADVANTAGES AND CONSTRAINTS

The problems in the development of the biofertilizer sector usually are associated with low demand due to lack of awareness and understanding of biofertilizers. In many cases production remains a challenge, not only because of its cost, but also because of the restricted demand and the poor delivery mechanisms that could be associated with the particular requirements for handling and storage conditions. The product shelf life, the quality of carrier materials, the storage conditions (e.g. temperature), handling (e.g. transportation), as well as the presence of contaminants affect the field performance and, consequently, the adoption rate. It is thus important to improve the shelf-life of locally formulated biofertilizers in various storage conditions to ensure product viability over a significant time period.

Infrastructural constraints

Facilities for production

Non-availability of suitable facilities for production is a major infrastructural constraint. In addition, inadequate availability of inputs and unavailability of inputs at appropriate time impose another problem. Employing microbiologists in production units to monitor the production and developing cold storage facilities in production centers is a good approach do improve production infrastructure.

The biofertilization suffers from inadequate marketing facilities and unavailability of regular information regarding the use of biofertilizers, which imposes uncertainty and risk among farmers.

Equipment

This shortage of essential equipment, power supply, etc. leads to increase in labour, since the production process in this case is slow and time consuming.

Laboratory, production, storage space

Space availability for laboratory, production, storage, etc. is very important. To expand biofertilizer production, extra land is needed for growing, for example, green manure crops. The lack of provision of subsidy and trading of biofertilizers at reasonable price are other important issues. However, the increasing demand for biofertilizers and the awareness among farmers in the use of biofertilizers have facilitated the biofertilizer manufacture and encouraged the entrepreneurs to get into biofertilizer production.

Storage of inoculant packets

Lack of facility for cold storage of inoculant packets is a problem that threatens the quality of biofertilizers, since they have to be stored in a cold place, away from direct sun or hot wind. The inadequate storage facilities may expose biofertilizers to high temperatures, which are unfriendly conditions.

Financial constraints

Funding

Non-availability of sufficient funds and problems in getting bank loans. The total use and price of inorganic fertilizers are continuously increasing. Meanwhile, their use efficiency is still low, and pressure on their application is coming from regulation/environmental concerns. Alternatively, biofertilizers (which are renewable) offer high use efficiency, relatively low price and minimal environmental impact. Currently, their financing is getting better.

Sale returns

The biofertilizer industry is vulnerable to less returns by sale of products in smaller production units. This is a major problem to face, since organization and operation of large production facilities is multifaceted due to scientific, economic, social and environmental problems that have to be handled.

Physical and environmental constraints

Seasonal demand for biofertilizers

Biofertilizers demands are of seasonal character, and so are the requirements for biofertilizers supply, and consequently, the biofertilizer production and distribution are done only in a few months a year. The biofertilizer producers face a challenge to design improved formulations tailored to local conditions and to supply them in a mode that satisfies the spatial and temporal variability of crop responses. Thus, extensive research on the technology to develop formulations that could satisfy these requirements is necessary. Without such research, the producers will not be able to benefit from the full potential of biofertilizers.

Cropping operations

Biofertilizers application is generally dependent on the other cropping operations demanding simultaneous activities. The short span of sowing/planting in a particular locality must be considered as well. Thus, biofertilizers must be applied in appropriate doses following a recommended method. Any use of adhesives of poor quality and with strong doses of plant protection chemicals will diminish the biofertilizer application efficacy.

Soil characteristics

Soil characteristics like salinity, acidity, drought, water logging, etc. are of vital importance. High soil temperature or low soil moisture, extreme acidity or alkalinity in soil, poor availability of phosphorus and molybdenum and presence of high native population or presence of bacteriophages, should all be considered, since they affect the microbial growth and crop response. For instance, the field performance of biofertilizers, e.g. *Rhizobium* inoculants, is affected not only by the characteristics of the plant (crop genotype) and the inoculant (the microbial strain), but also by the environmental conditions (i.e. soil and weather), as well as the agronomic management.

The soil pH affects the microbial population, i.e. the survival of the strain, and the nutrient availability. This effect and the relationships to the availability and survival of beneficial microorganisms in the biofertilizers applied to soil can be summarized in the following way:

Indicator	pH decrease	pH increase
Population of beneficial microorganisms (*Rhizobia*)	Low	High
Strain survival	Low	Low at pH > 8.5

A healthy population of microorganisms beneficial to plant growth is difficulty to support at low pH. Legume response to inoculation in soils with high acidity is week. Limited availability of nutrients such as P and Mo negatively affects nodulation and reduces the *rhizobia* population, thus having a negative effect on BNF. In mineral soils, the pH range of maximal P availability is quite small (pH 6.5–7.0). For Mo the situation is relatively acceptable for 5.5 < pH < 7.5, where the availability of Mo increases with pH, particularly at pH levels > 7 and drastically decreases at pH < 5.5. High reactivity of phosphate with aluminum, iron and calcium, and the subsequent precipitation makes it unavailable to plants. In field conditions with acidic pH and low phosphorus, the nodulation process is adversely affected. In such situations, lime could be used to improve the pH.

The effect of soil pH, however, depends on the type of biofertilizers. Several field experiments using cyanobacteria on different types of soils found that urea N inputs could be reduced by 25–35% with application of this biofertilizer in the cultivation of rice in acidic and saline soils. However, the product was less effective in calcareous and neutral soils. Hence, the efficacy of a biofertilizer depends on whether the microbial strain can survive in field conditions. Consequently, there is a need to understand the optimum pH for each type of biofertilizer in the various agro-ecological conditions.

The availability of nutrients is another important soil characteristic that has to be considered. This is particularly true for phosphorus (P). It has been shown that application of inorganic P fertilizers in combination with biofertilizers increased soybean yields by ≈ 47% over the negative control in soils with low P content. Furthermore, *rhizobial* activity and BNF is enhanced by increased availability of P. Hence, P is among the limiting nutrients for legume BNF in most plants and selected biofertilizers have shown the ability to improve the plant P uptake. This means that a reasonable approach to improve BNF efficiency through improved P availability and uptake is to perform co-inoculation of effective *rhizobia* inoculants and biofertilizers. Thus, in arid saline soils where the availability of P and K (potassium) is limited, use of phosphate-solubilizing bacteria (PSB) showed improved availability of the nutrients. Following the improvement of the performance of chemical P fertilizers by PSB, some companies have promoted increased sales of chemical fertilizers alongside biofertilizers. Combination of biofertilizers and low-cost fertilizer materials such as rock phosphate may represent an important market opportunity.

Soil drought represents a stressful environment for plants to survive. Biofertilizers application can prove to be of benefit in drought-prone areas, since it enables the crops to survive through improved water-use efficiency. This potential of biofertilizers is a promising tool to augment seasonal drought episodes that significantly contribute to yield gaps. For instance, field trials in Africa have shown that *rhizobia* inoculation improves the yield of alfalfa, fenugreek, cluster bean, field pea and common bean grown in drought conditions.

The putative mechanisms of action of selected biofertilizers to improve crop resistance to drought are as follows:

Biofertilizer	Mechanism of action	Benefits
Mycorrhiza (AMF)	Enhance the host capability for osmotic adjustment.	• Continued water uptake even in dry soils (and soils becoming dryer) contributing to plant survival in drought conditions; • Increased photosynthesis and better osmotic adjustment under drought stress.
***Rhizobium* (BNF)**	Production of phytohormones	• Changes in root morphology and physiology resulting in increased water and nutrient uptake; • Enhanced nodulation, increased dry weight of nodules, better nitrogen fixation and crop yield.

Human resources and quality constraints

Staff competence

Inadequate human, financial and material resources can compromise the production and application of biofertilizers. Lack of technically qualified staff in the production units is a serious problem. This constraint is in direct connection with the lack of proper training and adoption of technical qualifications for production of biofertilizers. Improving the technical and human capacity for quality control of biofertilizers has also been identified as critical for adequate biofertilizer market realization. Supportive government policies therefore appear important to ensure that only high-quality biofertilizers are legally sold.

Educational and training in biofertilizers

In general, the main problem is lack of proper training in organic farming and inadequate knowledge of field functionaries about organic farming. Additionally, lack of suitable training in the production techniques and skills about improved methods of biofertilizers making; lack of awareness about the concentration, time and method of biofertilizer application; lack of knowledge about different pesticides are other important issues that have to be considered in the view point of human resources and quality constraints.

Technical training on the production and quality control to the producers; rendering technical advice and projects to manufacturers; organizational training to the extension workers and farmers to popularize the technology; to arrange better and wider dissemination of information are measures that should be considered.

Production techniques

The most important difficulties arise due to ignorance on the quality of the product by the manufacturer due to lack of quality specifications and requirements by both the production management and consumers.

The governmental support for the production and use of biofertilizers may lead to promising results. Thus, various Asian countries have achieved increased use of biofertilizers through support of the government. For example, in Thailand, the production and use of biofertilizers drastically increased as a result of the support of the Ministry of Agriculture to the sector. A similar government initiative was reported in India.

Many countries have mandated the national biotechnology institutions to address the biosafety issues to ensure that products are safe to plants, animals, humans and the environment, while creating an enabling environment for innovation. The trends in investment in biofertilizer production are indicating positive results. However, given the risk imposed by the short shelf-life and the lack of guarantee of offtake of biofertilizers, the production resource generation is very limited.

Quality specifications and quick quality control methods

Quality control and regulation of biofertilizers is important to ensure conformity to prescribed standards, product safety and efficacy. The sale of poor quality biofertilizers through corrupt marketing practices results in loss of faith among farmers. Poor quality biofertilizers can be expected in the market when the quality control framework is not well-defined, resulting in poor field performance. Adherence to specified quality standards by manufacturers is important to ensure only adequate quality products are allowed at the market. Recurrent monitoring of products in the market is important to ensure product quality in the full commercialization chain.

An assessment on biofertilizer products revealed that a great number of the product formulations did not match the product labels due to the absence of the active ingredients or the presence of contaminants. Enforcement of quality standards could significantly contribute to mitigate this constraint. Well-defined requirements for quality would also facilitate the approval process of biofertilizers.

The non-availability of quality provisions and quick quality control methods is the reason why biofertilizer production and specifications are vulnerable to compromising. For instance, in

South Africa, the first commercially manufactured inoculant was produced in 1952. However, due to poor quality products on the market, in the 1970s an independent quality control system was introduced to ensure that the products could match the best quality inoculants produced in other countries.

Quality standards at par among different countries could facilitate the regional trade. One approach is to align the standards with those in countries with significant history of biofertilizers use, such as India, South Africa, New Zealand, France, Australia and Canada among others. In that way, the consumer protection will be improved, while facilitating trans-boundary trade. For instance, in these countries, *rhizobium*-based inoculants should contain at least 5×10^7–10^9 colony forming units (CFU) of the active ingredients (i.e. microorganism strains) per gram of the biofertilizer product. Meanwhile, no contaminants should be detected at 10^5 dilutions. In Australia, Canada, China, New Zealand, Thailand, the USA, as well as most of the countries in the EU, self-regulation of the biofertilizer industry has been established. Here, the industry pays for the quality control. In countries like Canada, France and Uruguay, the government plays a role in the quality control of biofertilizers. For instance, in France, despite the long history of biofertilizer use in agricultural production, manufacturers are still required to generate sufficient data to support the quality, efficacy and safety of novel products.

Regulation

Lack of effective regulation on biofertilizers is among the greatest contributors to low availability and adoption of the products. Research to improve the agricultural application of biofertilizers is often disrupted through lack of awareness, infrastructure and human resources, as well as the absence of a supportive regulatory and policy framework. The potential benefits of biofertilizers can remain largely unexploited due to inadequate policy and regulatory framework. Low demand for biofertilizers can be possibly a result of bad regulatory environment.

Effective regulatory environments can significantly reveal the potential of biofertilizers use. To ensure that proven technologies do not compete with poor-quality biofertilizers in the marketplace, effective regulations for improved quality control are required to promote fair trade and market growth for biofertilizers. Lack of appropriate regulatory framework about the quality of the products leads to poor facilitation of production, distribution and use of biofertilizers.

Another obstacle in the use of biofertilizers is the difficult procedures in registering new products. Poor management of fertilizers and supplements (e.g. biofertilizers) registration can rise obstructions to innovation and limit the accessibility to novel products that otherwise would improve farmers' competitiveness. Most EU, North American and some Asian countries have established appropriate regulations in order to control such difficulties and create a favourable business environment for biofertilizers.

For example, the Canadian Food Inspection Agency (CFIA) has well-structured and precisely defined procedures accepted by the industry for the registration of biofertilizers. This is a good practice in clear administrative processes that allow biofertilizer businesses to operate in a secure environment and to attract new investors in the biofertilizer industry.

However, in many countries, no such administrative guidelines have been made available through regulations, resulting in difficulties in the introduction of new biofertilizer products on the market. There is a need for a common framework covering policies, laws, regulations, standards and institutional arrangements to guarantee the prospect of the biofertilizers industry. The key constraints that such a framework will combat include:

- Inadequate or incomplete policies and guidelines for regulation of biofertilizers and biopesticides;
- Multiple and often overlapping regulatory mandates by responsible authorities;
- Limited capacity, including staff, skills and laboratory for product monitoring;
- Inadequate enforcement of quality control for biofertilizers and biopesticides;
- Lack of biofertilizer- and biopesticide-specific regulations, standards and guidelines;
- Weak institutional arrangements with limited collaboration between relevant authorities.

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BIOFERTILIZERS TECHNOLOGY: AWARENESS, MARKETING AND FUTURE PERSPECTIVES FOR SUSTAINABLE DEVELOPMENT

Contents

"Sustainable development is development that meets
the needs of the present without compromising
the ability of future generations to meet their own needs"

Brundtland Commission, 1987

AWARENESS ON BIOFERTILIZER TECHNOLOGY

Biofertilizers technology as an inalterable part of sustainable agriculture has to fit the basic requirements for its main dimensions. The biofertilizers technology has to be:

- *Appropriate*: to suit the social and infrastructural situations of the end-users;
- *Economically feasible and viable*: to be applicable by all farmers, regardless of their financial status and position, concerning the return on investment;

- *Environmentally friendly*: enriching the environment or, at least not harming the existing agro-ecological conditions;
- *Stabile*: the positive aspects of the technology must remain stable in long-term perspective;
- *Efficient*: mode of utilization of inputs to convert them into useful and eco-sound outputs;
- *Adaptable*: adaptable to existing local conditions;
- *Socially acceptable and sustainable*: acceptable by different societal segments and satisfying personal needs;
- *Administratively manageable*: practically implementable under certain bureaucratic structure;
- *Culturally desirable*: fits the various cultural patterns of society;
- *Renewable*: use and re-use without significant additional inputs;
- *Productive*: rate and amount of production per unit of land/input; yield per unit of area (or labor input, or investment) as a dimension of sustainable agriculture.

However, successful promotion of biofertilizers technology in sustainable agriculture depends on implementation of programmes for raising awareness among the biofertilizers producers and consumers. Biofertilizers are apparently an environmentally sound and farmer-friendly renewable source of low cost agro-input. However, bioinoculants, especially those regarded as broad spectrum biofertilizers (*Azotobacter*, *Azospirillum*, phosphate-solubilizing bacteria and arbuscular mycorrhizal fungi) have not received the deserved attention. The reason for this is mainly due to the inadequate awareness of the extension workers and the farmers about the benefits of biofertilizer technology. This unawareness regards the biofertilizers' utility, short shelf-life, lack of ready availability in time and in the desired quality, inconsistency in results with their application. Other problems in the adoption of the technology by the farmers are due to the different methods of inoculation applied. A complication rising unawareness is the fact that no visual difference in the crop growth immediately after biofertilizer application is observed in comparison with that of inorganic fertilizers. In addition, there are socio-psychological constraints that lead to unawareness of biofertilizer technology: lack of motivation form extension agencies; low credibility of source of biofertilizers; farmers' belief that chemical fertilizers are more effective than biofertilizers; lack of use of biofertilizers by fellow farmers or their application being not permitted in farmers' culture.

Lack of awareness of biofertilizers is a major challenge for farmers, the private sector (i.e. agro-dealers), extension services and policy makers. Insufficient understanding of the technology obstructs the diffusion of innovation that could have otherwise been facilitated by awareness creation through dissemination of information by different channels and stakeholders. The awareness of the key stakeholders in biofertilizer technology can also be improved by national and international research organizations, as well as by the biofertilizer industry through participatory demonstration trials. After that those stakeholders could, in turn, train farmers in their communities. Demonstration trials are a good approach to increase awareness and the use of novel products by farmers; they are more useful when there is participation by various stakeholders. *Inter alia*, government support may play an important role in promoting the increased use of biofertilizers among farmers and market growth for the products. In some Asian countries, for instance, biofertilizers are supported by the government through national projects on development and use of the technology. Zonal production facilities, state departments and state agricultural facilities, public sector firms and cooperatives also produce biofertilizers. Private industries obtain subsidies from the government to cover the cost of plant and equipment for production. Farmers can get awareness of the biofertilizer technology through efforts to increase the availability of the products, research and extension for education and effective marketing strategies.

Considering these obstacles, it is apparent that, to raise awareness in biofertilizer technology, proper education of the extension personnel, dealers and farmers about their significance and economic feasibility of application is needed. Thus, extensive knowledge, practical training, adoption and perception are obligatory elements of putative approaches to better understanding and application of biofertilizer technology.

MARKETING CONSTRAINTS

By 2018, the worldwide market for biofertilizers is anticipated to exceed a market worth of US$ 10.2 billion. The top consumers of biofertilizers are Europe and Latin America, mainly because in the countries from these regions, there are stringent regulations imposed on chemical fertilizers. These are followed by Asia-Pacific, which control more than 35% of the market. Market growth together with the effective regulation of biofertilizers, are crucially important for increased availability and use of biofertilizer products. To ensure market growth of biofertilizer products, several important constraints have to be overcome.

Instability of the inputs and outputs markets

The minimum availability and adoption of agricultural inputs including biofertilizers may be considered as (at least partial) explanation for the instability of the inputs and outputs markets. In general, when farmers obtain a value/cost ratio higher than three to four, the willingness to adopt a novel agricultural technology increases as a result of the market opportunities.

Lack of developed marketing channels and infrastructure

Poorly developed marketing channels and infrastructure, due to limited involvement of the private sector in the distribution of inoculants and the limited farmer awareness about and access to inoculants, affects the biofertilizer market negatively. Countries that have succeeded in enhancing the biofertilizer market growth have implicated a strategy focused on reduction of distribution costs, and consequently, the costs of the products. For instance, with the increased soybean cultivation in Brazil in the 1960's, application of biofertilizers (i.e. *Rhizobium* inoculants) was immediately adopted. Use of *rhizobia* inoculants in North America is a practice that has been continuing for more than a century. The European Union encourages the use of biofertilizers by advising farmers to optimize the application of chemical fertilizers or replace them partly or completely with biofertilizers that are considered environmentally friendly.

Initiatives for promotion of biofertilizer business sector

The government purchase of large portions of biofertilizer products for distribution to farmers can ensure a continuous market for producers. Associations formed by manufacturers to coordinate the commercial sector issues in the development of government policy, are another effective instrument to encourage the biofertilizer business. In addition, non-governmental organizations and international research centers may also contribute to the increased use of biofertilizers. All these cumulative activities by the government, research institutions and industry players have put the biofertilizers branch at the forefront in the sustainable agroindustry.

On the contrary, weak linkages with private sector manufacturers, local stock holders, NGOs and small-holder farmers; the poor support of production, distribution and use may negatively affect the availability and adoption of biofertilizers. Therefore, the biofertilizer market growth will require a strong public–private partnership and enough commitment to improve. Lessons learned up to now combined with sufficient awareness creation may be useful to build the partnership to increase the awareness and understanding of the technology. As the profitability of biofertilizers is demonstrated through participatory demonstration trials and output markets, the demand is expected to increase, and consequently, the biofertilizer (i.e. input) market.

FUTURE PERSPECTIVE OF BIOFERTILIZERS

Uncontrolled over-application of chemical fertilizers by farmers during intensive agricultural practices has led to excess nutrients (particularly P) accumulation in soils, which, as a result, makes the soils dead. That is why, nowadays, the production of efficient and sustainable biofertilizers for crop plants, wherein inorganic fertilizer application can be reduced significantly to avoid further pollution problems, represents major research interest. It comprises undertaking short-term, medium and long-term research programmes combining the efforts and scientific potential of soil microbiologists, agronomists, plant breeders, plant pathologists, nutritionists and economists to work together.

The most important and specific research needs should highlight following points:

Selection of effective and competitive multi-functional biofertilizers

Microorganism(s) with multifunctional properties and biofertilizers containing more than one microorganism are currently gaining special attention. Although currently most biofertilizer products consist of a single function microorganism such as nitrogen-fixing bacteria, emphasis is given to the production of bacterial isolates that could be developed as multifunctional biofertilizer microorganisms. The multi-strain consortia confer additional characteristics to the biofertilizer they comprise in respect to improvement of crop plants growth and performance, as well as in enhancement and maintenance of soil fertility.

There is evidence that a multifunctional consortium of different strains of *Rhizobium*, phosphate-solubilizing bacteria and fungi, arbuscular mycorrhizal fungi, and free-living nitrogen-fixing *Azotobacter* strains improves the noduling ability, nitrogen content and herbage yield (up to two-fold) of subabul seedlings (*Leucaena leucocephala*) in comparison with the application of each component of the consortium alone.

On the market, there are approved products comprising multi-strain consortia that express a defined positive effect. Two such products are Bio-N® and Bio-Spark®.

Bio-Nitrogen or Bio-N® is an organic/multi-microbial inoculant fertilizer for rice and corn. It was developed by the National Institute of Molecular Biology and Biotechnology (BIOTECH, the Philippines), in the early 1980s. It contains two species of the nitrogen-fixing bacteria *Azospirillum* isolated from the roots of the grass *Saccharum spontaneum* L. It can fix and transform atmospheric nitrogen into a form usable by crops, enhance shoot growth and root development, make plants resistant to drought and pest attack, and increase the yield and milling recovery of rice. Bio-N® was originally developed for corn plants. After field tests on the preparation efficacy for rice and corn and high value crops, its application was widened. Further research helped for prolongation of its shelf-life from three to six months, and currently efforts are concentrated on finding an alternative microorganism carrier, different from the soil-dust charcoal.

Bio-Spark® was initially developed as a composting agent. Further, it was strengthened to become a biofertilizer and bio-control agent. Bio-Spark® is a product of more than two decades of research and experimentation. In 2002, the Trichoderma series was registered as a biofertilizer with the Fertilizer and Pesticide Authority (FPA) under the brand name BioCon®. With a new investor in 2010, BioCon® was renamed BioSpark Trichoderma®. BioSpark® is a multi-microbial inoculant which consists of three different *Trichoderma* species (*T. parceramosum*, *T.*

pseudokoningii and a UV-treated strain of *T. harzianum*). The fungus is an effective biological control agent against soil-borne pathogens and biofertilizers, as it enhances the growth of plants. The further intensive R&D work resulted in significantly improved quality and marketing of BioSpark®. Its shelf-life was increased from six months to two years.

Another approach for assembling multifunctional biofertilizer preparations is to use indigenous microorganisms that have all the desired characteristics and are present in compost. Among these important characteristics are plant-growth-promoting, phosphate-solubilizing and antagonistic actions towards pathogens. Thus, multifunctional biofertilizer products based on composting are designed and produced applying the following methodological approach:

- Isolation and screening for indigenous microorganisms at each stage of the composting process, that confer at least two important characteristics, e.g. ability to solubilize phosphate and to produce indole-3-acetic acid (IAA);
- Development of these indigenous microorganisms into biofertilizer products;
- Evaluation of the effects of the products on the growth of a model plant and the contribution of N_2 to the plants in a greenhouse trial. Selection of combinations of strains that significantly enhance plant growth through promoting nitrogen-fixing effects or solubilizing insoluble inorganic phosphate compounds or hydrolyzing organic phosphate to inorganic P or stimulation of plant growth through hormonal action such as production of IAA.

Such combinations of microbial isolates that could be developed as multifunctional biofertilizers could be a good opportunity for sustainable agriculture.

Quality control systems for the production of inoculants and their field application

The interest in biofertilizers is also increasing due to their potential for use in sustainable agriculture. However, many of the products that are currently available worldwide are of poor quality. The formulation of an inoculant is a multistep process that results in one/several strains of microorganisms included in a suitable carrier, providing a safe environment to protect them from the harsh conditions during storage and ensuring survival and establishment after introduction into soils. A key issue in formulation development and production is the quality control of the products, at each stage of the production process.

The successful application and use of biofertilizers for the agricultural system is restricted by several limitations:

Non-reliable efficacy: the efficacy of most biofertilizers is doubtful, since their mechanism of action in promoting growth is not well understood, despite the extensive research in this direction.

Effect of abiotic factors on biofertilizers efficacy: it is still not clear how variations in soil type, management practices and weather affect the biofertilizer efficacy.

Field trials performance: It is still difficult to test inoculants in the field as routine experiments.

The proper quality control mechanism of biofertilizer production and application covers the whole experimental process: from microorganism isolation, through laboratory screening of the isolated strains for plant growth; greenhouse screening for plant growth promotion; field screening of the most effective microbes in cropped soil; readjustment and refining of inoculants; environmental impact test and, finally, production.

Since quality is the parameter on which the acceptance or rejection by the end-users, the farmers, depends, it is one of the most important factors influencing the progress of the biofertilizer industry.

The quality specifications of biofertilizers differ from country to country and may contain the following parameters:

- The microbial strain(s) used; the quality of biofertilizers is usually defined in terms of two important characteristics: presence of a recommended strain in the required quantity and in active form.
- Microbial density at the time of manufacture and at the time of expiry: the number of selected microorganisms in the active form per gram or milliliter of biofertilizer. The guidelines used are limited to the density of the available microorganisms and their viability and preservation.
- The permissible contamination; it is important to set control schemes that account for putative contaminating microorganisms.
- The expiry period;
- The pH, the moisture and the carrier;
- The final biofertilizer product has to manifest the major effects for quality management. These effects are used as indicators for the biofertilizer properties. The list of the major effects must include those of the guaranteed activities of the biofertilizer. Thus, there must be a system that allows distinguishing between the resident microorganisms, targeted microorganisms and the supplementary compositions on the effects of the biofertilizer. If the final results of the three experimental schemes are the same or cannot be confirmed statistically, then the product is just an organic matter. This means that the effects of microbial products have to originate from the guaranteed microorganisms and this should be presented in details as a prescription.

Quality has to be controlled at various stages of production as well: during the mother culture stage, carrier selection, broth culture stage, mixing of broth and culture, packing and storage. In China, for example the main quality parameters of biofertilizers are as follows:

- Appearance;
- Living target bacteria: fast and slow-growing *Rhizobium*, nitrogen-fixing bacteria, Si bacteria, organic/inorganic P bacteria;
- Multi-strain biofertilizer;
- Water content;
- Size;
- Organic matter;
- pH;
- non-target bacteria (contaminants);
- shelf-life.

The quality control of microbial products in favour of the customer needs a strong quality management system operating. The control management is very essential and must be performed continually. The procedure of biofertilizer quality control includes the following steps:

- Guaranteed identification of the strains;
- Guaranteed cell density of the strains;
- Assessment of the main activities as effect indicators of biofertilizers; regular inspection for quality control by the competent authorities;
- Evaluation of the effect on target crops;
- Registration under the regulation.

The quality of biofertilizers can be ensured by taking into account the following quality control constraints: legislative, environmental, technical and lack of awareness. In addition, for capacity building of the personnel engaged with quality control initiatives, regular trainings have to be organized by national/regional centres for organic farming. Training modules for laboratory analysts for field level officers and fertilizer inspectors have to be designed and implemented as a part of the quality control systems for efficient production and application of inoculants.

Study of microbial persistence of biofertilizers in soil environments under stressful conditions

The assessment of the persistence and traceability in soil of the strains applied with biofertilizers can be a big challenge. There are several important reasons for this.

1. The huge and complex population of microorganisms present in the soil and the rhizosphere.
2. The high variability of the microbial communities which reflects ecological, environmental and structural soil characteristics.
3. The large variety of agricultural management systems.

That is why one cannot choose a single qualitative and quantitative approach to trace the persistence of bio-inoculants in the soil because of the variety of organisms forming the biofertilizers. This difficulty, consequently, raises the questions about the methods to be considered suitable for monitoring the persistence of different inoculated strains. The methodological approach is of crucial importance for evaluation of the success of inoculation, consequently, the biofertilization.

The situation is further complicated due to the significant spatial and temporal variability of crop responses to biofertilization. It is due, to some extent, to the poor understanding of where and when to apply biofertilizers. On the other hand, in soils that experience stress conditions, the effectiveness of the products may be different. A biofertilizer has to be tested in variable conditions including abiotic stresses such as drought, soil acidity or low soil fertility to develop adequate recommendations for use.

During the past two decades, phenotypic and PCR-based methods have been developed to better characterize the structure, dynamics and diversity of soil microbial communities. For detection of microorganisms released in the environment, molecular methods based on PCR techniques that use natural genome polymorphism have largely facilitated and allowed discrimination at the strain level of natural and introduced organisms, minimizing the costs and the time efforts.

The PCR-based methods are predominantly molecular DNA fingerprinting methods, mainly qualitative and not quantitative. The non-culture-based methods that are usually used for assessment of the biodiversity of soil microbial communities include traditional molecular fingerprinting, sequencing or a combination thereof. However, the traditional molecular fingerprinting method based on universal bacterial primers has been found insufficient to discriminate between non-native and native microorganisms. To overcome this problem, community level fingerprinting (e.g. T-RFLP) combined with phylogenetic strain identification applying the culture-dependent approach is used as a modern approach to highlight differences in community structure and at the same time to successfully track inoculants.

The molecular marker-assisted approach, such as T-RFLP, DGGE, TGGE, appears to be particularly useful for monitoring purposes. The combination of two non-culture-based methods can assess the persistence of microbial inoculants introduced in the soil, on the one hand, and evaluate the possible changes occurring at species level for the native strains, on the other hand.

BIOFERTILIZERS TECHNOLOGY: AWARENESS, MARKETING AND FUTURE PERSPECTIVES FOR SUSTAINABLE DEVELOPMENT

Agronomic, soil and economic evaluation of biofertilizers for diverse agricultural production systems

The positive effect of biofertilizer application depends on many factors. Similarly, the evaluation of the biofertilizer application is also complex. The mechanisms involved in plant promotion may be both host-plant-specific and strain-specific. Plant-growth-promoting microorganisms, when released into the soil, are subjected to competitive conditions that may severely reduce their beneficial effects. That is, the beneficial effects due to the application of a specific biofertilizer may differ significantly under different agro-environmental conditions, questioning the efficacy of microbial-based products.

To overcome such awareness, it is important to consider which factors affect the efficacy of biofertilizers on crop productivity. The factors mostly affecting the efficacy of biofertilizers are related to the plant (agronomic), the soil and the economy of the products.

Factors related to the plant

Plants can exercise a significant effect on the strain(s) comprising the biofertilizer and on their efficacy in promoting the growth and yield. This is undoubtedly related to the plant physiological status and phenological phase of growth. Plants can modify the release of compounds from their roots depending on their nutritional status. This act results in changes (quantitative and qualitative) in the nutrients deposit in the rhizosphere. The changes themselves vary in time and space regarding the position of the root and the growth stage, causing selection of specific rhizosphere bacterial communities.

Plant roots excrete exudates that contain compounds with either stimulatory or inhibitory effect on rhizosphere microorganisms. Such compounds affect the microbial capacity of establishing beneficial relations with the plant. For instance, under P-deficiency conditions, plants release more chemical signals stimulating hyphal branching and colonization of AMF in comparison with P-sufficient conditions.

Plants can also influence the functions of soil microorganisms, such as nitrification. It is shown that increased release of genistein, a phenylpropanoid compound, significantly stimulates total AMF hyphal length, probably due to its participation in the chemical signaling leading to AMF root colonization. Phenolic acids, also exuded by roots, are responsible for the shift in soil microbial communities.

It has been suggested that rhizosphere microbial communities respond to other rhizosphere carbon pools (e.g. microbial exudates) as well. Thus the coexistence of native strains and the strains inoculated with the biofertilizer with the plant host makes the role of rhizodeposits in shaping the rhizosphere microbial community very complicated.

Despite of this complex picture, root exudates are likely to be of great importance in initiating the rhizosphere effect in very young seedlings and on emerging lateral roots. In this respect, the application of biofertilizers on seeds and seedlings would increase the efficacy of the treatment.

Factors related to soil conditions

Biofertilizers, known as microbial products, act as nutrient suppliers and soil conditioners that lower the agricultural burden and conserve the environment. Good soil conditions are imperative to increased crop production, as well as human and/or animal health welfare.

Several biotic and abiotic factors pose challenges in the successful application of commercial biofertilizers and are responsible for the efficacy of the biofertilizers as a field practice. On the other hand, there are several tools and actions which can be utilized and implemented to improve the field efficacy of biofertilizers. To guarantee the efficacy of a biofertilizer in a particular soil with a specific variety of crop is, thus, a complex task, which shall be considered by researchers, manufacturers, agricultural advisors and farmers when designing and applying a specific biofertilizer: a challenge to transform the fertilization with these products into a common practice for twenty-first century agriculture.

Abiotic factors

The shaping of bacterial and fungal soil communities is strongly dependent on soil chemical (pH, nutrient content) and physical (texture) characteristics. Soil pH has been found to be the most important factor influencing the bacterial community structure at the ecosystem level. In general, higher diversity is associated with neutral soils and lower diversity, with acidic soils. This is reasonable due to the relatively narrow pH growth tolerance of bacterial taxa. The field surveys of AMF communities in a wide range of soil pH suggest that it is also the major driving force for structuring fungal communities, thus affecting the colonization potential and efficacy of all kinds of plant-growth-promoting microorganisms included in biofertilizers.

Other abiotic factors that influence the AMF adaptation are soil temperature and nutrient availability and they can strongly influence the effect of the AMF symbiosis on plant growth.

Interaction with native soil microorganisms

Mathematical simulations showed that the most significant factors affecting the survival of plant-growth-promoting microorganisms, and thus the ability of providing beneficial effect to plants, are the competition with autochthonous bacteria, the compatibility with the exuded compounds by the plant host (rhizodeposition) and their ability to utilize them.

When introduced into the soil, the biofertilizer strain(s) begin to compete with the autochthonous microorganisms. The understanding of the ecological interactions among soil microorganisms and the impact of those microorganisms included into biofertilizers with the soil microbial populations are still limited. Lack of knowledge about these complex interactions does not allow to effectively predict the effect of inoculants introduced with the biofertilizers.

Despite these shortcomings, the research community puts great efforts in evaluating these interrelationships and their impact on biofertilizer efficacy, both in the short- and long-term, using a variety of methodological approaches. Some of the exploited methods are analysis of soil microbial biomass, soil microbial activity, soil microbial community structure and diversity. Using these techniques, it has been demonstrated that inoculation with biofertilizers containing different plant-growth-promoting microorganisms (e.g. fluorescent pseudomonad, symbiotic and free-living nitrogen-fixing bacteria, AM fungi, etc.) affects various taxonomical or functional groups of autochthonous soil microorganisms in different ways. The application of inoculums based on nitrogen-fixing bacteria can either increase or strongly reduce the local bacterial community structure and diversity, even when the inoculation is carried out with a multi-strain consortium. A symbiotic nitrogen-fixing strain has been shown to particularly affect a specific group of Proteobacteria. Many studies have confirmed a high degree of specificity of the bacterial species associated with AMF. Inoculation with AMF also significantly affects the general development of rhizospheric bacterial and fungal biomass. Once established successfully, introduced AMF have been shown to decrease the species richness of indigenous AM fungal communities in most roots.

A key factor accounting for biofertilizer efficacy is the selection of strains that express features supporting the colonization process of the root environment. In this respect, quorum sensing confers an enormous competitive advantage on bacteria, improving their chances to survive (e.g. through biofilm formation) and the ability to explore more complex niches by moving in the soil through motility. In other words, at least a minimum population level of the initial PGPR inoculum needs to be available to promote plant growth.

The efficacy of biofertilizers is also mediated by protozoa, particularly by naked amoeba, which is the most important bacterial grazer in soil. An increase in the bacterial and fungal feeding nematodes population has been observed after application of a biofertilizer composed of both AMF and PGPR. The wheat rhizosphere colonization by two *Pseudomonas* species and *Bacillus subtilis* was substantially reduced by three species of nematodes (*Caenorhabditis elegans*, *Acrobeloides thornei* and *Cruznema* sp.).

The observed relationships between indigenous and introduced microorganisms depend largely on the techniques used to assess the dynamics of soil microbial communities. The modern metagenomic approaches combined with culture-based methods for microbial quantification could clearly identify the number of microbial taxa. However, there are several important issues that still need to be resolved:

- to recognize which functions are attributable to a specific microorganism or group; the study of genes coding for important enzymatic activities or key genes in the interaction process between the inoculant and native microbial population may contribute to gain knowledge about them;

- to identify the metabolic potential of soil microbial communities in response to inoculation;
- to find the link between the effects on the soil microbial communities structure and the functional capabilities of soil microbial population;
- to identify possible functions for the application of biofertilizers specifically designed for particular soil/crops.

Economic conditions

The growth in the organic food market is a major driving force for the increasing trends in the global biofertilizers and biopesticides market. The reason for this advancement is due to the fact that future organic industry is strongly dependent upon the crop promotion and protection products free of chemicals.

The global market for biofertilizers in terms of revenue was estimated to amount to about 5 billion USD in 2011. The Asia-Pacific region was responsible for approximately 34% of the total demand in 2011. According to a detailed analysis of the current market and of the scenarios for its development in different continents, it is forecasted to double by 2017, actively in Latin America, India and China. The global market for biofertilizers is expected to exceed a market worth of USD 10.2 billion by 2018. Latin America is currently among the top consumers of biofertilizers: in Mexico, a programme to support the introduction of nitrogen-fixing biofertilizers based on *Azospirillum* was carried on 1.5 million hectares. According to estimates of the Indian National Biofertilizer Development Center (NBDC) and the Bio-Tech Consortium of India Ltd (BCIL), about 350.000–500.000 tons of biofertilizers are potentially required for Indian agriculture. European and Latin American countries are the leading consumers of biofertilizers, owing to the stringent regulations imposed to chemical fertilizers, which tend to be replaced by biofertilizers. The global bio-pesticide market was valued at $1.3 billion in 2011 and is expected to reach $3.2 billion by 2017. North America dominated the global bio-pesticide market, contributing for about 40% of the worldwide demand in 2011. Europe is expected to be the fastest growing market in the near future owing to the stringent regulations for pesticides and the increasing demand for organic products.

Global biofertilizer market revenue share, by product segment (2012)

Product segment	Global biofertilizer market revenue share (%)
Nitrogen-fixing	78.7
Phosphate-solubilizing	14.6
Others	6.7

However, slow effects of biofertilizers over chemical fertilizers and low adoption of biofertilizers by end-users is anticipated to hinder the growth of the market.

Nitrogen-fixing biofertilizers were the ones mostly consumed in the industry in 2012, accounting for over 78% of the global demand. These biofertilizers are undoubtedly agriculturally useful being applied to improve crop yield and they involve several potential benefits in environmental application. Furthermore, the demand for bio-based soil treatments due to the increasing environmental concern is also expected to stimulate the demand for biofertilizers over the next few years. In addition, increasing consumption for leguminous and non-leguminous plant products is also expected to augment the demand for nitrogen-fixing biofertilizers in the near future.

Phosphate-solubilizing bacteria are expected to show the fastest growth over the next few years because of their potential use in agriculture, namely in developing cost-effective and eco-friendly multifunctional biocontrol agents and biofertilizers. The market for other types of biofertilizers such as potash-mobilizing and zinc-mobilizing ones is saturated due to the low demand from the farmers.

The demand for biofertilizers is segmented at the market in accordance with their mode of application. The highest demand is that for seed treatment, accounting for approximately 72% of the global demand. Biofertilizers are extensively used in seed treatment due to technological advancement and rising environmental concern about the application of chemical fertilizers.

The biofertilizer demand was significantly high in North America in 2012, accounting for 32% of the global demand, owing to the presence of a large industry of genetically modified (GM) crops in the region, especially in the USA, where biofertilizers are widely used in the treatment of crops. The rest of the world ranked as the second largest region in the industry. The reason for this is the rising demand for natural food products, the environmental hazards associated with chemical fertilizers and the promotion of biofertilizers to create awareness among the society.

Asia-Pacific is expected to boost the demand for biofertilizers because of the growing demand for organic food coupled with intensive organic farming in the region. Furthermore, national governments of emerging economies such as China and India are promoting the use of biofertilizers through tax incentives and exemptions, and grants for the production and distribution of biofertilizers.

BIOFERTILIZERS TECHNOLOGY: AWARENESS, MARKETING AND FUTURE PERSPECTIVES FOR SUSTAINABLE DEVELOPMENT

An economically significant share of the fertilizer market is already allocated to nitrogen-fixing biofertilizers, phosphate-solubilizing biofertilizers, potash-mobilizing biofertilizers and other biofertilizers like zinc and sulphur-solubilizing biofertilizers. A bottleneck step in the progress of the biofertilizer industry and market growth is the lack of awareness about the concept of biofertilizers, the low rate of adoption by the farmers and the presence of low-quality products in the market that hinder its development. It would thus be important to define a legal framework on biofertilizers to protect both the reliable manufacturers of biofertilizers and the farmers utilizing an effective product from a market which allows low-quality products.

The marketing of biofertilizers should be regulated assuring a minimum quality standard of the final product. Improvement of quality standards for production and establishing a clear legal framework that guarantees both manufacturers and farmers are needed to sustain such potential economic development.

Considering the fact that 60–90% of the total applied fertilizer is lost and only 30–50% of applied N fertilizers and 10–45% of P fertilizers are taken up by crops, the application of biofertilizers can play a key role to develop an integrated nutrient management system, sustaining agricultural productivity with low environmental impact. The general goal is to reach the same crop productivity obtained without biofertilizers, but with a significant reduction of mineral fertilizers use, rather than to expect the application of biofertilizers to result in an increased yield over respective uninoculated controls. Biofertilizers have the potential to help reduce the buildup, leaching or runoff of nutrients from fields when used in the framework of an integrated nutrient management system, participating in nutrient cycling and benefiting crop productivity.

More stimuli for a wider and effective use of biofertilizers can be derived from recent knowledge on microorganisms and technological development. Use of strains cooperating with autochthonous microorganisms, or exploiting the synergies with microbial communities, as well as the inclusion of protozoa in the formulation of biofertilizers could also play a key role in the development of new kinds of biofertilizers.

Biofertilizers are profitable to farmers; they offer higher nutrient use efficiency, benefit–cost ratio, reduced requirements for chemical fertilizers and environmental benefits. As long as the cost of inorganic fertilizers is quite high and less profitable, biofertilizers will play a significant role when well-understood and correctly applied. Good practices of profitability of biofertilizers in various countries where they have been successfully applied may be useful to support policy and farmers' decisions related to incorporation of biofertilizers into their agricultural systems.

In Brazil, great savings estimated to US$ 3 billion per cropping season are realized with the reduced need for N fertilizers. Inoculation with *Rhizobium* has resulted in cost savings of US$ 1.3 billion in production cost. Soybean and other legumes are inoculated with rhizosphere bacteria instead of applying chemical nitrogen fertilization. Such microbial inoculants increase the nutrient use efficiency.

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The nutrient use efficiency can be enhanced by use of plant-growth-promoting rhizobacteria (PGPR) or co-inoculants of PGPR and arbuscular mycorrhiza fungi (AMF). The fertilizer efficiency of all biofertilizers is ≥ 90%, as there are very minimal losses due to leaching and fixation. Reducing the application rate of inorganic fertilizers when used together with biofertilizers may result in fewer nutrient losses and, consequently, in both economic savings and environmental protection without negatively impacting the yields.

Farmers generally apply excessive amounts of chemical fertilizers as a result of the low nutrient use efficiency. The cost of excessive inorganic fertilizer inputs in North America is estimated at US$ 2.5 billion per year. Farmers in Europe and North America have applied generous amounts of chemical phosphorus and nitrogen fertilizers for a long period of time. Besides the high price, this practice has negatively affected human health and the environment; hence the need to make agriculture environmentally and economically sound. Biofertilizers therefore offer a good opportunity to minimize such negative impacts on the environment and human health. For example, under the intensive farming system in Egypt, prevention of potential loss of N through leaching and significant increase in maize yield was achieved with the application of half the recommended N rate and biofertilizer, i.e. *Azospirillum*. Reducing the application rate of chemical fertilizers following the integration of biofertilizers for similar crop yields is expected to result in better economic return given that biofertilizers are considered cost effective.

Biofertilizers are many times cheaper than chemical fertilizers with a cost–benefit ratio of more than 1:10. It is reported that the application rate of chemical fertilizers could generally be reduced by 25–50% for nitrogen and 25% for phosphorus when appropriate biofertilizers are used without negatively affecting the yield performance. Mono cultures continue to dominate the market but mixed cultures are picking up fast and may surpass the single-strain inoculants in the next 5 to 7 years.

Transferring technological know-how on biofertilizer production to the industrial level

Improvement in crop production due to application of biofertilizers has been reported extensively. At present various biofertilizers are produced in large scale industrially and are available for field application. For instance, inoculants using *Rhizobium* and *Azotobacter* are produced industrially following a production technology comprising three important steps:

1) Development of strains;

2) Upscale of biomass;

3) Preparation of inoculants.

The biofertilizer production comprises blending aseptically pure bacterial broth with high cell density and sterilized carrier (e.g. peat, charcoal and/or lignite) to obtain a moist powdered formulation having high population of desired microbes. It is generally recommended for products free from contaminants to have a microbial load of approximately 10^7 cells per gram carrier. It is thought that this formulation can give optimum results of plant growth promotion in the designated crop following the recommended method of application.

The main bottleneck in the biofertilizer production at industrial level is that bacterial strains are usually developed and maintained by research laboratories and not by the production units. Further, in order to use efficient strains, research efforts must be concentrated on obtaining region-, soil- and crop-specific strains and make them easily available to the entrepreneurs in the industrial production units for scaling up of biomass yield.

As biofertilizers are live microbial preparations of very high cell density, the desired microorganisms have to be carefully monitored during the production process. It is logical, since the quality of inoculants in a biofertilizer is one of the most important factors resulting in their success or failure, acceptance or rejection by the farmers. The quality means the presence of the right type of microorganism in an active form and in desired numbers. The production stages that require quality control are:

- Preparation of mother culture;
- Carrier selection;
- Broth culture stage;
- Mixing of broth with carrier;
- Packing;
- Storage.

Testing of the culture is usually done by taking a sample from the finished product for comparison with a standard specification at the time of mixing of the broth with the carrier.

Biopesticides and biofertilizers are two important cornerstones that need intensive research to improve the quality mainly to achieve food security for the growing population and restore soil fertility. Nature has provided wide possibilities for research in these fields which need to be explored. The development of new biopesticides with multiple modes of action against pests and of biofertilizers with multi-crop growth-promoting activities is most important for sustainable global agriculture. These two study trends need to be prioritized in agricultural research by universities, research organizations, R & D wings of manufacturers for technology development to the farming community. The technologies so developed need to be transferred worldwide to achieve maximum benefits to the society.

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Establishment of "Biofertilizer Act" and strict regulation for quality control in markets and application.

Common Agricultural Policy (CAP)

The Common Agricultural Policy (CAP) and its system of European Union agricultural subsidies and programmes require farmland to be maintained in 'Good Agricultural Condition' and encourage application of particular land management activities to benefit the environment. Furthermore, some countries have included the principles of "humus/organic matter management" in these requirements and check it in the frame of the cross-compliance obligations.

CAP in EU is built on the following pillars:

- Subsidizing production of basic foodstuffs in the interests of self-sufficiency.
- Emphasis on direct payments to farmers as the best way of guaranteeing farms' income, food safety and quality, and environmentally sustainable production.
- EU (considering its 27 member-states and a number of farmers increased by nearly 70 percent) has made funding available to modernize farms, food processing and marketing structures, and to encourage environmentally sound farming. A special three-year post-enlargement funding package tailored specifically to the needs of these farmers is now providing € 5800 million to help early retirement, less favoured areas, environmental protection, afforestation, semi-subsistence farms, producer groups and for compliance with EU food, hygiene and animal welfare standards.

Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91

1) **Organic production** is an overall system of *farm management and food production* that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes. The organic production method thus plays a dual societal role, where it on the one hand provides for a specific market responding to a consumer demand for organic products, and on the other hand delivers public goods contributing to the protection of the environment and animal welfare, as well as to rural development.

2) The **share of the organic agricultural sector** is on the increase in most Member States. Growth in consumer demand in recent years is particularly remarkable. Recent reforms of the common agricultural policy, with its emphasis on market-orientation and the supply of quality products to meet consumer demands, are likely to further stimulate the market in organic produce. Against this background, the legislation on organic production plays an increasingly important role

in the agricultural policy framework and is closely related to developments in the agricultural markets.

3) The development of organic production should be facilitated further, in particular, by **fostering the use of new techniques and substances** better suited to organic production. There is a need for development of an organic-based biofertilizer for organic farming. Organic farmers are no more allowed to use manure from conventional farming.

What should be done for better sustainable future?

The applicable regulatory bodies, the policy makers, the scientific community, the product proponents, and the farmer associations/organizations should concentrate their efforts to:

- Develop and/or review existing fertilizer and pesticide policies to include biofertilizers and biopesticides;
- Enact and/or review laws on fertilizers and pesticides to include biofertilizers and biopesticides;
- Review of existing regulations on fertilizers and pesticides to include biofertilizers and biopesticides;
- Develop standards for biofertilizers and biopesticides. These should include Standards Operating Procedures (SOPs) and norms on quality, safety, efficacy, testing, labeling and registration;
- Establish institutions, facilities and human resources to facilitate the production and testing;
- Encourage regional integration efforts for harmonization of policies, laws, regulations and standards;
- Disseminate information to stakeholder groups and ensure access to approved biofertilizers and biopesticide products.

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EU POLICIES IN ORGANIC FARMING

Contents

ORGANIC ACTION PLAN – ACTIONS, AXES AND PILLARS

The European Action Plan for Organic Food and Farming (EU Commission 2004, COM (2004)415 final) introduces the basic tools for the European-wide growth of the organic sector. Action 6 is connecting the Action Plan to the instruments of the Common Agricultural Policy (CAP) and proposes full use of the Rural Development programmes in order to help organic farming in the Member States.

EU POLICIES IN ORGANIC FARMING

The enforcement of Action 6 of the Organic Action Plan requires reference to all Member States' Rural Development programmes. This reference should be organized at the level of basic discussions on the programme after through determining the aims for the execution of the activities in the organic sector in the Member States (regions) and finally, through finding the way to reach these aims by specific measures and a substantial budget for the sector.

This budget is confined, because the prevailing 1st pillar payments of the CAP claim that a part of two-thirds of the total EU agricultural budget represents national funding and in addition - co-financing is included. Thus, the Rural Development programmes devoted to organic farming have to undercut with a lot of measures decreasing the resources support for the sector. This will happen if the programme does not provide a set of measures initiated to support organic agriculture. The major parts of the programme put aims on the further execution of organic farming. These aims are realized by keeping the existing field area, increasing the number of farms, working out a product scale, and enhancing product quality by supporting processing and marketing projects.

In the course of executing Action 6, the Member States and regions can be separated in three classes:

A) Group of Member States' programmes lacking in their review and declaratory

- Declaration for organizing the Rural Development programmes - link to the EU Action

- Layout, serving as bypass for execution of Action 6.

B) Group of Member States bounding their view and vindication for some measures to the adequate Community documents and in this way - to the EU Organic Action Plan.

C) Group of Member States that has put organic farming as a priority for the Rural Development programme.

Thus, treating the Organic Action Plan and settling organic farming as a priority, the last two classes can be accepted as Member States implementing Action 6. Anyway, this does not certainly guarantee the programme quality regarding the organic farming.

The Rural development measures are allocated along three thematic axes:

- Axis 1: Improving the competitiveness of the agricultural and forestry sector. Here, measures for farm modernization, the setting up and use of advisory services, participation in food quality schemes, adding value to agricultural and forestry products, etc. are foreseen

- Axis 2: Improving the environment and the countryside. Here, agri-environmental programmes, natural handicap payments, etc. are planned.

- Axis 3: Improving the quality of life in rural areas and encouraging diversification of the rural economy. This axis includes measures for diversification into non-agricultural activities, tourism activities, conservation and upgrading of rural heritage, etc.

EU POLICIES IN ORGANIC FARMING

The Member States have implemented measures for organic farming by accepting the references of the strategic guidelines - only in Axis 2 (“Improving the environment and the countryside”), said in the Agri-environmental programme. Promotion of the organic land area is proposed nearly by all programmes, but mainly for discussions. Supplementary measures for organic farming can be track down only in some of the national and regional Rural Development programmes. Nevertheless, most of the programmes have treated organic farming as one of the priorities for certain measures as follows.

- Axis 1: Specifically, measure 121 "Farm modernisation", measure 123 "Adding value to agricultural and forestry products", measure 132 "Supporting farmers who participate in food quality schemes", and measure 133 "Supporting producer groups for information and promotion activities for products under food quality schemes".

An advantage for organic farms or projects is proposed only in a several programmes in Europe.

The analysis reflecting stipulations in Action 6 of the ongoing national and regional Rural Development programmes indicates that most Member States do not or only partly are carrying out Action 6. In fact, just few of the Member States possess opportunities to help organic farming to be used and can be considered enough performed.

Thus, the basic features for adequate execution of Action 6 of the European Organic Action Plan within the national and regional Rural Development programmes are listed in Table 1.:

Table 1. Basic features for adequate execution of Action 6 of the European Organic Action Plan

Activities	Recommendations for realisation
Larger share of 2nd pillar budget in CAP budget in the Member States (especially in „old“ Member States)	Next programming period
Effective use of Rural Development budget with targeted use for the organic sector by defining ambitious aims and delivering appropriate budgets for reaching the aims.	Current programming period
Effective support in agri-environmental programmes (no programme without organic measure, for conversion and maintenance)	Next programming period
Defining the organic farming measure as the top-level measure in the programme, with a considerable gap in the level of support to other measures.	Current programming period
No discrimination for organic farmers in agri-environmental measures, e.g. lower support level for organic land area in the same measure, maximum support level per hectare/per farm too limited to give incentive to conversion to organic farming	Current programming period
Setting priority for organic projects and farmers in Axis 1, Axis 3 and Axis 4 (LEADER) measures to support and develop the organic sector from «field to fork».	Current programming period

The analysis of the data showed an evaluation of all the Member States' and regional Rural Development programmes. It is made considering the budgets for the organic sector, and serves as a basis for new Organic Action Plan. It also outlines the performance of reform of the CAP for future Rural Development programmes development.

The European Commission have issued recommendations to the Member States for optimal exploitation of all instruments available to support organic farming within their Rural Development programmes. It is advising the development of national or regional Action Plans to be used as an approach for this exploitation. The main focus in these action plans should be put on:

- ✓ Implementation of new quality schemes to stimulate the demand side;
- ✓ Organization of activities that preserve the long-term benefits for the environment and nature protection;
- ✓ Encouragement of organic farmers to convert their whole farms into organic ones;
- ✓ Assurance of equal opportunities to organic and non-organic farmers for receiving investment support;
- ✓ Developing initiatives to stimulate producers in facilitating the distribution and marketing through integration of the production chain by (contractual) arrangements between its main players;
- ✓ Support to extension services;
- ✓ Training and education in organic farming for all engaged with production, processing and marketing;
- ✓ Making organic farming the preferred management option in environmentally sensitive areas.

The development of the Organic Action Plan, The European Rural Development Fund for the period has been established together with the EU Community strategic guidelines for rural development.

For Axis 2 measures - chapter 3.2 of these strategic guidelines a recommendation has been issued that stresses upon "*consolidation of the contribution of organic farming*", because "*organic farming represents a holistic approach to sustainable agriculture. In this respect, its contribution to environmental and animal welfare objectives could be further reinforced*".

Additionally, in the course of development of the national strategies the member States were advised to consider the EU level strategies: "*In working out their national strategies, Member States should ensure that synergies between and within the axes are maximised and potential contradictions avoided. Where appropriate, they may develop integrated approaches. They will also wish to reflect on how to take into account other EU-level strategies, such as the Action Plan for Organic Food and Farming, ...*"

Although the application of the above mentioned instruments supports the organic farming and the implementation of Action 6 of the Organic Action Plan, the quality of this support varied considerably and needs evaluation. The evaluation and the potential obstacles for further development of the sector are defined and performed through the measures of the Rural Development programmes. In fact, the evaluation is necessary to analyze whether the European Commission was successful with one of the most crucial actions of its Organic Action Plan.

ALLOCATION OF BUDGETS TO THE MEMBER STATES

The role of the environment and organic farming for the Member States economies and societies is estimated through evaluation applying the following indicators:

- Allocation of budgets for Rural Development programmes (Pillar 2) (absolute values)
- Allocation of budgets for Rural Development programmes (Pillar 2) (in relation to Pillar 1)
- Relationship of the budgets of Pillar 2 Axes 1, 2 and 3.

These valuable indicators have been applied and analysed for the EU-27. The evaluation was considered important since the EU Pillar 2 budget has been harshly reduced for the financial period 2007-2013, in contrast to the Pillar 1 budget that remained intact. On the other hand, the requirements to be fulfilled by the Rural Development programmes had to take under consideration the severe problems with the climate change and the implementation of nature preservation global programmes (e.g. Natura 2000). That is why the Pillar 2 reduced budgets needed carefully planned targeted use to guarantee the effectiveness of the programmes.

Another important indicator that have to be considered are the budget allocated to the organic farming measures in the agri-environmental programmes and the share for organic projects in other Rural Development measures.

Pillar 1 - Pillar 2 relationship

The CAP budget is divided in two main parts: Pillar 1 for market and direct aids, Pillar 2 for rural development. However, this division is quite unfavorable for the rural development, because of the following main reasons:

- ✓ The EU budget for pillar2 is much lower than for pillar1:
- ✓ Based on the EU budget for 2007, the two budgets (pillar 1 : pillar 2) ration is 77% : 23%. Speaking about total budget (EU plus national co-financing) this ratio is 67% : 33%.
- ✓ The Pillar 2 budgets require national co-financing (on the contrary, the EU funding for Pillar 1 is 100%). This fact which restricts both the interest and the ability of Member States to implement ambitious Rural Development programmes, since it is not easy to assure national co-financing.
- ✓ Pillar 1 payments only require minimum standards to be fulfilled (cross compliance), and therefore they are available to most farmers throughout Europe without the requirements these farmers to assure contribution through improvements on an economic or ecological level.

Actually, the relevance of Rural Development programmes in the Member States is estimated through the distribution of the budget for the Pillars 1 and 2. The lower financial values for Pillar 2 indicate that considerable financing for it would ensure a broader range of measures that are better financially equipped and therefore more attractive to the farmers.

The effective rural development measures require certain standards, e.g. environmental standards for participation in agri-environmental programmes. That is why, keeping in compliance with these standards lead to increased public acceptance of financial support for agriculture and rural areas.

In addition to the relation to Pillar 1, Pillar 2 absolute budget values are also rather small.

New Member States show a significantly different distribution of Pillar 1 and 2 budgets. As a rule, the Pillar 2 budgets are at least equal to the Pillar 1 budgets. As the Pillar 2 budgets are co-financed by the Member States and regions, the proportions shift towards a minimum share 60:40 (pillar 2: pillar 1). The only old Member States exhibiting a similar profile are Austria (46%) and Portugal (43%), based on EU funded budgets, and Finland (62%), Austria (60%), Luxemburg (59%) and Portugal (49%) based on a calculation with national co-financed budgets.

Pillar 2 Axis 1: Axis 2: Axis 3 ratio: inside the Rural Development programmes

The Rural Development programmes budget has to be planned in a way to support financially a wide range of measures, such as support for advisory systems and quality production, farm investment, agri-environmental programmes, infrastructure measures on community level, etc. The good financing of these measures is a prerequisite for their effectiveness. The Rural Development regulations have shaped a framework within which the Member States had the opportunity to tailor their national and regional programmes considering the local circumstances and conditions. Thus, each development programme will set out priorities that are accustomed to the country/region specific conditions. In this way, the different programmes will have an important impact on the further development and orientation of agriculture and rural areas.

EU CAP AND BIO-BASED PRODUCTS

The chemical fertilizers became free for all throughout the world in the last century. This trend resulted in immediate enlargement in the crop yield, thus lead to promotion of the profit for the farmers. Meanwhile, in recent years a serious environmental breakdown is noticed due to chemical fertilizers' permanent field application and overuse. The practice of the chemical fertilizers is related with water and soil pollution, loss of beneficial microorganisms and insects and in this way - in overall reduction of soil fertility. This motivates the modern day farmers to show interest in more eco-friendly products like bio-fertilizers that hold promising future in reducing soil quality problems with optimum crop yield.

The EU CAP promotes employment of bio-based products along with organic farming. It furnishes up to 30% of the budget as direct green payment to farmers preserving the sustainable agricultural practices. Moreover, awareness regarding the environment and demand for soil fertility and organic foods propel market sales.

Favorable regulations, especially in Europe and North America, are intended to be a key exploiter of the global industry. Also, the claim for high agricultural output to answer to human needs is appraised to achieve industrial revenues. Besides, minor product costs in parallel to affected spear would impulse industry demand from 2016 to 2024.

Meanwhile European quest for good food and good farming is rising. Human population, as well as farmers and citizens, are eager for innovation and need to receive better food and farming policies based on agro-ecological approaches.

Currently, EU policymakers have recognized the dual role of organic farming: from one hand, it is necessary to meet the consumers' demand for high quality products and from another - to ensure some public goods.

All these involve, for instance, the preservation and retrieve of water and soil quality as a result of organic land management practices.

This perception is coming out in the early 1990s, when organic farming was legally defined under EU Regulation (EEC) No 2092/91. During this time, the organic farming support payments for transformation and maintenance were established under the CAP.

For the meantime, the confession of organic farming has also expanded to other EU policy domains, such as research and some areas of market progress.

Nevertheless, it is still important in many policy areas the necessity to support climate for local and organic food chains. The EU citizens are also maintaining the EU organic market opportunities valued at EUR 20.8 billion in 2012. Thus, regardless the consumer demand progress in many EU countries, provision of such organic foods is not enough. The EU citizens prefer organic production of food, and the majority of farmers have to be encouraged for application of such methods in order to produce more organic products. So, researchers and policymakers now also confess the power of agro-ecological practices and innovation.

It is proofed that the favorable climate is crucial for organic farming and for this reason, farmers need public support for use of agro-ecological methods linked with strong demands for organic products production. At the same time, they also need to receive the policymakers' support for the development of this sector.

Here the outlines of some opportunities and challenges impacting the new and existing EU policy for organic food and farming, and the agri-food sector, as well as ways that can help to make Europe more organic are given.

FOSTERING ORGANIC AGRICULTURE IN A GREENER AND FAIRER CAP

Greening direct payments for all farmers

For the first time, public good delivery constitutes a significant part of both direct payments and rural development. Under Pillar 1 of the CAP, direct payment eligibility depends on farmers undertaking three basic agronomic practices - crop diversification, the protection of permanent grassland and the allocation of 7 % of farmland as ecological focus areas. Collectively, these are known as the greening component. This new component represents 30% of national funding for Pillar 1.

Furthermore, under Pillar 2 Member States are legally required to spend at least 30 % of their rural development budgets on environmental measures, including commitments in support of organic production and agri-environmental climate protection practices, which go beyond the Pillar 1 greening.

The introduction of greening marks the beginning of a process towards normalizing public good delivery across the entire CAP. Organic farming is deemed to be also a compliant greening factor. This acknowledges the public good delivery aspect of organic farming as the only EU-wide certified, systemic approach to sustainable agriculture. The recognition can be seen as a strong political signal from EU policymakers that they view organic farming as a priority model of agricultural sustainability, and as an active contribution to the protection and enhancement of biodiversity, as well as for climate change mitigation and adaption. On the other hand, the low level of ambition of the greening measures as well as the introduction of questionable exemptions will severely curtail the potential of greening to drive public good delivery. For instance, in the European Commission's original proposals the greening component referred to all farms. However, in the final political agreement the measures are targeted primarily at arable farmers and will probably have very little impact on livestock farming. The concept of equivalency, whereby practices undertaken as part of agri-environmental measures or special certification schemes exempt farmers from greening requirements, also weakens the greening component. Ultimately, therefore, achieving a genuine paradigm change in agricultural sustainability will require corrections and improvements to be made in subsequent reforms.

Organic farming boosts greening

The implementation of CAP regulations regarding the perspective of organic farming defines the recognition of impact on greening. The opposing effect could be expected if Member States use the recognition as a reason for neglecting the support of organic farming under Pillar 2. Thus, the Member States have to guarantee a more positive and functional promotion for the headway towards sustainable agriculture in Europe. It is considered that greening concept is coherent with strong support for organic farming under Pillar 2.

EU POLICIES IN ORGANIC FARMING

The support for the organic farming is founded in the demands set up under EU Regulation (EC) No 834/2007, and also through national legislation. It influences the coverage of the greening objectives. Thus, the Pillar 2 payments must therefore ensure explicit sustenance for organic farming, with admission playing as a positive signal of the EU's obligation to sustainability that can help to drive agro-ecological conversion throughout Europe.

Recent sustainability in rural development

The orientation of Pillar 1 regarding public goods provision is a welcome progress; measures under Pillar 2 are the basic exploiter for the growth of organic farming and considerable sustainability in rural areas.

During the new rural development programming period of 2014 to 2020, the organic farming is regarded as a step in its own right, giving opportunity to certify organic farmers or groups of farmers for a period of five to seven years, on a per-hectare basis. Payments are designed to compensate farmers for additional costs incurred and income foregone, and to cover transaction costs such as increased management efforts, certification costs and training and advice. Today, most authorities in the Member States offer organic support payments under their national or regional rural development programmes (RDPs). However, support levels differ between and within Member States, and they often fail to adequately cover all the extra costs, or to take into account the reduction in yields organic farmers might face. Therefore, organic farming support payments must represent a significant top-up compared to conventional farm support payments, in order to provide farmers with strong incentives to convert to and maintain organic farming. In the new programming period, organic farmers are also still eligible for optional agri-environment-climate payments that go beyond the requirements of organic production, such as the preservation of indigenous animal breeds or the conservation of plant genetic resources. However, the provision of combination payments is at the discretion of the Member States; it can be made organic-specific or may apply to all farmers, varying significantly across national and regional RDPs. To stimulate more far-reaching agro-ecological approaches, organic farming systems should be clearly prioritised under new agri-environment-climate schemes.

EU ORGANIC FOOD AND FARMING POLICY

RDP measures combined with organic farming support

New RDPs policy also proceeds to propose different opportunities to join organic farming aid with other RDP measures, like farm funding, diversification, advisory services, information and promotion activities, and producer groups.

Definite assistance for organic farming is now more apparent in a number of measures. The organic farmers now receive financial support for a 20 % higher rate in comparison to the previous situations, which improve farm sustainability and performance of processing, marketing and farm product development.

Also, the organic sector can also be helped by EU dissemination activities. But the new CAP Regulations have to be maintained by the Member States' farm advisory systems. Other initiatives are also convenient to strength organic sector such as: planning of non-agricultural activities; assistance in the formation of producer groups; support for measures related to the environment and climate change; short supply chains and innovation performance.

Organic farming is substantially mentioned or prioritized through measures under RDPs for 2007-2013 in some Member States. Besides, a substantial variation in supply under these measures between different countries and within EU states occurs. Many authorities do not succeed to overpass the classical payments model in agri-environmental area and to implement a more holistic model, combining organic support payments with other RDP measures. Meanwhile, the substantial visibility of organic farming regarding other RDP measures, like investments and advisory services, gives new opportunities for prevailing of organic farming in RDPs. boosting agro-ecological innovation in the new European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-AGRI).

The prevailing of the organic farming in rural development, pointed out by the EU leaders is of great importance and concerns the need for agri-ecological innovation to change European agriculture in more sustainable way. The priority in the next programme period will be innovation, established by the newly build up structure EIP-AGRI. This new EU policy instrument is also a main subject of Horizon 2020, as well as in the rural development policy until 2020.

The goal of EIP-AGRI is to build up a link between research and farming practice by enforcing stakeholders from different areas of the agri-food system. It aims to boost farmers, businesses, researchers and advisers to divide concept and trials, to implement innovative decisions, and to put the results of research projects into practice. Within the frame of the Rural Development Regulation, the EIP-AGRI claims the necessity for step ahead in the evolution of agri-ecological production systems, focusing on the crucial role of organic farmers at the core of innovation activities supported by the new RDPs.

It is considered that EIP-AGRI proposes considerable resources to foster the development of agri-ecological technology using the strengths of organic farming and accounting weaknesses, thus grasping new opportunities for innovation

The EIP-AGRI work provided will be performed by specific operational groups. It is intended these groups to set up the link between different stakeholders in order to reveal practical problems, which will be accounted as a voluntary measure in the Rural Development Regulation. In such way, the national and regional authorities instead EU officials will determine the objectives and the content of the EIP-AGRI in the Member States. For this reason, these authorities have to support the operational groups that will be responsible for solving organic and agri-ecological problems in the new initiative. It is important that EIP-AGRI will also act as a tool to facilitate the exchange of information, knowledge and expertise between projects, sectors and borders. It will connect farmers, advisors, agri-businesses, researchers as well as and civil society to create a network - the EIP Network, facilitated by the EIP-AGRI service.

Recently, a 20-member focus group on optimising organic arable yields, headed by the European Commission, is working in this area. The group is cooperating various different stakeholders, and is looking for the ways to improve yields on less productive organic farms, in a way to match better production levels of other farms having similar farming systems.

Thus, the focus group gathers existing knowledge (from scientific reports and projects, as well as practical experience) launching innovative solutions. Also, it indicatse specific areas needed for innovative research and marks the topics and criteria for work of future operational groups, as well as the approach for knowledge sharing.

Cohesion and Structural Funds: a new common framework

The 2014-2020 programming period introduces opportunities to facilitate priorities and visibility performance of organic farming, both - under RDPs and different EU policy frameworks. It is a consequence of the fact that they are linked to a new EU instrument called the Common Strategic Framework (CSF). In it, clear investment priorities are determined in respect to the financial planning period 2014-2020 in the Member States and their regions.

Thus, an effort is being made to link rural development with the Cohesion Fund and other EU structural funds. As a result of this effort, the combination of funds with other opportunities to boost EU economic growth and jobs till 2020 together with national priorities will be achieved. The CSF will be also joined to other EU policy instruments such as CAP direct payments, Common Fisheries Policy (CFP) and the EU Framework Programme for Research and Innovation, Horizon 2020.

Meanwhile, partnership agreements between the European Commission and the national and regional authorities for the next seven year period are provided. With such institutional recognition of the social, economic and environmental benefits of organic food and farming, the establishment of these agreements gives opportunities to prevail organic farming across the new RDPs and other EU policy frameworks.

CAP expend 2014-2020

Agricultural policy is the only sector that is totally funded by the EU, with the funds exhausted on annual direct payments and market measures (100 % financed by the EU). The recall is released to multi-year rural development measures, which are also co-financed through national and regional budgets.

For more of a decade, Pillar 2 measures have stated the CAP for the perspective competitiveness and sustainability of farming enterprises and for greater economic variegation and quality of life in rural areas.

At the same time, Member States have also been liable to embed their disposable finance for rural development through the so-called modulation - moving funds from Pillars 1 to 2.

Regardless the pattern for provision of considerable help for rural development during the last ten years, Pillar 2 use up is still just a part of Pillar 1. In the seven years period: 2014-2020, rural development will report for just 9 % of the total EU budget, in parallel to the 29 % given for direct payments and market measures.

A simile of forms for 2013 and 2020, for example would have a decrease of -18 % for rural development (from EUR 13.9 billion to EUR 11.4 billion) in comparison to -13 % for direct payments and market measures (from EUR 43.2 billion to EUR 37.6 billion).

If free will cadence is engaged, the decrease for 2020 would rise to -19.7 %. Now Member States have opportunity to exchange 15 % of their direct payments and rural development funds from Pillar 1 to 2, but also in the *vice versa* - from Pillar 2 to Pillar 1. Through this reverse modulation, some Member States can even move up to 25 %. Eight Member States also have opportunity to tune the percentage for specific years during the programming period.

Member states of 9 are still not clear how will solve to employ this choice, with some likely to make full use of the possible cadence alternative, while others will choose modulation to close the gap in Pillar 2 spending, which results from budget cuts.

Until the new CAP has a serious accent on public goods provision between Pillars 1 and 2, the low level of purpose with respect to greening, coupled with the threats of reverse modulation and cuts to the Pillar 2 budget, could seriously undermine support for organic farming. However, other measures could potentially contribute to the development of more sustainable food and farming in Europe.

EU ORGANIC LEGISLATION AND POLICY DEVELOPMENTS

Besides the support under the CAP, EU legislation on organic food and farming has proceeded to develop since EU Regulation (EEC) No 2092/91, which was stated in the early 1990s. The growth process also involved a full checkup of the Regulation, culminating in the acceptance of EU Regulation (EC) No 834/2007. From its reception, rules on the performance have been agreed to detail the organic production, as specification of rules on organic wine, organic yeast and organic aquacultures.

EU organic regulations look for to execute a coherent approach to consumer protection, suspending devious contest and providing common standards for organic production, labelling and marketing in the EU.

Meanwhile, private and other national organic standards, based on the EU claims imaging the specific cultural, structural, geographic and climatic diversity of different Member States and regions, motivate establishment of innovation in organic standards across the sector.

As they form the only EU-wide sustainability label for food, organic standards and certification can facilitate sustainable agriculture, through emphasizing the increase of sustainability across the whole agri-food sector.

The organic farming policy and legislation starting by the European Commission in 2012 culminated in the progress of a new EU Organic Action Plan in 2014, and the substitute of EU Regulation (EC) No 834/2007 in the next EU legislative period 2014-2019. It raises the growth of the EU framework for organic food and farming by achieving a balance between policy bidding and legislative needs.

This could influence the partial and uncertain gathering of data and the anticipating of the production by market quest, to the lack of peculiar organic inputs such as seeds, young animals and protein feed, as well as the administrative load that discourages smaller-scale farmers and operators.

In fact, the current Regulation came into force in 2009. Hence, the goals, objectives and principles of the existing Regulation are not still entirely revealed by the evolution of further rules. Also, any complementary improvements to the regulatory and policy framework have to be understood in the sense of existing progress in organic farming. This process is facilitated through tied in and concerted solution between EU organic legislation and the new EU food and farming policy structure till 2020, such as the CAP and Horizon 2020.

Organic legislation fits the objective

The legislative goals and objectives are linked with chance of considerable variations in respect to time and challenges regarding organic food and farming across the EU. It is important to know, that the small improvements to EU legislation or new regulation based on organic principles or market forces, done by EU policymakers can cause significant effect on the organic sector's future in Europe.

Such evidence is that, a market-driven way could emphasize too much influence on market-orientated outcomes, go after fewer strict legal requirements and spending organic principles. For instance, if any discharge agreed to Member States under the current Regulation, or introduction of option of national ministries allowing imports happens, a serious adverse effect on the realization of sustainable organic agriculture could occur. This will tolerate the risk of contest deformation between Member States. In order to make organic production close to the principles set out in the Regulation, an approach of standards strengthening is keeping. However, it is necessary flexibility or exemption rules available to Member State to be maintained in order to fit the purpose for sustainable development of organic farming. For this reason, sector realities in different Member States and regions must be considered. So long as some delivery is no longer needful, then others will call for changes in line with the evolution of standards that the organic sector has performed since the onset of the Regulation. Anyway, the prompt elimination of all exclusive rules would be unthinkable today, since their amount stays crucial for definite production sectors and in geographical areas where the organic sector is still in its beginning.

Thus, combination of the different ways is necessary to assure a good account within the basic norms of organic farming and the long-term evolution and enlargement of the European organic sector.

Alterations, like input of group certification systems (currently only accepted in non-EU developing countries), or the demand that processors and traders have measured the environmental performance of their activities or present opportunities, are considered.

A set of certification systems in the EU for instance, would allow groups of small-scale farmers to receive certification as single unit, thus reducing the bureaucratic load of certification.

In addition, better environmental output insistence for processors could influence the sustainability standards minding by organic growers and livestock producers in order to move EU organic food to an even wider concept of sustainability.

Regulation (EC) No 834/2007 has been an important driver of the organic sector in Europe.

A new regulation should proceed to favor the growth of the sector by fostering a process-orientated approach that progress standards in the line of the basic organic farming principles.

Consumers and producers should work together to help the progress of the sector with EU and national policy frameworks giving to this dynamic through new EU and national organic action plans.

A NEW EU ORGANIC ACTION PLAN

The EU food and farming policies provokes interest of organic farming, and policymakers begin to value the multi-layered importance of organic systems and sustainable food and agriculture.

This began from the making of high quality food products and the provision of public goods, to job creation and the promotion of the agri-food sector and rural economies. Yet, a pursuant organic policy framework with a compound of policy measures was still necessary to use the benefits ensured by organic production. Following the application of a number of actions set out in the 2004 EU Organic Action Plan, such as specific standards for organic wine and aquaculture, the Commission's notice of a new EU Action Plan in 2014 was an acceptable initiative.

A principle-driven approach helped to direct organic production nearby to the principles set out in the Regulation, while strengthening the standards. Nevertheless, it was taken in mind that if this would necessary change of units of mobility or release rules available to Member States, the approach would not be fit for purpose and could prevent the sustainable progress of organic farming. Sector reality in different Member States and regions was taken correctly into account. So long as some changes were no longer necessary, others had to be involved in agreement to standards increase that the organic sector had offered because of the Regulation. Besides, the immediate removal of all the exceptional rules, considered necessary today, some of them remain critical for certain production sectors and in geographical areas where the organic sector is still in its infancy.

For this reason, a combination of different ways is necessary to assure a convenient relation of the basic principles of organic farming as well as the durable progress and expansion of the European organic sector.

Changes, such as the introduction of group certification systems (which are currently only accepted in developing countries outside Europe), or the requirement that processors and traders measure the environmental performance of their activities, present opportunities.

Group certification systems in the EU for example, would enable groups of small-scale farmers to gain certification as single entities, thereby decreasing the bureaucratic burden of certification; and greater environmental performance requirements for processors could build on sustainability standards delivered by organic growers and livestock producers in order to move EU organic food to an even wider concept of sustainability.

Regulation (EC) No 834/2007 has been an important driver of the organic sector in Europe. A new regulation should continue to support the development of the sector by enabling a process orientated approach that advances standards in the direction of the fundamental organic farming principles. Consumers and producers should work hand in hand to support the growth of the sector, with EU and national policy frameworks contributing to this dynamic through new EU and national organic action plans.

This should foster the proceeding growth of the organic sector until 2020. The definite outcomes of the 2004 Action Plan also give momentum for growth in respect to a pursuant organic policy framework at EU level. It also involves description of achievements, as well as an assessment of the issues needed to be performed.

For example, it is admitted that better contacts are necessary between national organic actions and national and regional RDPs, as said in Action 6 of the 2004 Action Plan. In this way an establishment of considerable agreement of policy frameworks for the organic sector in Member States will be sustain (Sanders et al., 2011). This is a field, in which more work still needs to be performed in order to direct organic farming in new RDPs by 2020.

Thus, the EU and national organic action plans have to be developed supplementary for achievement of the right outcomes in Member States and regions. Therefore, all action plans up to 2020 should be supported fully from all EU policy frameworks.

In this respect, they should:

- ✓ perform considerable exploitation of all convenient instruments and measures under the CAP in the following directions: support of organic and agri-environmental measure payments; support of knowledge transfer and innovation, market development and capacity building;
- ✓ develop organic approaches in EU research programmes and innovation tools, aiming to promote substantial transition to agri-ecological approaches;
- ✓ connect the organic regulations with the horizontal legislation more effectively, such as labelling and the regulation of farm inputs;
- ✓ enhance the stock of quality protein feeds by increasing local protein feed production and using different protein sources;
- ✓ promote the disposability of organic seeds and propagating material by funding long-term breeding programmes for locally adapted and organic plant varieties that enhance agri-diversity and optimize the yield potential of organic farming;
- ✓ set up new sign share events for organic products, linked with the EU organic logo, and promoting organic farming in educational programmes and green public-sector provision;
- ✓ refine the gathering of organic data, currently collected by researchers and Member States' authorities, are not harmonized enough to be used effectively by policymakers and stakeholders;
- ✓ enhance legislation to protect the organic sector from GMO contamination;
- ✓ support the registration of organic, traditional plant protection substances under horizontal legislation.

EU RESEARCH POLICY AND ORGANIC FUNDING SCENARIOS

The ideas of organic issues have been involved systematically into the EU research policy framework during early 1990s. Up to the 1980s, research activities on organic farming had been performed predominantly by private research institutes, with the first EU projects on organic farming funded in the 1990s. After that the EU budget for organic research has risen from EUR 767 000 in 1993 to more than EUR 6 million in 2013. In such way, EU became an important investor in organic research, as well as in the development of the sector. Thus, it is decisive to realize the different EU policy tools for research and innovation, and how they can be affected.

The EU's most important funding instrument for research for the period 2014-2020 is the EU Framework Programme for Research and Innovation Horizon 2020, with a total budget of almost EUR 80 billion. As outlined above, the support for agricultural innovation implemented under the EIP-AGRI comes from both Horizon 2020 and the new RDPs. Horizon 2020 addresses three key areas: scientific excellence, industrial leadership and societal challenges. The last of these is particularly important for the agricultural sector (especially the issue of food security, sustainable agriculture and forestry, marine, maritime and inland water research, and the bio-economy). With at least 5 % of the total Horizon 2020 budget (EUR 4 billion) allocated to address societal challenges for the next seven years, the budget for these research areas has almost doubled compared to the previous programming period.

New instruments under Horizon 2020 include multi-actor projects and thematic networks. They will be used to fund specific projects contributing to the EIP-AGRI.

- Multi-actor projects are intended to involve different stakeholders (researchers, farmers, advisors, enterprises, educators, NGOs, administrations and regulatory bodies). They are targeted at the needs and problems facing farmers and other practitioners. They also seek to foster participatory research - something with which the organic sector already has broad experience, for example, through on-farm breeding programmes. Keeping in mind the sector's long history of strong collaboration across disciplines and between researchers and producers, the multi-actor approach presents good opportunities. Moreover, many of the calls for multi-actor research projects are expected to be specifically relevant to organic agriculture, for example calls related to soil quality and function, or genetic resources and agricultural diversity.
- Thematic networks, on the other hand, will focus on specific themes, mapping the current state of existing scientific knowledge and best practice. The networks will help to develop materials that are easily accessible and facilitate knowledge exchange. Like the multi-actor projects, thematic networks should involve all the relevant stakeholders, and provide a platform for actors in the organic sector to exchange their knowledge at EU level.

The EU's big investments in research are still managed by Member States. Research funds of relevance to organic farming and sustainable food and agriculture include CORE Organic, ERA-Net SUSFOOD and the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI). The EU supports these examples of Member States pooling national research funding. The aim is to establish greater coherence between EU and the national research policies.

ORGANIC PERSPECTIVES OF THE BROADER EU POLICY FRAMEWORK

While EU organic legislation and polices, such as the CAP and policies on research and innovation, affect organic farming directly and indirectly, other EU policies also have significant implications for the development of agro-ecological approaches. A paradigm shift towards sustainability in EU food and farming also depends on EU rules and regulations that empower rather that impede the growth of small and local businesses and sustainable consumption. Organic farmers have always been pioneers of sustainability of food and agriculture, offering solutions that not only benefit the rest of organic sector, but which can also inspire the entire food and farming sector.

Small and local farm businesses adaptation

A lot of organic farms are involved in on-farm processing and direct marketing. As these activities help them to create own added value through use the 2004 EU Food Hygiene Package. But it has been found that the process is difficult for the farmers of many Member States, as they put added costs to answer these strict requirements. Some farmers have had as a result to give up processing altogether. Also, Member States often do not properly implement the flexibility measures, allowing adapted rules and lesions for primary producers. The later are engaged in direct supply chains involving small quantities of primary products, or for local retailers supplying directly to consumers.

The introduced hygiene requirements influence the processing sector and cause bigger confirmation of processing facilities. For instance, many small butchers have been forced out of business due to economic constraints and difficult hygiene rules. This limits the opportunities for organic farmers to deliver their products to certified organic processors within an appropriate distance of their farm.

Food and farming are free from GMO

The majority of European consumers throw out genetically modified organisms (GMO) in food.The risk of GMO pollution of food is still not definitely concerned by the EU authorities. Thus, the organic sector still stands high costs in ceasing the risk of such contamination. Such events happened in Spain, where GM maize has contaminated organic fields, and some farmers have lost their organic certification and their premium prices. As a consequence, many of these organic farmers have ceased to cultivate maize in those traditional regions, which cause loss of local maize sorts.

In order to prevent the soy and maize from such contamination processing companies, working with soy and maize reported in 2009 costs of about EUR 20-86 per tonne.

Marketing of seed and planting material legislation

The plant genetics is working in favor of the organic farming through disposal of a broad range of species responding to consumer demand and to different geographic conditions. The growing environmental challenges in Europe such as resource depletion and climate change are of crucial importance for special care towards plant genetic resources and their preservation. Therefore, it could be possible to market new varieties and populations adapted to low input and local conditions.

EU legislation on the marketing of seed and planting material definitely restricts market access to registered plant varieties. There are strict criteria for registration and certification of plant reproductive material. This policy affords to market concentration in seed companies and loss of genetic diversity in crops. In this way, the legislation needs to be tailored to support farmers' rights and facilitate the conservation and further development of genetic resources and the diversity of crops.

Encourage young people to start organic farming

Farming community in Europe is growing old very fast. Investigations in this respect show that in 2007 1 farmer under the age of 35 relates with nine farmers over 55. Beginning from 1975 and lasting to 2007, the total farm numbers for Belgium, Denmark, Germany, Ireland, France, Italy, Luxembourg, the Netherlands and the UK decrease with more than 2.6 million, measured with loss of 83 000 farms per year. Of these, almost 1.8 million were in Italy and France alone. Substantial renewal is crucial for the evolution of economically viable rural areas and the preservation of diverse cultural landscapes, and for high quality food production, biodiversity and food cultures. Younger farmers also need access to land. As no EU-wide framework can provide an all-in-one solution, a coordinated mix of policy measures is needed, which takes into account the CAP and other EU policies and encourages young people to take-up farming. This should include a common understanding between Member States of land use policy.

Consumers are able to make informed food choices

To choose the proper food depends on many factors: from cultural proficiency to the information maintaining the transparency of production process. The EU legislation on food information to consumers can ensure consumers with detailed information about ingredients.

The organic logo is an important element linked to a certification pattern based on a broad number of sustainability aspects. The use of different labels and logos is now also actual for debate at EU level.

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BIO-FERTILIZERS MARKET SIZE

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INTRODUCTION

Soil contamination and environmental hazard from the imprudent and disproportionate application of agrochemicals on crops has been a key issue for the industry in recent times. Additionally, the risk to human health has also led to stringent regulatory framework around the use of synthetic chemicals in agriculture.

The main driving issues in fertilizer production and consumption are:

❖ All living organisms rely on a safe and healthy supply of food and nutrients, including nitrogen, phosphorus and potassium (NPK) for proper growth and development.

❖ Fertilizers are used for producing healthy and abundant plant crops.

❖ Some estimates have indicated that without commercial fertilizers, there would be a global food deficit equivalent to one-third of the current availability.

❖ Plants require 14 essential nutrients for healthy growth and, if the soil lacks any one of these, plant growth can be limited.

❖ The three macronutrients that are essential for food production and quality are NPK.

❖ In many part of Europe and Africa soil erosion is seen due to lack of soil organic matter.

Biofertilizers have emerged as the most feasible solution to these issues and have been gaining considerable market acceptance since the time they were first introduced. Biofertilizers, in addition to providing an eco-friendly option, also maintain the soil and crop health with increased efficiency.

The driving factors of the global biofertilizers market are increased demand for organic food products, promotion of biofertilizers by various government agencies to create awareness among the masses and environmental hazards associated with chemical fertilizers. The global biofertilizers market is controlled by many factors such as lack of awareness about the concept of biofertilizers, which is restraining the growth of the industry. Various advantages of chemical fertilizers are another factor, which is holding back the customers from making a switch to biofertilizers. Low rate of adoption is due to some application disadvantages associated with biofertilizers. Leading manufacturers are focused on expansion of the business in the domestic market and setting up new plant for increasing production capacity as well as product line.

MARKET OUTLOOK FOR FERTILIZERS PRODUCTION

Global state of art in fertilizers production

In June 2015 FAO/Fertilizer Organizations Working Group reviewed the prospects for fertilizer demand until 2015/19 and the supply and request balances. It was estimated that world demand for total fertilizer is expected to grow at 1.6 percent per annum from 2015 to 2019.

In response to the economic slowdown in many emerging and developing countries, persistent low international prices for most agricultural commodities, and dry conditions across South Asia, Southeast Asia, Latin America and Africa, world fertilizer demand is expected to contract by 1.0% in 2015/16, to 181 million tones (Mt) nutrients. Drops are seen of similar magnitude for the three nutrients: -1.0% for N, to 108 Mt; -1.0% for P, to 41 Mt; and -0.8% for K, to 32 Mt. Aggregate demand in 2015/16 is anticipated to rebound in the three regions where it contracted in 2014/15: Eastern Europe & Central Asia (EECA), West Asia and North America. The sharpest decline is expected in Latin America, reflecting unfavorable economic, political and weather conditions in Brazil and Argentina. African demand was hit by widespread El Niño impacts and cuts to fertilizer subsidy budgets in several countries. The poor monsoon in South Asia strongly influenced the 2015/16 winter season.

The outlook for 2016/17 is more optimistic in view of slightly improving market conditions, the expected more favorable weather, and a better political and economic situation in some sizable markets. Global fertilizer demand in 2016/17 is seen as rebounding (+2.9%) to 186 Mt, with growth rates of relatively similar magnitude for all three nutrients: +3.0% for N, to 111 Mt; +3.0% for P, to 42 Mt; and +2.3% for K, to 33 Mt. Fertilizer demand would remain almost unchanged in North America and would increase elsewhere. Demand growth in EECA is seen as firm, as grain exports are expected to benefit from the current weakness of regional currencies. Thanks to prospects for normal monsoon rains, demand in South Asia would fully recover from the downturn in 2015/16. Driven by recent political change in Argentina, demand would firmly rebound in Latin America but would not fully recover owing to persistent recession in Brazil. Expected increases in the rest of the world would be smaller.

In the absence of major economic or policy changes in the main fertilizer-consuming markets, the current context supports moderate fertilizer demand and growth prospects in the years to come. According to the baseline scenario, world demand would rise on average by 1.6% per annum (p.a.) between the base year (average of 2013/14 to 2015/16) and 2020/21. Aggregate global demand is projected to reach 199 Mt at the end of the outlook period. K demand would expand firmly (2.3% p.a. to 37 Mt); P demand would grow more moderately (1.7% p.a. to 45 Mt); and N demand growth would continue to progressively decline (1.2% p.a. to 117 Mt). This rebalancing of the N: P: K ratio reflects progressive adoption of better fertilizer management practices by farmers. The highest growth rate would be in Africa (3.6% p.a.). Demand would also expand firmly in Latin America (2.9% p.a.), South Asia (2.9% p.a.) and EECA (2.8% p.a.). Latin America would benefit from the competitive advantage of Brazil and Argentina on the global soybean, maize and sugar markets. Similarly, EECA has the potential to increase its share of global cereal trade. South Asian demand is strongly influenced by fertilizer subsidy regimes, whose evolution is highly unpredictable; high uncertainty is therefore associated with forecasts for this region. In East Asia, fertilizer demand growth is forecast to slow further (0.9% p.a.), as Chinese N and P demand is likely to reach a plateau by the end of the outlook period. Demand in developed countries is anticipated to rise marginally, with stronger prospects in Oceania. With N and P demand in China levelling off, about half the world market can be considered ‘mature’. In volume terms, South Asia, East Asia and Latin America would account for 33%, 22% and 22%, respectively, of the global increase in total fertilizer demand anticipated in the next five years.

European market of fertilizers

As regards the EU the total production of fertilizers in 2007 was close to €17 billion, up from €13.6 billion in 2004. In the EU however, the overall use of mineral fertilizers has declined in recent decades. A 2015 study by the European Parliament’s Policy Department B, Structural and Cohesion Policies, indicates that mineral fertilizer use declined by 20 % from 1995 to 2012, mainly as a result of a decrease in the use of phosphorus and potassium-based products. In the fertilizer sector 1,058 enterprises were acting, with a total of 100,000 employees. Spain, Italy and France have the largest number of enterprises (228, 187 and 175 respectively) but small and medium size companies dominate in all three countries. In contrast, 54 companies in Germany, have in total over 10,000 employees and represented more than 17% of the total production value of the EU. Some of the newer Member States (i.e. Poland, Romania, Lithuania and Bulgaria) also have a small number of large sized companies employing on average more than 2,000 employees each. However, the level of production in these countries is rather low and output does not represent more than 3% of the total EU value of production (with the exception of Poland with 8%). Besides Germany, France represents 13% of the total EU production, Netherlands, UK and Italy (each with around 10%) also account for significant parts of the European market.

BIO-FERTILIZERS MARKET SIZE

The fertilizers industry - especially the segment related to the production of the main nutrients - went through a consolidation phase during the late 1980s and early 1990s as a response to changing market conditions and other factors such the costs of the basic raw materials. Currently, the fertilizers industry in Europe can be grouped in three main categories:

- ❖ A small number of large multinational companies (not more than 7) with interests in the broader chemical sector and with global networks that focus on transforming the basic elements (nitrogen from air, phosphorous and potassium from mines) into a rather small range of straight or compound mineral fertilizers that are used for major food crops. The use as fertilizers is one category of the possible uses of their chemical products. Some of them sell their products in bulk to smaller companies for blending or further processing but most are also involved in the marketing and sale of their products for agricultural, professional or even consumer use. Available data indicate that 4-5 companies represent more than 80% of total production although there are still some independent national players in specific countries (e.g. Poland, Romania, and Greece).
- ❖ A number of mainly medium size enterprises (independent or subsidiaries of the larger manufacturers in the previous group) are focused on the production of complex liquid fertilizers and other specialty fertilizer types. They cover both the professional (agricultural) market but also the so-called hobby sector (gardening) selling directly to consumers. Many of them are focused on the national markets but significant proportion of these companies (more than 30%) also export to other European countries and some also outside the EU. However, data that are more precise are not available.
- ❖ A large number of small firms - estimated over 800 of the total of 1058 according to Eurostat - are directed exclusively on blending of fertilizers bought from large companies to cover specific needs predominantly in their local market (vineyards, fruit, and vegetables). The number and market focus of these firms varies among countries. The great majority of them focus on local or national markets17 and very few of them export to other European or third countries. The level of sophistication of the production processes may vary greatly in this sector.

Use of mineral fertilizers in agriculture represents the main segment of the market (Fig. 1). In 2008, the total consumption in agriculture in the EU27 was 17.9 million tons of nutrients (11.2 million tons N, 3.1 million P and 3.6 million K). France, Germany, the UK and Poland consume the largest quantity (nitrogen, phosphate and potash) representing together close to 50% of the total. However, the current levels of production in Europe do not match demand. Europe is a net importer of nitrogenous and phosphatic fertilizers (mainly from Russia, Ukraine, Morocco and Egypt) although it has a positive trade balance as far as potassic fertilizers are concerned.

At the same time, there has been a significant overall decrease in the use of fertilizer's among EU15 Member States during the last decade (a 14% fall). In contrast, in most EU12 Member States total consumption increased between 5% (Hungary) and 24% (Latvia). To a certain extent, this reflects changes in the intensity of the use of fertilizer's and soil improvers (expressed in terms of spending per hectare of arable land) and the different levels of horticulture and permanent crops in total agricultural land use. The economic crisis led to an even more dramatic decrease in the total consumption of fertilizer's. It fell further by 23.5% over the period 2008 and 2011 because of collapsing agricultural prices and falling agricultural production. The consumption of nitrogenous fertilizer's in the EU27 decreased by 13.5% while that of phosphoric and potassic fertilizers fell approximately by 40%.

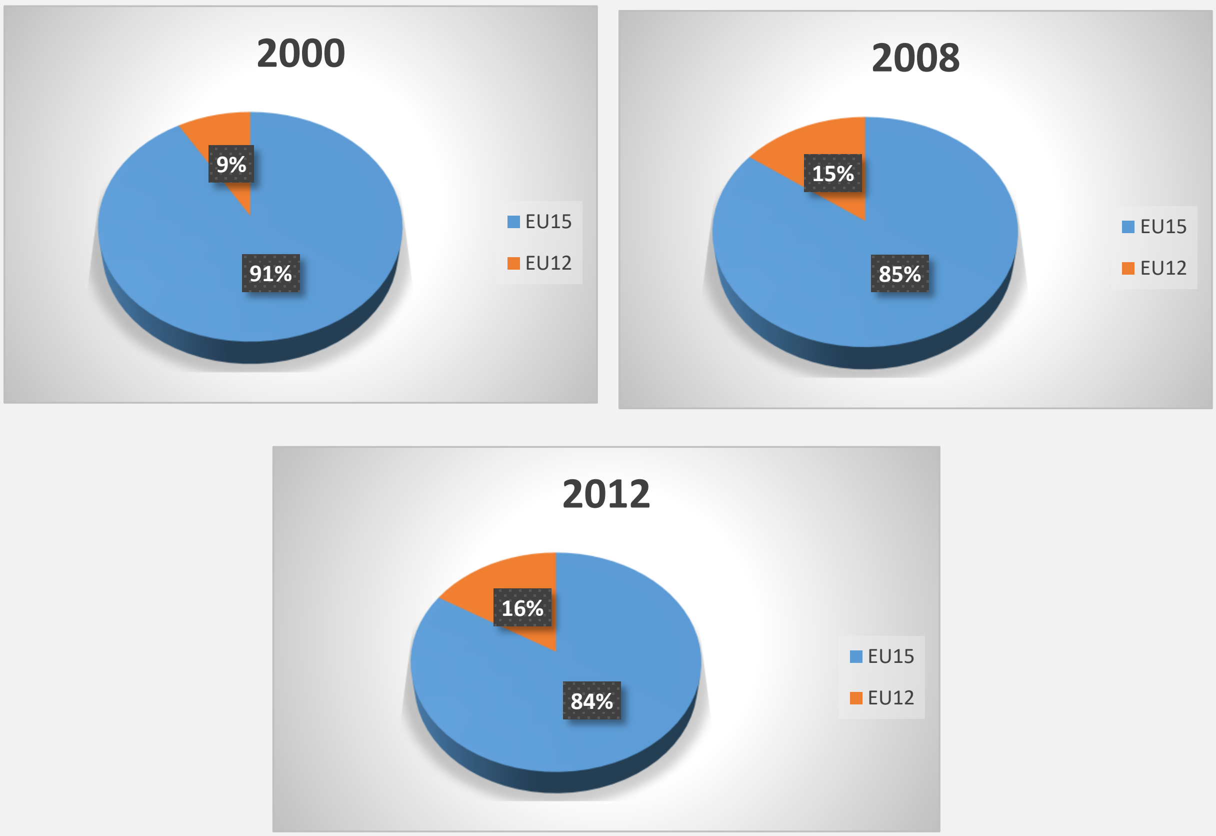

Fig.1. Development of fertilizers consumption in the EU

Finally, concerning the price of fertilizers, while the level of demand is an important determinant, energy, raw material and transportation costs also play a very important role in the cost of the production of fertilizers and their respective prices. Natural gas represents 50-70% of total production costs of nitrogenous fertilizers. The increase in the price of natural gas during the last years led to an increase in the price of fertilizers in the EU27 by around 25% and an even higher increase is expected (almost doubled in comparison to 2000).

Transition to biofertilizers production and application

Current data showed that in many developing countries, fertilizer applications are imbalanced, i.e. farmers apply too little phosphate and potash in relation to nitrogen, especially in Asia and Europe. In other countries, the „mining" of soil nutrients is severe, and yields have fallen as nutrients removed by the crops are not replaced. This problem is most serious in sub-Saharan

Africa, the Caribbean and parts of Asia. Most high-quality agricultural land is already in production. The marginal benefit of converting new land diminishes. Available land and water resources are declining in many developing countries. Future food production growth will primarily depend on further intensification of agriculture in high potential areas and to a lesser degree in low potential areas. Variations in fertilizer production and imports by China, the world's largest consumer, continue to have a major impact on world consumption. In the last decade, China has decreased its fertilizer imports by 18 percent and increased its production by about 14 percent. In EU counties (e.g. Denmark and Italy), the organic matter in soil is significantly reduced to below acceptable levels (3%).

Global competition for resources is increasing worldwide. Concentration of phosphorus mines and gas fields outside the EU makes the EU fertilizing product industry and the European society dependent and vulnerable on imports, high prices of raw materials as well as the political situation in supplying countries. The transition to nutrient recycling would therefore be a key element to increase the European food security.

The production of inorganic fertilizer is high energy intensive. It has been estimated that 2% of the world's energy production is devoted to the production of inorganic nitrogen fertilizers. In 2007, the global inorganic fertilizer industry (including nitrogen and phosphorus fertilizers) generated 465 million tons of CO_2. Nutrient recycling would contribute to mitigation of climate change via less energy demanding technologies which can combine sometimes the production of alternative energy sources (e.g. digestion of bio-wastes generating biogas and heat) thereby contributing to a transition towards a low-carbon and more sustainable economy.

Disrupted nutrient recycling is a problem for Europe and all over the world. Phosphorus and nitrogen are lost across environmental media during food production or are wasted instead of being used for plant nutrition. The leaks of nitrogen and phosphorus from human activities have led to ecological deterioration of surface water via eutrophication and "dead-sea" bottoms in coastal oceans along the EU coastlines close to mined phosphorus factories. The total losses to water and landfill are substantial and would account for 30% to 35% of the annual usage of phosphorus.

By maintaining the value of the raw materials and energy used in products from extraction to recycling, the transition towards a more circular economy can promote innovation, increased competitiveness in the sector and lead to job creation.

All of these factors anticipated the fast development of biofertilizers market size. Shift towards adopting renewable products, by periodically phasing out synthetic, toxic products and reducing carbon footprints are likely to drive this demand.

BIO-FERTILIZERS MARKET SIZE

Biofertilizers use

Although biofertilizers were first commercialized in North America and Europe, there is increasing preference towards their use in parts of Asia Pacific and South America. North America was the largest market for biofertilizers, followed by Europe. Together these markets accounted for over 50% of the global revenue. Growing preference towards organic food coupled with growing awareness regarding the hazards associated with chemical fertilizers and atmospheric pollution has resulted in high consumption in the region. In addition, strict regulatory scenario has forced many farmers to adopt biofertilizers in place of their chemical counterparts and this is expected to boost the demand for biofertilizers over the next decade. The "Common Agricultural Practice" limits and restricts the use of synthetic fertilizers while promoting the use of biofertilizers and organic farming.

Asia Pacific was the third largest market for biofertilizers, with increased demand from regional markets such as India, China and Taiwan. Asia Pacific is expected to witness double-digit growth over the period 2013 to 2020 due to the increased consumer preference towards organic food and growing agricultural activities. However, contrary to other regions, the key application of biofertilizers in Asia Pacific is soil treatment but not seed treatment.

South America is expected to show fastest growth over the next seven years on account of the growing agricultural activities in Brazil. The region is expected to grow at a CAGR of 16.4% from 2013 to 2020. Nitrogen fixing biofertilizers were the largest product segment in 2012 and accounted for over 70% of global revenue. Growing demand for nitrogen fertilizers is perceived to be a key factor for nitrogen fixing biofertilizers market development.

Nitrogen fixing biofertilizers are excessively consumed in the regions of South Asia and South America due to increased agricultural activities.

Phosphate is the second most widely used nutrient in fertilizers and witnessed consumption of over 40 million tons in 2012. However, the phosphate provided to plants in the form of chemical phosphate fertilizers is immobilized rapidly and becomes unable to plant.

Seed treatment was the largest application of biofertilizers and accounted for over 70% of the market in 2012. Treating seeds with biofertilizers helps them sustain bacteria and virus attacks and also helps increasing the yield. In addition, biofertilizers help in harnessing atmospheric nitrogen and making it available to the plant. Seed treated with biofertilizers are capable of increasing phosphorous content of soil by solubilizing it and improving availability.

Soil treatment is the other primary application of biofertilizers and it involves the spraying of biofertilizers over the agricultural land. It increases the fertility of the soil and improves the yields of the planted crop.

BIO-FERTILIZERS MARKET SIZE

Biofertilizers market segmentation

The biofertilizers market has been segmented based on product, applications, and regions. In terms of product, over the period of seven years between 2015 and 2022, the market has been divided into three major segments:

1. Nitrogen fixing biofertilizers
2. Phosphate solubilizing biofertilizers
3. Other biofertilizers, including potash mobilizing and zinc solubilizing ones (Fig. 2).

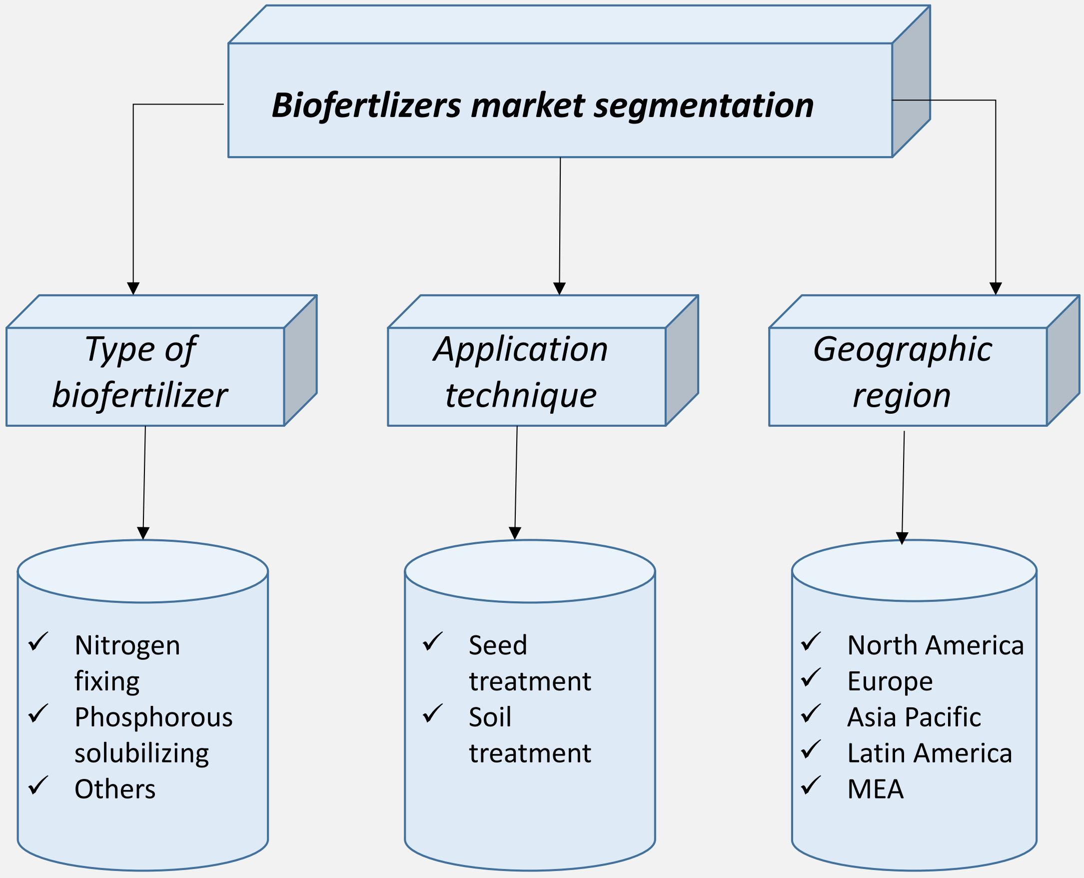

Fig.2. Biofertilizers market segmentation

Nitrogen fixing biofertilizers are made up of mixed strains of various nitrogen fixing bacteria such as *Rhizobium*, *Azospirillum*, *Acetobacter* and *Azotobacter*, and help improve nitrogen yield of the soil. Phosphate solubilizers are employed as control agents for agricultural improvement.

In terms of application, the market is divided into two major segments including seed treatment and soil treatment.

BIO-FERTILIZERS MARKET SIZE

Product and application has been segmented on a regional level in terms of revenue (USD million), where 2014 has been considered as the base year with a forecast period of seven years between 2015 and 2022.

Biofertilizers market size and growth prospects

The biofertilizers market is projected to grow at a CAGR of 14.08% from 2016, to reach USD 2,305.5 million by 2022 (Fig.3). The market is driven by factors such as: i) increase in demand for fertilizers due to the rise in global food production and ii) development of new biofertilizer manufacturing technologies. The high growth potential in emerging markets and untapped regions provide new growth opportunities for the players in the biofertilizers market. On the other hand, some factors restraining the biofertilizers market are lack of awareness and low adoption of biofertilizers coupled with poor infrastructure.

Global biofertilizers market is expected to witness substantial growth over the period 2015 – 2020 on account of providing physical barrier against pests. In addition, these products protect plants against pathogens and enhance absorption of zinc and phosphorous. In addition, use of biofertilizers in agriculture aids the decomposition of organic residues and stimulates overall plant development and growth. Growing need for high agricultural yield in order to meet increasing population demands has triggered the use of biofertilizers because of low environmental impact. Increasing need for organic foods among consumers in expected to have a positive impact on the biofertilizers market over the next five to seven years. Moreover, rising of chemical fertilizer prices coupled with commercial response to growing food cost is expected to be one of the key drivers for biofertilizers market over the period 2015 - 2020.

BIO-FERTILIZERS MARKET SIZE

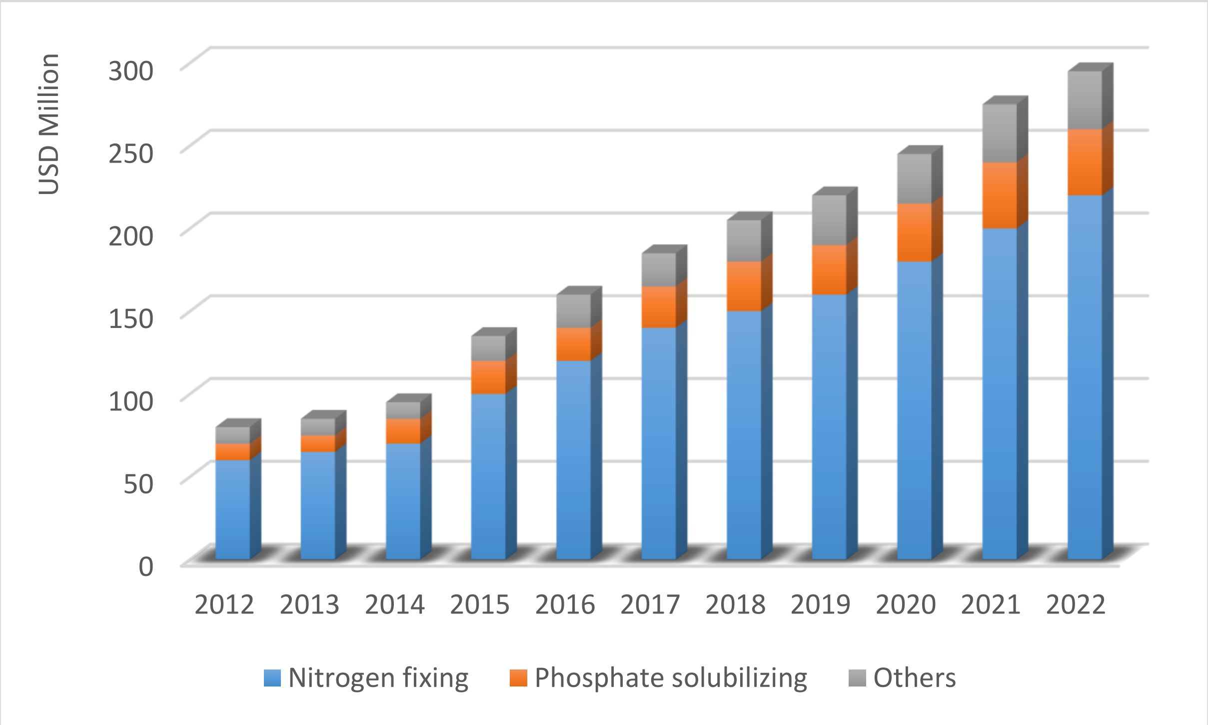

Fig.3. Global biofertilizers market revenue, 2012 – 2022 (USD Million)

Key issue in bioertilizers market growth and acceptance is the industry value chain. It consists of raw material producers & suppliers, biofertilizers producers, distribution channels, and end-users (farmers, domestic cultivators). Biofertilizers are produced through various sources such as ley crops, frying oils, potato peels, manures, slaughterhouse wastes, organic domestic wastes, and food industry residues. There is also considerable presence of feedstock suppliers, who outfit the biofertilizers producers. For example, Swedish Biogas is an integrated company that manufactures biofertilizers as a byproduct of biogas production. The company also supplies raw materials to independent biofertilizers producers. Most of the raw material suppliers incur costs in terms of logistics, i.e. raw material procurement and delivery to manufacturers. With most of the raw materials being bio-waste, profitability of suppliers is high which is estimated at approximately 10% of value addition.

Majority manufacturers of biofertilizers are integrated across different stages of the value chain as the demand of the product is largely dependent on growth of the end-use industries. 90% of total biofertilizers manufactured is used in the production of corn, rice, and maize.

Organic food and beverages are naturally derived products, without comprising synthetic chemicals, and food additives. Key product forms of organic foods include organic fruits, vegetables, meat products, naturally derived alcoholic beverages such as wine and beer. Increasing consumer awareness regarding the adverse impact of inorganic food on human health has resulted in industry trend shift towards promoting organic food market and is expected to remain one of the key factors for biofertilizers market over the forecast period.

Biofertilizers market share by product

Nitrogen fixing biofertilizers dominated the market, accounting abput 79% of global revenue in 2012 (Fig. 4). Nitrogen-fixing biofertilizers are used for leguminous as well as for non-leguminous crops, especially, when growing rice and sugarcane. The nitrogen-fixing segment growth is attributed to the fact that nitrogen-fixing biofertilizers are the most commonly used biofertilizers, across the globe. Substantial R&D efforts have been carried out in the last couple of decades, along with increasing awareness of the farmers; these are the major reasons driving the growth of this market. Rising importance of nitrogen fixation to increase intake of numerous compounds such as nucleic acids and chlorophylls in plants is expected to have a positive impact on market over the forecast period. However, market presence of synthetic fertilizers is expected to pose a credible threat to nitrogen fixing biofertilizers demand over the next seven years.

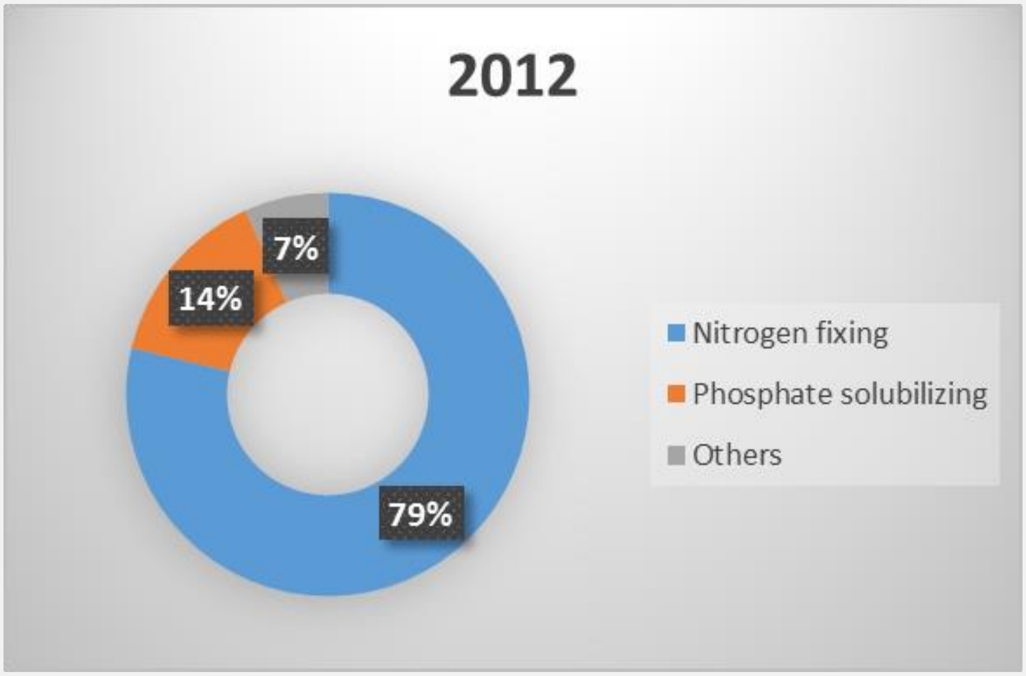

Fig.4. Global biofertilizers market revenue share by product

Phosphate solubilizing bacteria accounted for 14% of global biofertilizers market in 2012 and is expected to account for about 18 % of revenue share by 2022. These products are majorly used to convert low molecular weight organic acids into soluble nutritional product forms. Other product types include potash mobilizing, zinc and sulfur solubilizing biofertilizers. Above-mentioned product forms jointly held 7 % of global biofertilizers market revenue in 2012.

One of the upcoming trends expected to stimulate the growth prospects of this market is the introduction of liquid biofertilizers. Last are liquid formulations containing the desired microorganisms, micronutrients, and chemicals promoting the formation of resting spores. This helps the biofertilizer to attain a longer shelf life and tolerance to adverse conditions - shelf life of nearly two years, and tolerance to high temperatures and ultra-violet radiations. Furthermore, the microbe density in such biofertilizers is higher in comparison to solid biofertilizers. They are applied using power sprayers, fertigation tanks, hand sprayers, and as a basal manure mixed along with farmyard manure. These liquid biofertilizers also have a very high enzymatic activity, leading to the high adoption rate amongst farmers.

The global liquid fertilizer market is expected to grow at a CAGR of around 3% by 2020. The depletion of soil quality has pushed the use of fertilizers that helps farmers to increase the crop yield by three to four times. The surge in crop acreage and the growing requirement to boost crop production are stimulating many farmers to use liquid fertilizers as plants can immediately absorb these substances thus offering faster outcomes. Small-scale farmers are also purchasing liquid fertilizers to reduce their dependency on weather conditions and get an increased yield even in damp, wet, or windy weather. Additionally, there is also a rise in the demand for the proper use of fertilizers as the degradation of soil quality is leading to micronutrient deficiency in crops worldwide.

APAC (Asia Pacific) will be the fastest-growing region in the market during the period 2012 – 2020 due to the increase in hydroponic system field areas, availability of fertilizers at subsidized rates, and rise in mechanization, which has resulted in the increased adoption of technologies such as liquid fertilizer sprayers. Some of the major fertilizer-consuming countries in the region include Australia, Indonesia, Malaysia, the Philippines, Thailand, Vietnam, Japan, South Korea, China, India, Pakistan, and Bangladesh. The demand for fertilizers will see tremendous growth in the region owing to the surge in programs that promote balanced fertilizer use.

Biofertilizers market share by application

Seed treatment was the largest application segment, accounting for 72% of global biofertilizers market revenue in 2014 (Fig. 5). Plant seeds are treated with biofertilizers in order to prevent bacteria and virus attacks that reduce crop yield. In addition, biofertilizers help in binding atmospheric nitrogen and making it available to the plant. Seeds treated with biofertilizers are capable of increasing phosphorous content of soil by solubilizing it and improving their availability.

BIO-FERTILIZERS MARKET SIZE

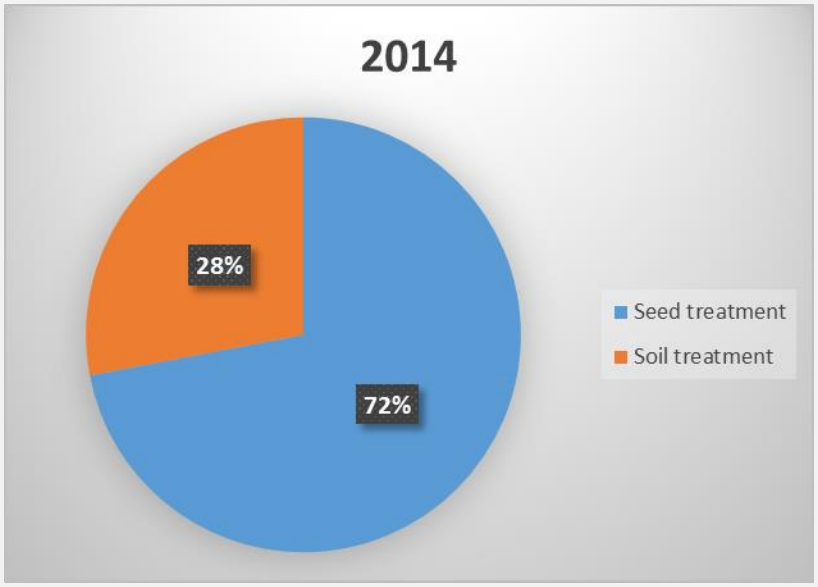

Fig.5. Global biofertilizers market revenue share by application

Furthermore, the growing demand for fertilizers to improve the production yield is boosting the sales of new fertilizer spreader across the globe. Vendors have come up with new models of spreaders with improved features such as extended spreading widths, intelligent speed monitoring systems to enhance spreading accuracy, and slow releasing fertilizer spreaders, which help in accurate fertilizer application and maintaining the quality of the soil. Other inventions in the spreaders include LED rear lighting systems, increased hopper capacities, and section shut-off systems. Such technological advancements and improved features will accelerate the volume sales of fertilizer spreaders during the forecast period. Technavio's market research analyst predicts the global fertilizer spreader market to grow at a CAGR of more than 6% by 2020.

Precision fertilizer spreaders will help to improve crop yields and ramp up production through calibration systems to regulate the quantity of fertilizer and mass flow controllers to monitor the amount of fertilizer required per subplot. Also, these spreaders will help in soil mapping, soil nutrient software packages to determine fertilizer application, and use satellite technology to guide fertilizer application. KUHN, AMAZONE, BBI, and Sulky are some of the popular brands for precision fertilizer spreaders in the market.

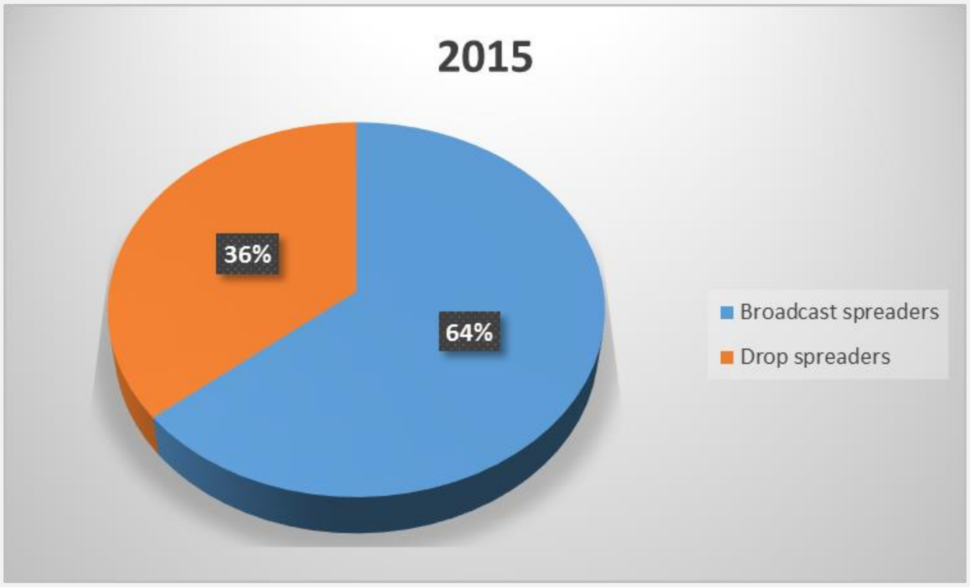

Fig. 6. Segmentation by product type and analysis of the fertilizer spreader market

The broadcast spreader segment dominated the market in 2015 and accounted for around 64% of the total market share (Fig.6). These spreaders are also known as rotary spreaders or centrifugal spreaders and are mainly used for spreading granular fertilizers. Consolidation of farmland will positively influence the growth of this segment in the coming years, as these spreaders are primarily suitable for use in large farms. Moreover, the vendors are introducing new broadcast spreaders with improved features such as balanced fertilizer distribution, GPS speed sensors to maintain the right speed, and pressure-based nozzle control systems to ensure a consistent pattern in the spreading of fertilizer.

Geographical segmentation of the fertilizer spreader market is between countries from Americas, APAC and EMEA. APAC will continue its dominance in the market during the forecast period and is expected to occupy more than 60% of the overall market share by 2020. Rising dependence on fertilizers for improved crop productivity is a major factor contributing to the region's high market share. The increasing focus on the quality of crop production has prompted farmers to use phosphorous and potassium fertilizers, thereby boosting the sales of fertilizer spreaders in the region.

Biofertilizers market revenue share by region

North America was the largest market, accounting about 32% of global biofertilizers revenue in 2014 (Fig.7). Positive agriculture industry outlook in the U.S. and Canada along with increasing awareness towards the application of eco-friendly products in farming is expected to have a favorable impact for biofertilizers market over the next seven years. Europe held 23% of global biofertilizers market revenue in 2014. The European Commission framed the „Horizon 2020 Strategy" in 2007, which aims at promoting consumption and production of eco-friendly products at domestic level. In addition, the commission also planned Action Plan 2020 for enhancing production of organic foods at domestic level.

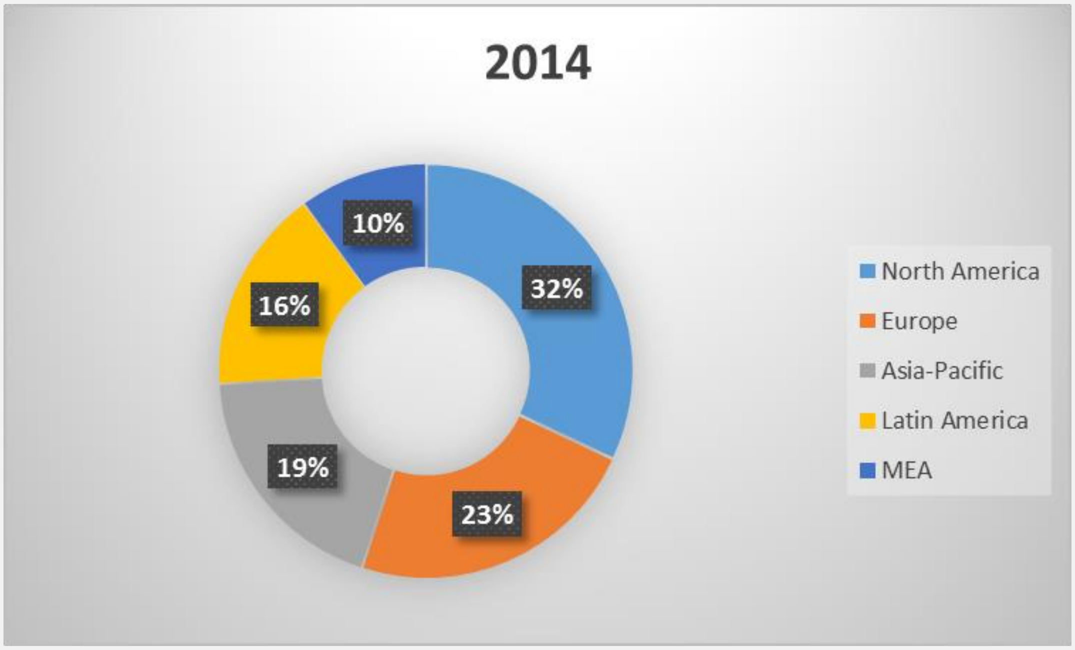

Fig.7. Global biofertilizers market revenue share by region

Prevalence of favorable government support for use of eco-friendly products and increasing production output of organic foods is expected to augment biofertilizers market growth over the forecast period. MEA and Asia Pacific are expected to remain promising markets over the next seven years. Lucrative opportunities in food & beverage market in Asia Pacific and MEA is expected to play a crucial role in enhancing agriculture output at domestic level and thus expected to increase application scope of biofertilizers over the next seven years.

Major companies in the sector of biofertilizer commercial production

Major companies in the sector include:

- Novozymes A/S
- Rizobacter Argentina S.A.
- Lallemand Inc.
- National Fertilizers Limited
- Madras Fertilizers Limited

BIO-FERTILIZERS MARKET SIZE

- Gujarat State Fertilizers & Chemicals Ltd.
- T Stanes & Company Limited
- Camson Bio Technologies Limited
- Rashtriya Chemicals & Fertilizers Ltd.
- Biomax
- Symborg
- Agri Life
- Kiwa Bio-Tech Products Group Corporation

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