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.

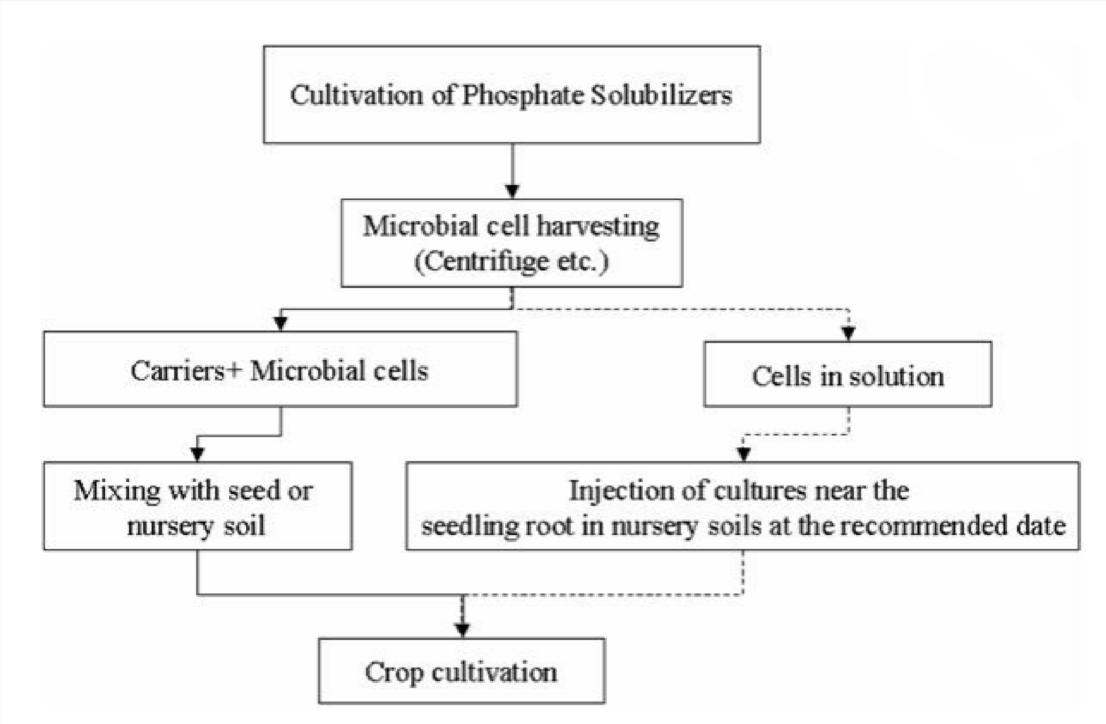

Fig. 1. Inoculation method of phosphate solubilizers

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 BIOFERTlLlZER 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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