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Use of biofertilizers in agriculture*

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Biofertilizer is generally used for all types of plant nutrient inputs which are of biological origin. Unlike chemical fertilizers and organic manures microbial biofertilizers are biologically active products containing one or more specific microorganisms like bacteria, bluegreen algae (Cyanobacterium) or fungi. These organisms are capable of nitrogen fixation, phosphate solubilization or phosphate mobilization. Besides accessing nutrients, for current intake and residual, different biofertilizers also provide growth promoting factors to plants, controlling soil borne diseases and improving the soil health and properties. The organisms help not only in saving but also in effectively utilizing chemical fertilizers and result in higher yield.

Key words: *Rhizobium, Azotobacter, Azospirillum,* phosphate solubilising bacteria, nitrogen fixation.

INTRODUCTION

The green revolution brought impressive gains in food production but with insufficient concern for sustainability. In India the availability and affordability of fossil fuel based chemical fertilizers at the farm level have been ensured only through imports and subsidies. Dependence on chemical fertilizers for future agricultural growth would mean further loss in soil quality, possibilities of water contamination and unsustainable burden on the fiscal system. The deterioration of soil health occurs mainly due to reduction in organic matter of soil coupled with diminishing microbial load. Replenishment of soil with these microorganisms by artificial means leads to the technology, namely, the biofertilizer technology. The Government of India has been trying to promote an improved practice involving use of biofertilizers along with fertilizers. The concept of biofertilizer science is an age old one. However, the commercial production of biofertilizer has been commenced in 1895 by a USA based industry. In the Indian subcontinent,this still remains in the juvenile stage.

Indian agriculture is still based on traditional farming practices. There is a strong temptation to spread the use of chemical intensive agricultural technology and practices to new areas offering more serious environmental consequences. There is a wide spread evidence that ecological farming can be high yielding than the green revolution method.

EFFECT OF CHEMICAL FERTILIZERS IN SOIL AND ENVIRONMENT

Excessive and imbalanced use of chemical fertilizers has adversely affect the soil causing decrease in organic carbon, reduction in microbial flora of soil, increasing acidity and alkalinity and hardening of soil. Excessive use of N-fertilizer are contaminating water bodies thus affecting fish fauna and causing health hazards for human beings and animals. Production of chemical fertilizers adds to the pollution. Use of biofertilizers in combination with organic manures offers a great opportunity to increase the crop production *vis-à-vis* sustainability. But one should keep in mind that biofertilizers should be of right type and have to be applied at the right time to derive maxium benefit. Carrier based or liquid formulation of these microorganisms are generally applied in the field, and regular practice of such biofertilizers may lead to the saving of about 30-35% nitrogenous and 20-25% phosphatic fertilizers.

WHAT IS BIOFERTILIZER ?

Biofertilizers are ready to use live formulates of such beneficial microorganisms which on application to seed, root 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. Nitrogenous biofertilizers harvest

*12th S.N. Banerjee Memorial Lecture delivered on January 06, 2010 atmospheric nitrogen and convert into ammonical form, which in due course is made available to the plants or is released in the soil. Phosphatic biofertilizers solubuilize fixed forms of phosphorus already present in the soil and make it available for use of plants. Composting biofertilizers are used for hastening the process of composting and for enriching its nutrient value. To overcome the deficit in nutrient supply and to overcome the adverse effects of chemical cultivation the scientific fraternity searches for alternatives to exploit all the available resources of nutrients under the theme of integrated nutrient management. Under this approach the best available option lies in the complimentary use of biofertilizers, organic manures in suitable combination with chemical fertilizers. This integrated approach of nutrient mangement not only ensures higher productivity but also ensures the good health of our soil and environment.

BENEFITS OF BIOFERTILIZER

The benefits of biofertilizer are many and some of them are mentioned here.

a. Increase crop yield by 20-30%
b. Replace chemical N&P by 25%
c. Stimulate plant growth
d. Activity soil biologically
e. Restore natural fertility
f. Provide protection against drought and some soil borne diseases.

TYPES OF BIOFERTILIZERS

There are several types of biofertilizer but most important of them are the following types :

***For Nitrogen* –** the N fixing microorganisms

Rhizobium for legume crops

Azotobacter / Azospirillum for non legume crops

Acetobacter for sugarcane

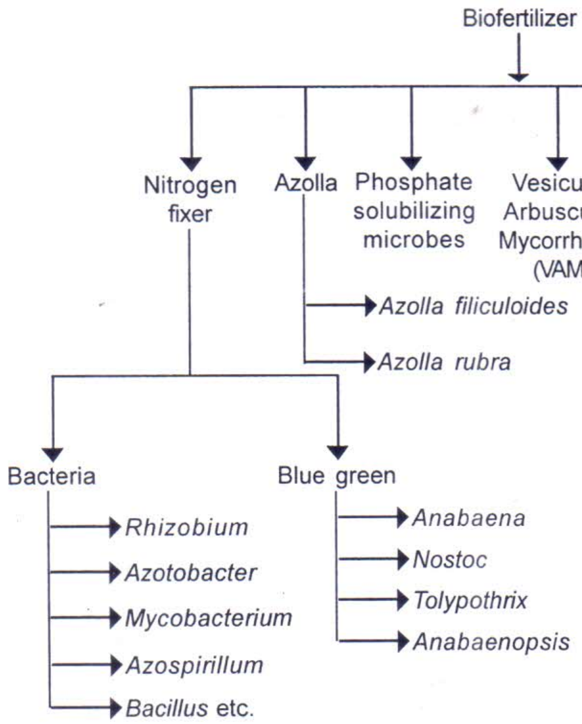
Azolla is used in paddy as green manure form and dual crop form

Blue green Algae (Cyanobacterium) is suitable for low land paddy

For Phosphorous – the P solubilizing microorganisms

Bacillus sp., *Pseudomonas* sp.,*Aspergillus* sp., *Penicillium* sp.

Michorrhizal inoculants - for P mobilizes (Phosphatika for all crops to be applied with *Rhizobium, Azotobacter*, *Azospirillum* and *Acetobacter*)



COMPONENTS OF BIOFERTILIZERS

Nitrogen fixing bacteria

Contained in the root nodules of legumes.

Free living

convert nitrogen from the atmosphere into ammonium (NH_4).

Phosphorous solubilizing bacteria

Solubilizing unavailable organic and inorganic forms of phosphorus (80%). Organic P (e.g., purine, pyrimidine, polyphenols) slowly mineralized by the action of phosphatases.

Inorganic P (e.g., Ca-P, Al-P) solubilized by the action of organic and inorganic acids. During microbial phosphate solubilization, organic acids are produced by microorganisms resulting in the lowering of pH from 7.0 to 3.0. The most commonly produced acids include citric, fumaric, lactic, malic, glyoxalic, succinic, tartaric and α-ketobutyric acid by *B. megaterium, B. circulans, E. freundii* and *P. striata. A. awamori* and *P. digitatum* biosynthesize only citric, succinic and tartaric acid. The nitrifying bacteria and *Thiobacillus* spp. produce nitric and sulphuric acid during the oxidation of nitrogenous or inorganic sulphur compounds which react with calcium phosphate and convert them into soluble form. The most efficient phosphate solubilizing fungi belong to genus *Aspergillus* and *Penicillium* obtained in tista soils, acidic lateritic soils and compost. *Aspergillus aculeatus* was best in solubilizing tricalcium phosphate (94%) followed by dicalcium (54.5%) and aluminium phosphate (31.8%). Ferric phosphate was best solubilized by *Asperigillus niger. Pseudomonas putida* has been reported to solubilize tricalcium phosphate to the extent of 50%. Temperature and salt tolerant strains of *Bacillus circulans* can solubilize more than 50% P_2O_5 as tricalcium phosphate at 45°C and 3.5% NaCl. Acid tolerant strain of genus *Bacillus* is reported to exhibit a solubilization of 45μg P ml^{-1}. The use of PSM, not only improves the phosphate nutrition of both the crop and soil but also increases the fertilizer use efficiency. Application of rockphosphate in neutral to alkaline soils in conjunction with efficient PSM is as good as the sole application of superphosphate. The practice of using microphos inoculants can easily be adopted by the farmers due to being cost effective and eco-friendly.

Azotobacter

The fixed N in *Azotobacter* cells is nitrified after its death and decay. Plants can utilize this nitrogen from *Azotobacter* plasma. It is found that only 30 per cent of total nitrogen of *Azotobacter* plasma can be utilized over a period of two years. Amount of nitrogen fixed by *Azotobacter* in soil is estimated to be in the range of 0.1 - 60 kg ha-1 annually to 10-40 kg ha-1 in soil and rhizosphere. *Azotobacter* has also been reported to assimilate amount of nitrogen which is equivalent to 1.3 - 2.0 kg of sodium nitrate applied to the soil. Berezova *et al.* (1938) are the first one to report the presence of auxin like substances in *Azotobacter* culture. Besides auxins, *Azotobacter* cultures are also found to produce various kinds of vitamins, e.g. B-group of vitamins, Nicotinic and Pantothenic acids, Biotin and Cynocobalamine, phytohormones like IAA, GA and Cytokinin . *Azotobacter* is a root symbiont and its particular strains are specific for particular plants growing under specific agro-climatic conditions. Wani *et al.* (1976) have reported that maximum increase (77.13%) in yield of "Suhashini" variety of paddy is obtained due to the application of *Azotobacter* isolated from the rhizosphere of the same variety. *Azotobacter* is also reported to improve the phosphate uptake of the plant by solubilizing tricalcium phosphate.

Azospirillum

Among the non-symbiotic nitrogen fixers, *Azospirillum* inoculation is economically profitable. *Azospirillum* exerts its effect mainly at early stages of plant development after optinal colonization of roots. Pure cultures of *A. brasilense* produce gibberellins, cytokinin - like substances and elaborate auxins from tryptophan. Phytohormones produced by *Azospirillum* stimulate the density and length of root hairs. Despite their N_2-fixing capability, the increase in yield caused by *Azospirillum* inoculation is mainly attributed to an improvement in root development resulting in increased water and mineral uptake. Inoculation with *Azospirillum* inoculation leads to enhanced uptake of mineral nutrients like. NH^+_4, $H_2PO^+_4$, K_+, Rb^- and Fe_2^+

Nutrients uptake enhancing fungus (Mycorrhizae)

Maximizing absorption area (Mycorrhizae can penetrate smaller crevices crevices than root hairs) of plants. Improve soil texture. Increase water uptake in plant. Increase mineral uptake (especially P, Cu & Zn). Sequester heavy metals (As, Pb). Limits uptake of Al, As, Ti, Ba & Cd.

Blue green algae (BGA) and Azolla

BGA are photosynthetic nitrogen fixer. They too add growth promoting substances including vitamin B-12, improve the soil aeration and water holding capacity.

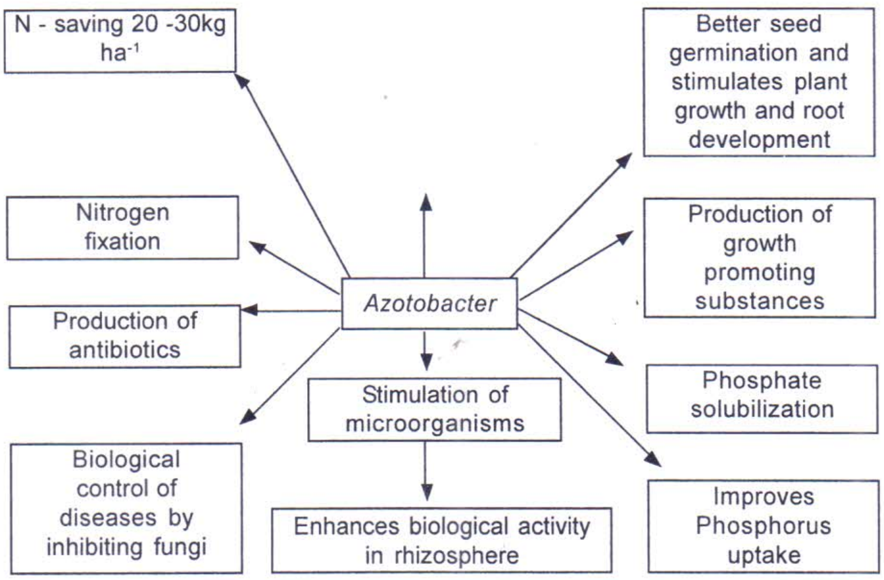
Azolla is an aquatic fern found in rice field. It has symbiotic relationship with BGA and can help rice and other crop through green manitoring of soil.

in the quality of the inputs recommended. Fourth impediment is 'lack of methodology' to prove productivity *vis-à-vis* production of particular crop or soil region attributable directly to usage of biofertilizers. The fifth bottleneck is indiscrimate use of micro nutrients and plant hormones. The sixth stumbling block is lack of coordinating machinery/ outfit.

Strain selection

The two criteria used in selecting a strain for mass production of *Rhizobium* inoculant are : the ability to form effective nitrogen fixing nodules with all the hosts for which the culture is the ability to form effective nitrogen fixing nodules with all the hosts for which the culture is recommended and the ability to process this trait under a wide range of field conditions. These two criteria coupled with a capacity to survive in soil saprophytically in the

Multiple action of *Azotobacter*



Constraints for biofertilizer production and its spread among farmers

The first stumbling block is farmers 'mindset' that exists post green revolution time-the deep rooted bias towards "chemical fertilizers". The second barricade is 'time' taken to bring about nutrition release to crops. The third obstacle is 'uniformity'

absence of the host would be additional advantage. The ability of an introduced rhizobial strain, a criterion which can be arrived at by careful laboratory work, field testing and serological studies. In case of *Azotobacter chroococcum, Azospirillum brasilance, Azospirillum lipoferum,* criteria selecting strains for mass production should be higher nitrogen fixation capacity as well as the capacity to produce growth regulator. In case of PSB, the criteria for strains in selecting for mass production are : the ability to solubilise fixed phosphate in higher scale.

Checking the broth

At the end of fermentation, the broth is checked for freedom from contaminants by the following methods.

i) pH-test, if above 8 or below 6, the broth is suspected to be contaminated,

ii) a Gram smear test from diluted broth culture for detection of Gram (+) contaminants, spore formers etc.,

iii) incubation of the broth on peptone agar medium – if abundant growth takes place within 2 days, suspect contaminant of this broth,

iv) streak the broth of YEM agar for verification of this pattern of growth of *Rhizobium.*

Counts of cells in broth

This is done by the normal plate count method to determine the viable count at 28^{0}C ($\pm$2^{0}C) incubation. When count in this manner, broths having viable cells higher than 10^9/ml may be used.

Storage of broth

It is not advisable to store the broth after fermentation for periods longer than 24 hrs. even at 4^{0}C since counts of viable cell begin to decrease.

Carrier based inoculants

Peat, lignite or charcoal is ground to fine powder capable of passing through 100 mesh sieve and heat treated followed by neutralization with $CaCO_3$ to raise pH of the carrier to 6.8. Dilute broth having rhizobial cell population in excess of 10^9 cells/ml is blended with the carrier (at the ratio 1:3) so as to bring the final moisture content of carrier to 35 – 40% on wet basis. The resulting product shall have at least 300 million (3 x 10^8) rhizobia per gram of carrier. At higher temperatures (25 – 35^{0}C) of carrier, a number of rhizobia falls below optimum when carrier based inoculants are stored beyond 2 – 3 months whereas storage at 4^{0}C, prolongs the shelf life of cultures up to 12 months.

WHY BIOFERTILIZERS ARE NOT SO POPULAR?

Inspite of several long lasting benefits the bio-inoculants are not very popular among the farming community. There may be several reasons for this, however some of the important ones are as given below :

Nutrient contribution is dependent on survival of organisms.

Low carbon content of soils - low proliferation.

Water scarcity - possibility of desiccation

Fluctuating soil pH - variable microflora.

Extreme temperature - in summer months.

Shelf-life of organisms.

Poor storage and transportation.

Lack of awareness.

Eagerness to look for instance effects.

FUTURE PROSPECTS OF BIOFERTILIZERS

The integrated approach of nutrient management not only ensures higher productivity but also ensures the good health of our soil and environment. In addition to the benefits, in public domain farmers save input cost in terms of chemical nitrogenous and phosphatic fertilizers. Introduction of liquid culture may help improving the efficiency of biofertilizers through longer life of organisms and minimum chance of contamination of strains. Grain quality is better.

SUGGESTIONS

If the CFU value is higher in 10^{-7} dilution, then the contamination at 10^{-5} dilution may be ignored, because high CFU will maintain the efficiency of biofertilizers and suppresses the contaminant. So we look after this item. Particle size at carrier materials is also important; as the size of particle increased the moisture retention capacity will be reduced by the carrier, so size should be maintained. Moisture level should be maintained within 30-40% (max.), otherwise the chance of contamination will be increased. Low moisture will also affect the CFU value. Inoculants should be stored at low temperature (10-15^{0}C),otherwise the CFU value will be reduced.

Average comparative analysis of different parameters using three methods

Using conventional method (per Bigha)			Using hem..Fertilizer as recommended by Govt. of W.B. with application of Bio-fertilizer (Azophos) (per Bigha)			30% N & 25% P reduction in case of chem. fertilizer with application of Biofertilizer (Azophos) (per Bigha)		
Name of fertilizer	Qty(Kg.)	Cost	Name of fertilizer	Qty(Kg.)	Cost	Name of fertilizer	Qty(Kg.)	Cost
N:P:K 10:26:26	125	1087.50	Urea	60	300.00	Urea	45	225.00
Urea	30	150.00	SSP	100	330.00	SSP	75	247.00
Different Micronutrient etc.		100.00	MOP	27	121.50	MOP	27	121.5
			Azophos (6 X 400g)	2.4	144.00	Azophos 2.4 (6 X400g)		144.00
		Rs. 1337.50			Rs.895.50 .			Rs. 738.00
Yield		3.60 MT			3.90 MT			4.40 MT

SPECIFICATIONS AND SUGGESTIONS

1. ***Rhizobium* standard**

i. Base — Carrier based or liquid based

ii) Viable cell count — CFU minimum 10^7 cell / g carrier material or 10^7 cell / ml of liquid material

iii) Contamination level — No contamination at 10^{-5} dilution

iv) pH — 6.5 – 7.5

v) Particle size in case of carrier based material — All materials shall pass through 0.15 - 0.212 mm IS sieve

vi) Moisture per cent by weight (max.) in case of carrier based — 30 – 40 %

vii) Efficiency character — Should show effective nodulation on all the species listed on the packets

** Type of carrier — Charcoal or similar favouring growth of the organism

2. ***Azotobacter* standard**

All the items are same as *Rhizobium* except item No. (vi) — Strain should be capable of fixing at least 10 mg of nitrogen / gm of sucrose consumed

Efficiency character

3. ***Azospirillum* standard**

All the items are same as *Rhizobium* except item No. (vii)

Efficiency character — Formation of white Pellicle in semi solid nitrogen free bromothymol blue media

** Types of carrier — The carrier such as peat, lignite, peat soil, humus, wood charcoal or similar materials.

4. **Phosphate solubilizing (PSB) standard bacteria**

All the items are same as *Rhizobium* except item No. (vii)

Efficiency character — The strains should have phosphate solubilizing capacity in the range of min. 30%, when tested spectrophotometrically. In terms of zone formation, min. 5 mm solubilization zone in prescribed media having at least 3 mm thickness within 4 – 6 days.

From the point of view of food quality and food safety organic agriculture in modern times was initiated in countries that reached highest level of chemical use and productivity in agriculture.

Organic agriculture gaining momentum among farmers in the country for reasons of the produce getting premium price and providing food safety to the consumers. This has gained importance since the time when the umbrella organization "International Federation of Organic Agriculture Movement" (IFOAM), has been constituted in 1972 at Paris to provide information and knowledge.

Consumption of Biofertilizers

Year	Project requirement (MT)	Availability (in MT)
2005-06	649.00	400.00
2006-07	750.00	465.00
2007-08	790.00	690.00
2008-09	910.00	800.00
2009-10	1050.00	950.00 (anticipated)
2010-11	1170.00	1050.00 (anticipated)
2011-12	1300.00	1170.00 (anticipated)

Source : Department of Agriculture, Government of West Bengal

The West Bengal government which is keen to spread organic agriculture has resolved to set up one bio village in each of the 341 blocks in the state in the next 2 years. The agriculture department has decided to carry out the programme on a shoestring budget and submitted a proposal to a finance department, asking for just 1 core for each year from 2009-10 and 2010 - 11. The shortage of man power at the government level is another problem to execute the programme.

Lastly it may be concluded that biofertilizers have various benefits. Beside accessing nutrients, for current intake and residual, different biofertilizers also provide growth promoting factors to plants and some have been successfully facilitating composting and effecttive recycling of solid waste. By controlling soil borne diseases and improving the soil health and properties. The organisms help not only in saving but also in effectively utilizing chemical fertilizers and result in higher yield.

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